Leading Article

Keratin gene mutations in human skin disease

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Introduction

The molecular biology of these monogenetic skin diseases has been approached in two ways. The first was initiated by ultrastructural observations postulating mutations in the structural proteins of the cell so that linkage and keratin mutations could be extrapolated. The second approach has been by the use of transgenic mice technology to produce the phenotypes of human blistering skin diseases with specific keratin gene mutations.

Over the last 10 years dermatology has been in the forefront of academic research examining the genetic basis of monogenetic diseases and has used the powerfully combined approaches of molecular biology, morphological studies, immunohistochemistry and biochemical studies. The key to these developments has been the polymerase chain reaction (PCR) which has made linkage analysis and gene sequencing possible. The target genes for the hereditary genodermatoses were identified using biochemical, immunohistochemical and positional cloning techniques which has given molecular genetics the power to unravel the nature of the keratin gene mutations. Keratin mutations in a number of genodermatoses (epidermolytic hyperkeratosis; epidermolysis bullosa simplex, Weber–Cockayne, Koebner and epidermolytic palmoplantar keratoderma) have now been determined and all have two common features. The first, epidermolysis or blistering, is a prominent histological feature. Secondly, the mutations all lie in four highly conserved subdomains common to all the keratins. These subdomains are important in filament assembly. Mutations at these critical points result in perturbation of keratin assembly, and result in epidermolysis, seen in both the affected individuals as well as in transgenic mice.

The keratin cytoskeleton

The keratins comprise a heterogeneous group of more than 30 distinct proteins. The cellular

keratins have a major protective and structural role which comprises 90% of the total protein of the differentiated keratinocytes. All keratins have the same basic structure (Figure 1) and are numbered on the basis of their migration by two-dimensional gel electrophoresis.¹ The common structural components of the keratin polypeptide (Figure 1) are a central α -helical rod which is comprised of four segments (1A, 1B, 2A, 2B), lying between each of these four segments is a non-helical linkage segment (L1, L12, L2), and in the type two keratins the α -helical rod is flanked at each end by a homology region (H1, H2).^{2,3}

The keratins (K) provide the intermediate filament network that forms the cytoskeleton of epithelial cells. Specific pairs of type I (K9–K19; small acidic proteins) and type II (K1–K8; larger basic or neutral proteins) keratins are coexpressed during various stages of epithelial differentiation. In the absence of the corresponding keratin partner, single keratins are degraded within the cell. The keratins expressed are fundamental to the cellular differentiation and phenotype of the epithelial cell.⁴ These coexpressed keratin polypeptides form dimers with a coiled-coil structure in a 1:1 ratio. The dimers polymerize to form tetramers. These tetramers in turn polymerize to form intermediate filaments (10 nm in diameter) of the cytoskeleton. The process of heterodimer formation is believed to result from molecular interactions in the highly conserved regions at the beginning and the end of the keratin α -helical rod domains.

In an analogous fashion to the switching from fetal to adult haemoglobin after birth, the keratin expression is influenced by the maturation and terminal differentiation of the keratinocytes in the epidermis from which the cells are derived. K8 and K18 are the primary epithelial keratins expressed in the fetus and in all simple epithelia. K5 and K14 are first detectable in embryonic based cells and are the primary keratins expressed by all keratinocytes in stratified non-secreting epithelia in which cells high levels of expression are seen.^{5–7} In stratified epithelia the keratin expression profile changes

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Figure 1 A demonstration of the clustering of known sites of point mutation (arrows) in the type I and type II keratins in epidermolysis bullosa simplex (EBS), epidermolytic hyperkeratosis (EH) and epidermolytic palmoplantar hyperkeratosis (EPPK).

with the passage of the keratinocyte from the basal to suprabasal layers of the epithelium.^{7,8} In the basal layer, whilst the keratinocyte is in contact with the basement membrane, K5 and K14 are expressed. In the suprabasal layers of the skin, K1 and K10 are expressed, and in the stratified squamous oral and genital mucosa K6 and K16 are expressed.¹ K6 and K16 are also expressed by hyperproliferative and hyperkeratotic epithelia such as on the palm and sole and during wound healing.¹

Disordered intermediate filament cytoskeleton

The first suggestion of keratin mutations being involved in the pathogenesis of the autosomal dominant skin diseases came from ultrastructural studies. This was facilitated by the realization of the genetic concept that recessive disorders were most commonly the result of enzyme deficiencies, whilst dominant disorders usually resulted from defects in regulatory or structural proteins.^{9,10} The reasoning behind this concept is that a reduction in enzyme concentration of 50% will not significantly affect metabolic function, but an alteration in the physical properties of 50% of a specific structural protein could result in major morphological disturbance as in sickle cell disease.

Epidermolytic hyperkeratosis

Epidermolytic hyperkeratosis (EH) is a group of inherited skin diseases which include bullous congenital ichthyosiform erythroderma (BCIE), ichthyosis hystrix of Curth-Macklin (IHCM) and ichthyosis bullosa of Siemens (IBS). In EH the basal cells are normal, and there is suprabasal tonofilament clumping which appears to be the primary event with secondary cytolysis of the suprabasal cells, blister formation and hyperkeratosis.⁹

BCIE presents soon after birth with erythroderma and severe blistering, the denuded areas healing rapidly with recurrent episodes of blistering on the background of erythroderma. With time, a verrucous hyperkeratosis becomes progressively more prominent with a linear distribution in the flexures.¹¹ Immunohistochemical staining with specific monoclonal antibodies to keratin polypeptides demonstrated tonofilament abnormalities only in the stratified epithelia in which keratins K1 and K10 are expressed, but not in cultured keratinocytes or non-stratified epithelia. Immunoelectron microscopy demonstrated that, among the keratins detected in the suprabasal epidermis, the clumped tonofilaments predominantly expressed K1 and K10.¹² Linkage studies of large pedigree with BCIE have mapped

to the keratin gene clusters on chromosomes 12q suggesting keratin gene mutations are also responsible for BCIE.¹³ Subsequently, keratin gene mutations in K1 and K10 have been confirmed in a number of mutation hot spots on the keratin polypeptide.^{14–19}

Epidermolysis bullosa

Epidermolysis bullosa (EB) represents a large and heterogeneous group of diseases characterized by blistering or erosions occurring in response to mechanical trauma. Electron microscopy has been fundamental in our understanding of these genodermatoses according to the initial plane of blistering three main groups of EB can be distinguished.^{20,21}

- a. In EB simplex (EBS) intra-epithelial blistering results from cytolysis of the basal cells.
- b. In junctional EB cytolysis occurs in the lamina lucida with resultant blister formation but no abnormality of tonofilament structure has been demonstrated.
- c. In dystrophic EB (DEB) there are no ultrastructural tonofilament abnormalities and cytolysis occurs below the level of the lamina densa at the level of the anchoring fibrils.

(I) EBS can be subdivided into three main groups: Weber–Cockayne EBS, Koeblers EBS and Dowling–Meara EBS.

Weber–Cockayne EBS is the commonest and least severe form of EBS and presents with blistering confined to sites of greatest trauma, such as the hands and feet. Children tend not to be affected until they start to walk and the blistering tends to be worse in warm weather. In Weber–Cockayne EBS linkage studies have mapped affected families to the keratin gene cluster on chromosome 12 and, as the disease is restricted to the palmoplantar epidermis, have suggested that site-specific keratins such as K9 or K16 may be involved.^{22–24}

The blistering in patients with Koebler EBS occurs within the first few weeks of life but again develops at sites of trauma. In Koebler EBS the tonofilament clumping and condensation observed in these patients was so minor that it was considered to be the consequence of the cytolysis rather than the cause of it.

Dowling–Meara EBS is the most uncommon and most severe form of EBS, and presents at birth or in early infancy with grouped and generalized skin blistering at sites of mild skin trauma. In addition there is an associated palmoplantar hyperkeratosis, nail dystrophy and oral ulceration.²⁵ Tonofilament clumping of the basal cells is a prominent histological feature of the Dowling–Meara subtype. In Dowling–Meara subtype of EBS, electron microscopic studies using fresh

tissue and cultured keratinocytes demonstrated tonofilament clumping in the normal skin, outer hair root sheaths, sweat ducts, and sebaceous glands.²⁶ All these epithelia express K5 and K14 whereas no tonofilament clumping was demonstrated in tissues not expressing these keratins. Immunogold electron microscopy confirmed that the basal cell tonofilament clumps were strongly labelled with monoclonal antibodies to K5 and K14, and only slightly reactive suprabasally to K10.²⁶

(II) Junctional EB is characterized by more severe blistering of the skin and mucous membranes, which can result in nail dystrophy and loss of nails. Unlike EBS the eroded areas heal slowly with scarring and it may be fatal in infancy. Abnormalities of kalinin/laminin 5 expression, a lamina lucida protein expressed by basal keratinocytes, have been demonstrated in Herlitz's junctional EB;²⁷ subsequently, kalinin/laminin 5 mutations have been demonstrated in Herlitz's junctional EB.^{28,29}

(III) DEB is usually apparent from birth with severe blistering occurring over the entire skin surface, mucous membranes, pharyngeal and oesophageal mucosa in response to minor trauma. The erosions heal slowly with much scarring resulting in syndactyly and oesophageal strictures. The split in DEB occurs at the level of the anchoring fibrils which are comprised of collagen VII and adhere the lamina densa to the underlying dermis.^{30,31} Immunofluorescent labelling with monoclonal antibodies to collagen VII have demonstrated reduced or absent collagen VII staining in these patients.^{32–37} A mutation in the type VII collagen gene (COL7A1) has subsequently been demonstrated in Hallopeau–Siemens DEB, which shows no detectable collagen VII polypeptide.³⁸

Elegant studies with transgenic mice have demonstrated that specific K14 mutations can produce the full range phenotypes of EBS seen in man. Furthermore, a strong correlation was found between the degree of keratin tonofilament disruption and the severity of the disease. In those phenotypes with only subtle disruption of the tonofilament cytoskeleton but in the absence of tonofilament clumping, cytolysis still occurred.^{39,40} These studies produced some of the best evidence for a structural role for the intermediate filament network in cellular integrity.

Keratin sequencing in Weber–Cockayne and Dowling–Meara subtypes of EBS have demonstrated K5 and K14 mutations in all the pedigrees thus far examined.^{17, 39, 41–47} The sites of the mutation within the keratin polypeptide show significant clustering at certain points that appear to be important in maintaining the integrity of the intermediate filament network.⁴³

Epidermolytic palmoplantar keratoderma (Vörner subtype)

Epidermolytic palmoplantar keratoderma (EPPK) is an autosomal dominant skin disease in which there is diffuse palmoplantar hyperkeratosis with an erythematous edge. The characteristic histological feature is that of perinuclear epidermolysis and suprabasal clumping of tonofilaments but without frank blister formation. Linkage studies have mapped the gene to the keratin gene cluster on 17q.⁴⁸⁻⁵⁰ On the basis of the restriction of the disease to the palmoplantar epidermis K9 was predicted as the probable site of mutation in these pedigrees.⁴⁸ K9 mutations have now been demonstrated in a number of pedigrees with EPPK.^{50,51}

Conclusions

In those diseases in which a keratin gene mutation has been demonstrated there are a number of ultrastructural similarities in common. Firstly, epidermolysis is a characteristic feature of all the genodermatoses in which a keratin gene mutation has been demonstrated. Secondly, tonofilament clumping is seen in many of these diseases. Thirdly, hyperkeratosis is a feature of EH, epidermolytic palmoplantar hyperkeratosis, and some types of EB (Dowling-Meara). This would be in keeping with the demonstrated keratin gene mutations having similar functional consequences for the biophysical properties of the intermediate filament network and cellular integrity.

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