KERATINS AND SKIN DISORDERS

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The epidermal keratinocytes express two major pairs of keratin polypeptides. One pair (K5/K14) expressed specifically in basal generative compartment and the other (K1/K10) expressed specifically in the differentiating suprabasal compartment. The switch in the expression of the keratins from proliferating to differentiating compartment indicates the changes that occur in the keratin filament organization which in turn influences the functional properties of the epidermis. Proper regulation of keratin gene expression and the filament organization are absolutely necessary for normal functioning of the skin. Keratin gene mutations can influence the filament integrity thereby causing several heritable blistering disorders of the skin such as epidermolysis bullosa, bullous ichthyosiform erythroderma, etc. Changes in the keratin gene expression may lead to incomplete differentiation of the epidermal keratinocyte, causing hyperproliferative diseases of the skin such as psoriasis, carcinomas, etc. This review briefly describes the changes in keratin structure or gene expression that are known to result in various disorders of the skin.

INTRODUCTION

The skin is one of the largest and perhaps the most complex organs of the human body and provides a protective interface between the organism and the environment. It is adapted to withstand a variety of physical, chemical and biological trauma, and acts as a thermoregulator and as a transducer of environmental information (Fuchs and Coulombe, 1992). Histologically, the skin is divisible into an outer epidermis, a middle dermis and an inner hypodermis. The epidermis is entirely cellular and is made of cells that are different both structurally and functionally (Bowden et al., 1987). Central to this variegated cell population are keratinocytes, the epithelial cells that produce keratin. Keratinocytes are generated by mitosis in the basal or germinative layer and migrate outwards through successive stages of differentiation (spinous, granular and horny layers) towards the surface where they are cornified and shed. The horny layer is composed of flattened and fully keratinized cells that are devoid of nuclei. Transition from the living granular layer to the anucleate horny layer is rather rapid (Fischer et al., 1991). In addition to keratinocytes, the epidermis contains three more cell types (Fig. 1), the neural crest emanated pigment cells or melanocytes, the bone marrow derived langerhans cells and the receptors of touch, the merkel cells (Priestly, 1993).

All eukaryotic cells contain a complex cytoskeleton composed of three different structural proteins: actin-containing microfilaments (6 nm in diameter); tubulin-containing microtubules (25 nm diameter); and intermediate filaments (IF) (10 nm diameter) (Achtstatter et al., 1986; Steinert and Bale, 1993). Based on information about gene structure and protein sequence data, the IF proteins are classified into six subtypes: type I and II—keratins; type III—vimentin, peripherin, desmin and GFAP; type IV—a-internexin and neurofilaments; type V—lamin; and type VI—nestin (Parry and Steinert, 1992; van de Klundert et al., 1993). Of all the IF proteins, keratin IFs are the most complex and are considered as the hallmark proteins of epithelial cell differentiation (Singh and Gupta, 1994b).
Keratinization of the epidermal cells is a tightly regulated multistep differentiation programme that results in cellular stratification. The basal keratinocytes divide and the resulting daughter cells migrate outwards and differentiate. Two major pairs of keratins get expressed in the epidermis—one expressed specifically in the dividing basal cells; and the other in the suprabasal cells that mark the beginning of cell differentiation and in the cells above (Fig. 1). During the epidermal cell migration, the induction of markers for a specific stage in epidermal differentiation is coupled with the repression of markers expressed at the preceding stage (Dlugosz and Yuspa, 1993). Epidermal differentiation also involves induction of proteins such as involucrin, epidermal and keratinocyte transglutaminase, filaggrin and loricrin, all of which are necessarily expressed in the upper layers of the epidermis (Fuchs and Green, 1980; Eckert and Rorke, 1989).

**Keratins**

The keratins are a complex family of proteins that form the IF system in epithelial cells (Moll et al., 1982). The keratin family consists of about 30 polypeptides each coded by a distinct gene (Fuchs et al., 1987). The keratin IF genes have been found to be localized on different chromosomes. In general, type I keratin genes are located in two clusters on chromosome 17, one cluster on the short arm and another on the long arm. The type II keratins are localized in a single cluster on chromosome 12 (Bowden, 1993). Keratin expression is highly heterogenous and varies according to the epithelial cell type, period of embryonic development, stage of epithelial cell differentiation, disease state and cellular growth environment (Eichner et al., 1984). Keratins have been shown to be the first differentiation proteins that are expressed in embryos (Brulet et al., 1980; Singh and Gupta, 1994a). Indeed, keratins have been identified to form discrete filaments in the 2 to 8 cell embryo stage in golden Hamsters (Planche et al., 1989).

The protective function of the skin in general is a result of the cellular arrangement within the epidermis and the building up of extensive keratin cytoskeletal network (Fuchs, 1993). The intermolecular protein interactions that take place in the early stages of the assembly of epidermal...
keratins are remarkably stable and thus enhance the stability of keratins (Fuchs, 1990). Epidermal keratins also impart mechanical integrity and structure to the epidermis (Coulombe and Fuchs, 1991). The proper regulation of keratin gene expression and the formation of functional keratin IF network are essential for normal epidermal function (Bowden, 1993). Tampering with keratin synthesis leads to alterations in cell movement or cell differentiation and hence the subsequent function (Vassar et al., 1991; Singh and Gupta, 1994a). About 50 different skin disorders are known (Fig. 2) to result from changes in keratin filament appearance and organization (Griffiths, 1991). It has been shown that defective filament organization in the basal cells of transgenic mice results in inadequate stratification (Vassar et al., 1991). Mutations in keratin genes are found to be responsible for several skin disorders (see below).

**Molecular organization of keratin filaments**

The common denominator of all IF proteins is a central $\alpha$-helical rod domain containing about 310–315 amino acid residues. However, the flanking non-helical ends are variable from one IF protein to another. These variable regions contribute for the variation in the molecular mass of IF proteins (Fig. 3). The $\alpha$-helical rod domain is highly conserved both in size and secondary structure. Keratins being obligatory heteropolymers, at least one type I (acidic) and one type II (basic-neutral) chains are required for filament assembly (Hatzfield and Franke, 1985; Singh and Gupta, 1994b). The basic subunit of keratin filaments is a heterotypic tetramer of two polypeptide chains of each type I and type II keratins. The IF dimer comprises of a coiled coil made of two parallel polypeptide chains in an axial register (Steinert, 1991) (Fig. 4). Fuchs et al. (1987) have shown that the tetramers (protofilament) are formed by an antiparallel array of two dimers. Two such tetramers form a protofibril and generally four such protofibrils assemble to form a filament of 8–10 nm (Fig. 5).

**Keratins in epidermal development**

Human embryonic epidermal development identifiable by histology, ultrastructure and biochemistry can be divided into (a) embryonic period (7–9 weeks), (b) epidermal stratification (9–10 weeks), (c) follicular keratinization (14 weeks), and (d) interfollicular keratinization (~24 weeks). Keratins are found as early as by the 2–8 cell embryo stage. In the embryonic period, the epidermis is represented by only basal and peridermal cell layers. An intermediate layer without any keratinization is seen in addition, in the epidermal stratification phase. In the follicular keratinization phase the epidermis stratifies into three layers with the formation of hair follicles which begin to keratinize and trichocyte keratins start to express themselves.
By interfollicular keratinization phase, basal, spinous, granular and cornified cell layers are all recognizable (Dale and Hollbrook, 1987).

Keratins in skin, hair and nail

Keratins constitute 30% of the protein in the basal cells of the stratified epithelium and >85% in fully differentiated squames. Epidermis expresses predominantly two pairs of keratin polypeptides. Basal cells express K5 (type II basic keratin of 58 kDa) and K14 (type I acidic keratin polypeptide of 50 kDa). As the basal cells divide and enter the first suprabasal layer a downregulation of K5 and K14 expression and the induction of K1 and K10 expression occurs (Fuchs and Green, 1980; Moll et al., 1982). The type II keratin K1 (67 kDa) and type I keratin K10 (56.5 kDa) are only synthesized in differentiating epidermal cells. This synthesis continues up to 4–8 spinous cell thickness and represents one of the earliest changes indicating the commitment of the cell to terminal differentiation. This switch in synthesis to differentiation-specific keratins correlates well with increased bundling of keratin filaments at the basal suprabasal transition (Steinert and Roop, 1988). Filament assemblies in vitro have demonstrated that K1 and K10 self-aggregate more than the K5–K14 pair, and that the K1–K10 pair are less easily solubilizable (Fuchs, 1990). Biochemically, type I and type II keratin pairs that are co-expressed in basal cells have peptide repeats rich in serine whereas those expressed in differentiated cells have glycine-rich
sequences (Roop, 1987). This change in the basal-suprabasal-specific keratin polypeptide expressions may be essential for filament stability. In a natural situation several accessory proteins also play a pivotal role in the supramolecular organization of keratin filaments.

The hairs and nails, which are epidermal appendages, exhibit a complex morphology. The outer root sheath of hair follicle is a keratinizing epithelium and expresses a different set of keratin polypeptides K6 and K16 in addition to K5/K14. The upper outer root sheath contiguous with the epidermis expresses K1 and K10 suprabasally but the expression of these keratins is reduced in the region just above the sebaceous gland (Abling et al., 1992; Bowden, 1994). There is yet again some evidence that K17 and K19 are also expressed in hair follicles. Nails also show a similar profile of keratin expressions. In addition, hair and nail contain distinct subset of eight major hair-specific keratins, four acidic and four basic (low-sulphur \( \alpha \) keratins) HaKa1–4 and HaKb1–4 and two minor keratins (HaX and HbX) (Heid et al., 1988a,b).

A fine balance must exist between the dividing and differentiating cells in the epidermis. Any modifications that result in altered expression or structural composition of keratin filaments may lead to pathological changes.

**Keratin and skin disease**

In this review, we would like to summarize some skin disorders that are the result of altered keratin expression or assembly. A great deal of information pertaining to the structural and functional properties of keratin filaments, filament assembly and network formations in health and disease is now available (Fuchs and Weber, 1994). Alteration in the expression of keratins experimentally or otherwise can result in drastic changes in a variety of cell functions (Gupta et al., 1992a,b; Singh and
Gupta, 1994a; Singh et al., 1994). Interestingly, most of the genetically determined keratin disorders in skin are associated with mutations in the central rod domain (Fig. 6), which is crucial for filament formation. Perturbations in the filament formation or filament assembly may provide some cues to the cellular machinery that regulates the keratin expression per se and its influence on the whole gamut of division/differentiation programme in the epithelial cells in general and of the epidermis in particular (Fuchs, 1993). In fact, in the mitotic phase, in some of the epithelial cells, the keratin filaments reorganize into condensed non-cytoskeletal spheroidally shaped structures distributed all through the cytoplasm (Horowitz et al., 1981; Knapp and Bunn, 1987). In addition to mutations, agents that can disrupt filament assembly through certain post-translational modifications such as phosphorylations, etc. (Celis et al., 1985; Gupta et al., 1990a) can influence keratin functions. Hormones such as estradiol and vitamin D3, growth factors such as EGF, drugs like retinoids, carcinogens or agents such as TPA may adversely influence filament assembly, thereby affecting the division and/or differentiation of epithelial cells (Gupta et al., 1990a).

The application of molecular biological techniques in recent times to the characterization of genes that encode for keratins and other structural proteins in epidermis has made it possible to understand the aetiopathogenesis of many skin diseases. The knowledge on the structure of keratin IF and their dynamics in living cells has made it possible to understand the variations in the constituent keratin proteins which are responsible for pathology.

The clinical severity of some examples of epidermolysis bullosa warrants development of non-invasive prenatal diagnostic techniques for identifying the potential risk of the disease (Christiano and Uitto, 1994). With the advances in molecular biological techniques it is now possible to develop certain DNA-based techniques for prenatal diagnosis of certain inheritable disorders of the skin. A change in the single nucleotide in (keratin) genes can now be detected using specific probes that are available. Specific defects in keratin expression, filament formation and assembly are thought to play a pivotal role in the causation of genetic disorders like Epidermolysis Bullosa Simplex (EBS), Bullous ichthyosiform erythroderma (BIE) and palmoplantar keratoderma [Vorner] (McLean and Lane, 1995). Altered keratin expression appears to be secondary in hyperproliferative epidermal disorders like psoriasis but such altered expressions still serve as important markers of epidermal differentiation status (Bowden, 1993). Further, keratin expression also changes in skin carcinogenesis, although such disorders are unlikely to be the result of primary defects in the keratin genes themselves. Keratin expression has also been found to be abnormal in keloids and hypertrophic scars (Ramakrishnan et al., 1995). It has also been proved that physical factors like light can influence the expression of keratins in a given situation (Horio et al., 1993; Smith and Rees, 1994).

**Epidermolysis bullosa**

Epidermolysis bulosa (EB) comprises a group of genetically distinct diseases characterized by blistering of skin and mucosae. The blisters may either result from minor mechanical trauma or apparently arise spontaneously. As many as 23 EB variants are recognized and delineated based on their clinical appearance, extracutaneous involvement, mode of inheritance and the level of the blisters (Coulombe and Fuchs, 1993).

Epidermolysis bulosa simplex (EBS) is generally transmitted autosomal dominantly although two rare variants with autosomal recessive inheritance exist. The dominant forms are most common. On the basis of differing clinical presentation, ultrastructural characteristics and keratin abnormalities, four major subtypes of EBS are identified. These are the Kobner, Dowling-Meara, Weber-Cockayne and Ogna variants. The four major EBS subtypes have a combined incidence of 1:25,000 (Fine et al., 1991).

It has been recognized that in EBS the cleavage occurs within the basal layer of the epidermis in a defined cytoplasmic zone located between the nucleus and the hemi-desmosomes. Characteristic ultrastructural features are seen in different forms of EBS. The presence of tonofilament clumps in the basal layer constitute an ultrastructural hallmark for EBS (Dowling-Meara) (Anton-Lamprecht and Snyder, 1982). These clumps are immunoreactive to antisera monospecific for basal layer-specific keratins (K5 and K14) (Vassar et al., 1991; Ishida-Yamamoto et al., 1994). Formation of tonofilament clumps precede blistering. Tonofilament clumping is not seen in Kobner, Weber-Cockayne or Ogna variants of EBS, although changes in the keratin filament network of epidermal basal cells have been described in some of them (Coulombe and Fuchs, 1993).

It is now clear that EBS may have a defect in keratins as its genetic basis (Coulombe et al., 1991).
Studies on the pathogenesis of EBS (Dowling-Meara) represent the prototype for much that has been learnt about other type of EBS. Keratin mutation studies involving EBS cell culture and transgenic mice experiments strongly suggest that the manifestations of at least some examples of EBS are due to perturbations in the architecture of K5 and K14 keratin filament network in the basal cells of epidermis. Point mutations in either K5 or K14 coding sequence have recently been discovered in several instances of EBS (Coulombe et al., 1991; Lane et al., 1992). These mutations are responsible for the manifestation of EBS is supported by two lines of evidence (Fuchs and Weber, 1994). First, chromosomal linkage analysis experiments have mapped the genetic defect in three different EBS kindreds to either the long arm of chromosome 17 (Bonifas et al., 1991; Ryyaner et al., 1991; Hovnayan et al., 1993) at a location known to contain a cluster of type I keratin genes including K14, or the long arm of chromosome 12 at a location where a cluster of type II keratin gene including K5 are located. Secondly, genetic engineering of the specific EBS (Dowling-Meara) point mutations in the cloned K14 cDNA, followed by testing in vivo and in vitro have proved that these specific mutations can cause the tonofilament clumping and shortening of keratin filaments that are the diagnostic markers of the disease (Coulombe et al., 1991; Letai et al., 1993). These data prove that aberrant heterodimer formation compromises filament integrity of the basal keratinocytes and results in disease expression (Singh and Gupta, 1994a). Transgenic experiments indicate that the severity of the clinical disease correlates with the degree of disruption of filament formation in vitro (Coulombe and Fuchs, 1993). Initial studies by Coulombe et al. (1991) have characterized the K14 mutations in two EBS (Dowling-Meara) patients. At amino acid 125 (the amino end of the rod domain of K14) they found arginine (Arg) to cystine (Cys) and arginine to histidine (His) mutations in half the K14 mRNAs. Subsequent studies (Rugg et al., 1993; Letai et al., 1993) have found that point mutations of Arg (331) to Cys or His in K5 and valine (270) to methionine in K14 are generally associated with EBS. Both these mutations localize in L12 linker region (Fig. 6) that bisects the á-helical rod of the intermediate filaments indicating that the structural integrity of keratin intermediate filaments may be defective in the basal cells. The non-helical linker region also plays a significant role in filament integrity (Chan, 1994). Lane et al. (1992) have reported a complementary mutation in K5, a change from glutamic acid to glycine in the helix termination peptide. The two conserved helix boundary peptides are essential for keratin filament assembly.
Bonifas et al. (1991) found genetic linkage of EBS (Kobner) to chromosome 17 and elucidated a point mutation (T–C) within exon 6 of keratin 14 gene. This mutation results in a leucine to proline change in keratin 14 polypeptide. Humphries et al. (1990) have demonstrated linkage in one of generalized EBS (Kobner) kindred to chromosome 1q although this linkage is less well elucidated. However, the derangements of keratin biology in localized EBS are not clear. In one with localized EBS (Weber-Cockayane), linkage to chromosome 12 near the K5 gene was found (Bonifas et al., 1991). Enhanced tonofilament condensation in the basal cell has been observed in localized EBS (Haneke and Anton-Lamprecht, 1982).

Bullous icthyosiform erythroderma (BIE)

BIE is a rare autosomal dominant disorder of cornification with a prevalence of approximately 1:100,000 to 1:300,000 (Bale et al., 1993). At least one half of the cases are sporadic and presumed to be the result of new mutations in keratin gene (Goldsmith, 1976). The disease manifests itself shortly after birth and is characterized by generalized erythema, scaling and blister formation, especially at sites of trauma and friction. With time blisters cease to appear and the skin becomes keratotic and even verrucous, particularly in the flexural areas, knees and elbows. Hyperkeratosis of palms and soles sometimes occurs.

With light microscopy BIE affected skin shows a tremendous thickening of stratum corneum and vacuolar degeneration of the upper epidermis (epidermolytic hyperkeratosis). Additionally, the epidermis may show the presence of coarse keratohyaline granules in the granular layer and epidermal acanthosis. Clumping of filaments is observed to begin with the first suprabasal layer under electron microscope. These aggregated filaments have been shown to be clumps of keratin intermediate filaments that contain the terminal differentiation specific keratins K1 and K10 (Ishida-Yamamoto et al., 1992).

Genetic linkage studies, gene sequencing and transgenic mice models indicate that K1 and/or K10 defects underlie the pathological basis of at least some examples of BIE. Linkage analysis has revealed linkage to markers on chromosome 12 q, the known locus for type II keratin gene clusters (Compton et al., 1992; Bonifas et al., 1992; Pulkkinen et al., 1993). Point mutations in either K1 or K10 genes of patients with BIE have been identified (Cheng et al., 1992; Chipev et al., 1992; Rothnagel et al., 1992; Yang J-M et al., 1994; McLean et al., 1994). That these mutations disrupt keratin filament network in a dominant negative mode is also supported by in vitro experiments (Cheng et al., 1992; Chipev et al., 1992). Transgenic mice expressing a mutant K10 gene have been found to show phenotypic features suggestive of BIE (Fuchs et al., 1992). Interestingly as in EBS (Dowling-Meara) the severest disease producing point mutations affect amino acid residues localized at either the beginning or at the end of the rod domains (Coulombe and Fuchs, 1993). In three of the six cases studied, the arginine residue in position 156 of the K10 coding sequence was found to be affected.

There are still several gaps in our understanding of the molecular basis of BIE. Explanations are sought to understand the epidermal hyperkeratosis and acanthosis that are characteristic of the disease. It also remains to be elucidated why BIE pathology is localized on certain anatomical sites in the body. It is not known whether this involves altered expression of regional specific keratins like K2 or K9 or interplay of epigenetic factors like mechanical trauma. Other candidate genes for BIE disorders in skin include profilaggrin/filaggrin, involucrin and transglutaminase. It has been hypothesized that filaggrin abnormalities may contribute to keratin filament clumping and fragility because filaggrin has been found to promote keratin filament aggregation in vitro (Dale et al., 1987). A recent study (Ishida-Yamamoto et al., 1993) suggests that while filaggrin abnormalities are not primarily responsible for keratin filament clumping, they may have a contributory role in the altered epidermal structure and function in BIE. Linkage analysis pointed to the involvement of keratin type II gene (12 q 11–13) in ichthyosis bullosa of Siemens a variant of BIE. Sequence analysis revealed the presence of mutations in K2 gene in patients with disease. Three different mutations were detected, one is the 1A domain and two is the 2B domain of the rod (Kremer et al., 1994).

Epidermolytic palmoplantar keratoderma (Vorner) (EPK)

This is the commonest type of hereditary palmoplantar keratodermas. Diffuse thickening of palms and soles becomes apparent at the age of 3 to 4 weeks. There is little or no spread of keratoderma on to the dorsal surface. Histologically all the characteristics of BIE are observed in the affected
Skin. Keratinocytes with an ultrastructurally abnormal tonofilament cytoskeleton are found in the histological specimens (Wevers et al., 1991). A genetic linkage analysis maps the defect to chromosome 17q as in EBS. Although the precise keratin defect in EPK is not known, K9 which is specifically expressed in the epidermis of palms and soles is thought to be defective (Reis et al., 1994).

**Other genodermatoses**

Dale et al. (1992) reported that the expression of differentiation specific keratins (K1 and K10) are greatly reduced and the proliferative specific (K5 and K14) and hyperproliferation specific (K6 and K16) keratins are generally enhanced in CHILD (Congenital Hemidysplasia with Icthyosiform erythroderma and Limb Defects) syndrome. In certain heritable disorders such as restrictive dermatopathy the skin expresses less of high molecular weight keratins and increased low molecular weight keratins such as 48 and 50 kDa.

**Skin disorders associated with keratinocyte hyperproliferation**

Skin abnormalities have also been described where keratinocyte hyperproliferation is either innate or incidental. Thus altered keratin expressions have been found in diseases as varied and different as psoriasis, lichen planus, lupus erythematosus, toxic epidermal necrolysis and Darier’s disease.

Psoriasis is a chronic inflammatory and proliferative disorder of skin and in its typical presentation is characterized by asymptomatic sharply demarcated plaques of bright red erythema covered with a profusion of silvery white scales. The precise aetiology of the disease is not known but it is generally understood that non-genetic factors influence disease expression in genetically predisposed individuals.

Many studies (Thaler et al., 1980, Thewes et al., 1991; Bernard et al., 1992) have shown that in a majority of patients with psoriasis, the suprabasal keratins K1 and K10 are down-regulated and hyperproliferative keratins K6 and 16 (which are normally found in high turnover mucosal epithelia) are induced. The significance in the expression of K16 has been seen in the early development of psoriatic lesions where K16 protein detection precedes mitosis suggesting that it may render the cytoskeleton more plastic to enable cell movement. The qualitative changes in keratin expression suggests that there is not only abnormal keratinocyte differentiation but also abnormal keratinocyte proliferation in psoriasis. The authors’ preliminary studies indicate that the low molecular weight keratins are pronounced in classical Lichen planus lesions compared to normal skin (unpublished observations).

**Wound healing**

Re-epithelialization is a pivotal event in wound healing. Basically, it involves migration and cornification of perilesional basal keratinocytes (Sten and Hepalmans, 1988) across the wound bed to cover it. In the initial process of wound healing the basal keratinocytes express K6 and K16 keratin polypeptides (Tians et al., 1993), unlike the normal skin which expresses K5 and K14 pair.

In instances where there is extreme damage to the skin for e.g. as in thermal or chemical injury or in people who are genetically predisposed, abnormal wound healing takes place resulting in the development of hypertrophic scars or keloids (Kischev et al., 1992). Recent studies in our laboratory indicate that in keloid skin and in post-burn scars, there is an abnormal increase in the 58 and 50 kDa keratin protein expression (Corresponding to K5 and K14 keratins) compared to hypertrophic scars and normal epidermis (Ramakrishnan et al., 1995). It appears that there is an increase in the message for K14 resulting in the presence of undifferentiated keratinocytes in keloids and post-burn scars (Ramakrishnan et al., 1995). This observation, although it includes keratinocyte matrix interactions in wound healing, still suggests that altered keratin expression might influence abnormal wound healing to a significant degree.

**Skin tumours**

Abnormal keratin expression are also found in epithelial tumours, benign as well as malignant. Indeed, keratins may serve as biological markers for epithelial new growth (Gupta et al., 1992a,b). Transformed cells could trigger keratin filament rearrangements to be able to overcome cytoskeletal constraints imposed by cytoskeletal organization, thereby shutting off the differentiation process (Knapp and Bunn, 1987). In general, many skin tumours express keratin profiles of simple epithelia i.e. K8, 18 and K9. Additionally there is suppression of differentiation specific keratins. Tazawa et al. (1992) reported that carcinomas of sweat gland origin express various combinations of simple and stratified epithelial keratins. Perkins
et al. (1992) found loss of K10 expression in some examples of basal and squamous cell carcinomas while in others, expression of K8 and K18 have been reported. In experimentally induced skin carcinomas in mice, Larcher et al. (1992) demonstrated K8 expression with a concomitant reduction in K13 expression. They suggest that increased synthesis of K8 can serve as a marker of terminal disease progression in the mouse skin.

The transformation of benign epidermal tumours to a malignant state generally involves the loss of K1 and K10 keratins (Kartasova et al., 1992). Some basal cell carcinomas have been found to express K17 and K19 (Markey et al., 1992). It also has been found that the transition of some squamous cell carcinomas of human and rodent origin to a more invasive metastatic spindle cell types also involves reorganization of cytoskeleton with functional loss of genes that control epidermal differentiation (Stoler et al., 1993). Sebaceous carcinoma is another skin tumour where the low molecular weight keratin (54 kDa) is preferentially expressed (Murata, 1993). In some dermatofibromas there is an increased expression of K6 and K16 along with altered expression of K14 (Stoler et al., 1989).

Doorbar et al. (1991) have reported that human papilloma virus (HPV) associated epithelial lesions, ranging from benign warts to invasive carcinomas, can destroy the cyto keratin matrix in the differentiating cells of the epidermis. HPV infected transgenic animal models have shown a marked reduction in the expression of K1/K10 (Tinsley et al., 1992). Pei et al. (1992) have shown that HPV-16 infected keratinocytes show greater expression of K13, K18 and K9 keratins. Proby et al. (1993) analysed the profiles of keratin expression in viral-induced warts and verrucous keratoses and malignant growths. They report loss of K1 and K10 and increase in simple epithelial keratins K8, K18 and K9 during advanced stages of malignancy. In contrast, K17 expression is found in much earlier stages in suprabasal hyper proliferative lesions of benign warts. Human epidermal keratinocytes in culture transformed with SV 40 down regulate the expression of K5, K6, K14/K15 and K16 and K17 and express instead K8, K18 and K9 keratins as compared to normal keratinocytes (Hronis et al. 1984).

Other factors influencing keratin expression

(a) Chemical agents. Like hormones, retinoic acid can also influence the expression of genes through certain retinoic acid receptor elements (Evans, 1988; Jing et al., 1990) and modulate proliferation and differentiation of epidermal keratinocytes in vivo and in vitro (Kopan et al., 1988). Retinoids are synthetic Vit A analogues and are used in a wide variety of dermatological disorders including tumours. Retinoids are found to alter the keratin gene expression and modulate filament formation by regulating the transcriptional or post translational modifications of keratin polypeptides. There have been conflicting observations on retinoid-induced altered keratin gene expressions. Rosenthal et al. (1993) have observed that acute or prolonged topical retinoic acid treatment of human skin in vivo affect the expression of K6 and K13 keratins but not K1 and K10. Fuchs (1990) has reported that the expression of differentiation specific K1 and K10 is blocked by Vit A. Retinoids may also influence expression of K6 and K16 (Kopan and Fuchs 1989). Many of the observed effects of retinoids are secondary (Stellmach and Fuchs, 1989) although there may be some alteration in levels of mRNA expression (Fuchs, 1990; Stellmach et al., 1991).

Other chemical agents and carcinogens like TPA and 1-Oleyl-2-acetyl glycerol block calcium mediated induction of K1 and K10 in mouse epidermal keratinocytes both at the protein and at the message levels, an effect that is blocked by bryostatin, a known inhibitor of protein kinase C (PKC). TPA mediated inhibition of K1 mRNA is also blocked by cycloheximide or actinomycin D indicating that PKC-induced protein may be involved in the regulation of keratin gene expression (Dlugosz and Yuspa, 1993) and epidermal differentiation. In the TPA induced epidermal tumours similar suppression of differentiation-specific keratins may occur. TPA may also modulate post translational modifications of keratin polypeptide and thereby influence filament assembly and integrity (Gupta et al., 1992a).

Several growth factors that are produced by stromal cells influence keratinocyte proliferation and differentiation. Epidermal growth factor (EGF) and transforming growth factor alpha (TGF-α) induce the activation of hyperproliferation associated K6 and K16 through specific nuclear proteins (Fuchs, 1990, Jiang, 1993). Calcium ions also play an important role in epidermal differentiation (Jaken and Yuspa, 1988). Calcium ions may alter the expression of differentiation specific keratins or alter the filament assembly and integrity. Studies in the authors’ laboratory indicate that hormones like estradiol influence the differentiation of target tissues through the
mediation of calcium ions (Gupta et al., 1990b). Vijayalakshmi and Gupta (1994) have shown that increased calcium levels in rodent vaginal epithelial cells regulate transglutaminase which in turn helps in cross-linking the keratin polypeptides during terminal differentiation.

(b) Physical agents. Certain physical agents such as irradiation also cause rapid alteration in the expression of skin keratins. Horio et al. (1993) have reported that in guinea pig skin, ultraviolet (UV) radiation increases the expression of basal layer specific keratins in suprabasal cells. The variation in keratin expression can be seen even at the message level. Smith and Reiss (1994) have observed wavelength dependent alterations in the expression of keratin mRNAs. They reported that in guinea pig skin, ultraviolet (UV) radiation increases the expression of basal layer keratin based diagnostic tools would help in prenatal diagnosis of certain heritable disorders of the skin, such as the ones discussed in this review.

CONCLUSIONS

The protective function of the epidermis is mainly dependent on the expression of specific keratin polypeptides and the keratin filament integrity. The variations in the expression of keratins from basal to cornified layers represent a functional organization rather than a mere histological stratification. Changes in the integrity of the keratin filaments or the expression of the keratin polypeptides that are result of gene mutations or alterations induced by a variety of chemical or physical agents adversely affect the functional properties of the keratinocyte, thereby resulting in various dermatological disorders. The precise knowledge about the factors that regulate tissue specific expression of keratin polypeptides may provide further insights into the understanding of these keratin associated skin diseases in general, and also pave the way for devising improved therapeutic strategies, either newer drug therapies or gene therapy. Developing keratin based diagnostic tools would help in prenatal diagnosis of certain heritable disorders of the skin, such as the ones discussed in this review.

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