LECTURE

Whats new in genodermatoses?

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Abstract. Recent genetic analysis of the genodermatoses, in particular the palmoplantar keratodermas, has identified the important role of proteins involved in the regulation and formation of epidermal cell junctions. There are four major types of junction, of which three have been demonstrated to be important in skin, and in which component proteins such as desmoplakin and connexins are mutated in epidermal disease. These are the gap junctions, desmosomes and adherens junctions. These junctions are responsible for cell-cell adhesion and communication, key properties to maintain the normal cellular phenotype and tissue architecture. (Keio J Med 50 (1): 35–38, March 2001)

Key words: connexins, desmosomes, junctions, genodermatoses

In past years, genetic investigation of the inherited skin diseases (genodermatoses) has identified disease-associated mutations in many of the structural proteins of the skin, for example, the keratins and also proteins of the basement membrane such as the integrins and laminins.[1,2] More recently, investigations into the genodermatoses, in particular the palmoplantar keratodermas (PPKs)[3], have identified the important role of proteins involved in the regulation and formation of epidermal cell junctions. These junctions are responsible for cell-cell adhesion and communication, key properties to maintain the normal cellular phenotype and tissue architecture. The stability of the junctions takes on increasing importance in an organ such as the skin, in which the cells are not only undergoing constant differentiation, but are also subject to the most severe stresses, in particular those of the palmoplantar epidermis. Component proteins which form three of the four major types of junction are mutated in epidermal disease. These are the gap junctions, desmosomes and adherens junctions. This review will discuss, firstly, the identification of mutations in proteins that form these three epidermal junctions and, secondly, mutations in proteins that regulate intercellular calcium and mediate junction formation.

Gap Junctions

Connexins are the major proteins of these intercellular channels, the largest of the four junctions.[4,5] Each gap junction consists of two connexons, one from each cell, which in turn is composed of six individual connexin proteins. Gap junctions allow communication between cells, providing a voltage gated channel through which metabolites and other small molecules of less than 1 kDa can pass directly between the cytoplasm of adjacent cells. This communication is thought to be important in differentiation and also tissue repair.

At present, the connexin family of proteins consists of eleven characterised human genes with a least seven expressed in the epidermis (Di WL and Kelsell DP: personal communication: Fig. 1). Of this gene family, missense mutations in two connexins have been demonstrated in autosomal dominantly inherited skin disease, connexins 26 and 31. The mutated connexin is proposed to interfere with the overall structure of the junctions, possibly by weakening interactions between the connexin subunits or by changing the gating sensitivity of the channel.

Connexin 26 has been implicated in Vohwinkel's syndrome, which presents as papular and honeycomb

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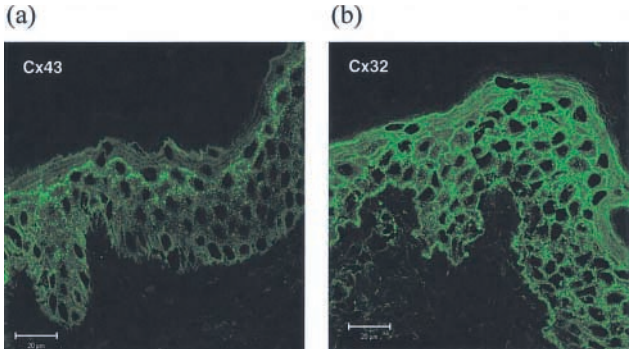

Fig. 1 Expression patterns of two connexins found in human epidermis showing characteristic punctate membrane staining. Fluorescent immunohistochemical staining of human epidermis using monoclonal mouse anti-connexin antibodies for (a) Cx43 and (b) Cx32.

keratoderma, with constriction of the digits often leading to autoamputation.[6–8] In some individuals, this skin disease is also associated with deafness. Germline mutations in Connexin 31 underlie some cases of Erythrokeratoderma variabilis.[9,10] This disease presents with two obvious morphologic changes, transient brownish/red hyperkeratotic plaques on the extensor surfaces as well as generalised hyperkeratosis. Some cases have associated PPK. There is a high degree of variability of severity of this disease, even within a family.

It is of further interest that recessive mutations in either Cx26 or Cx31 result in deafness with no epidermal abnormalities.[11,12] In addition, autosomal dominant mutations in these two gap junction proteins, distinct to those causing the above epidermal disorders, can also underlie non-syndromic hearing loss.[13,14] Further characterisation of different gap junction proteins is necessary to understand the effects of connexin mutations in the skin.

Desmosomes

Desmosomes are adhesive junctions, which are particularly prominent in the epidermis. The desmosome consists of several proteins, of which desmoplakin is the most abundant. The desmosomes anchor the intermediate filament network to the plasma membrane, and are important for the rigidity and strength of the cells.[15,16]

A mutation identified in desmoplakin is linked to an autosomal dominant striate palmoplantar keratoderma.[17] The patients show a linear pattern of skin thickening on fingers, palms and some areas on the soles. Light microscopy, together with ultrastructural analysis demonstrated loosening of the intercellular connections, as well as disruption of the keratin-desmosome interactions. An additional mutation in desmoplakin has been reported associated with a similar phenotype but there is variable penetrance with some mutation carriers showing no epidermal abnormalities.[18] All identified mutations appear to cause haploinsuffiency suggesting that dosage of the desmoplakin protein is critical for correct desmosome function in the palmoplantar epidermis. Plectin, another member of the plakin family, is an intermediate filament associated protein of the hemidesmosome and is mutated in the autosomal recessive syndrome of epidermolysis bullosa simplex and muscular dystrophy.[19]

It has also been shown that a dominantly inherited mutation in the desmosomal cadherin, desmoglein 1, causes a similar pattern of striate PPK.[20] This mutation causes deletion of the N-terminal part of Desmoglein 1 (Dsg1), responsible for calcium binding and dimer formation. This mutation is proposed to inhibit complete desmosome formation and weaken the structural integrity of the skin.

Like Desmoplakin, Plakophilin 1 is an intermediate filament binding protein within the desmosome. Mutations in Plakophilin 1 have been shown to cause the autosomal recessive disease, Hypohidrotic Ectodermal Dysplasia which results in skin fragility and abnormalities of the hair, nails and sweat glands.[21] The patient described in this study was a compound heterozygote for premature stop codon mutations occurring in the putative keratin filament-binding region of plakophilin. Immunohistochemistry of affected skin showed a complete absence of protein either due to the truncation of the peptide or as a result of nonsense mediated mRNA decay. Ultrastructural data revealed poorly formed desmosomes with fewer connections to the keratin filaments. As well as its role in cell adhesion, there have also been suggestions that plakophilin has a role in cellular signalling,[22] although it is unclear as yet if this role is important in the skin.

Adherens Junctions

These junctions attach to the actin filament network and consist of an intercellular complex of alpha, beta and gamma catenin, with a bridge composed of the classical cadherins between cells.[15] Recently, Naxos disease, a syndromic disease resulting in non-epidermolytic PPK, woolly hair and arrhythmogenic right ventricular cardiomyopathy, has been investigated with plakoglobin (γ-catenin) antibodies. Plakoglobin is present in both adherens junctions and desmosomes. Immunohistochemistry revealed an abnormal distribution of plakoglobin in the epidermis from four individuals with Naxos Disease (Hatsell SJ and Kelsell DP: personal communication: Fig. 2). A separate study has identified a deletion mutation in the plakoglobin gene in affected

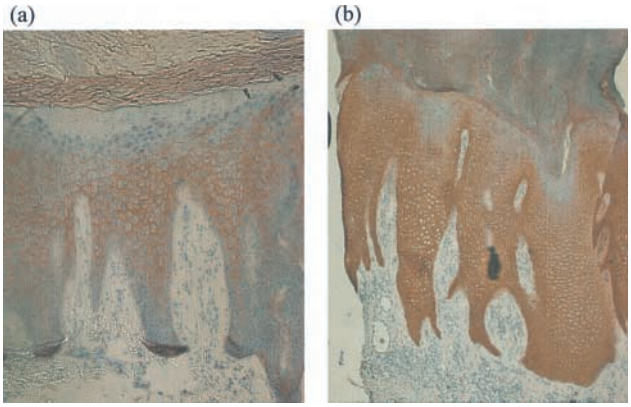

Fig. 2 Abnormal Plakoglobin (γ-catenin) distribution in Naxos disease. Fluorescent immunohistochemical staining of human palm skin using a monoclonal mouse anti-Plakoglobin antibody (PG-11E4) from (a) patient with Naxos disease and (b) unaffected individual.

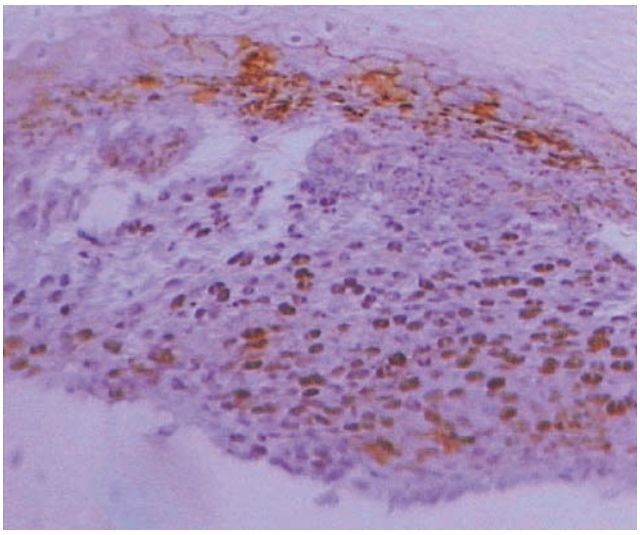

Fig. 3 Nuclear translocation of β-Catenin in a Pilomatricoma skin tumour. DAB immunohistochemical analysis using a monoclonal mouse anti-β-Catenin antibody showing nuclear staining in the tumour cells and membranous staining in the normal epidermis above the tumour.

family members,[23] which together with the immunohistochemistry data suggests that a molecular defect in plakoglobin underlies this disease.

In addition to having an important role in the formation of epidermal and cardiac muscle junctions, evidence is mounting that plakoglobin, together with other members of the armadillo (catenin) family have another role in cells as signalling molecules which activate gene transcription in response to extracellular signals, such as Wnt. This role has been comprehensively demonstrated for β-catenin, which is a key molecule in Wnt signalling during development, differentiation and other processes.[24] Aberrant localisation and expression of β-catenin has been reported in various tumour types, including pilomatricomas and squamous cell carcinomas supporting a key role for this pathway in epidermal differentiation and proliferation (Fig. 3).[25,26]

Calcium Pumps

A second recent area of interest has been the role that Ca^{2+} ATPases play in skin disease. These pumps are responsible for maintaining intracellular balance of calcium, and since calcium is a common signalling molecule, also for returning levels to normal after signalling events. Calcium signals are responsible for a number of different processes including the regulation of the cadherins present in adherens junctions and desmosomes. There is a calcium gradient across the layers of the epidermis, with calcium being four-fold higher in superficial rather than basal cells.[27] This gradient is thought to be important for the differentiation of keratinocytes, as well as lipid secretion.

Mutations in the Ca^{2+} ATPases, ATP2A2, were found to underlie Darier's disease.[28] This autosomal dominant disease is characterised by multiple keratotic papules and by histology which shows a loss of adhesion between epidermal cells, as well as abnormal keratinization. A comprehensive study of patients with Darier's disease has demonstrated that most mutations in this gene are likely to result in nonsense mediated decay of the mRNA leading to concurrent decrease in expression levels of the protein.[29] A second member of the Ca^{2+} ATPases, ATP2C1, has been shown to be responsible for Hailey-Hailey disease.[30] This is characterised by persistent blistering and erosion of the skin. This study has demonstrated, in cells and *in vivo* studies, that the regulation of cytoplasmic calcium is impaired in this disease, further demonstrating the importance of calcium regulation in normal epithelia.

Concluding paragraph

The study of these rare genodermatoses has identified a range of proteins and cellular mechanisms that are important in epidermal differentiation and proliferation. Recently, genetic investigations have identified mutations in genes that encode key proteins of the three main epidermal junctions. From the study of syndromic skin disease, gene mutations in junctional proteins have also been shown to cause deafness and cardiomyopathy which has opened up new areas of research into these debilitating non-epidermal disorders. As well as the continuing identification of new genes associated with skin disease and biology, for example, Cathepsin C in periodontal disease and PPK,[31]

future research will aim to develop a clearer understanding of how mutations in cellular adhesion and communication molecules affect epidermal differentiation and proliferation.

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