Keratin genes, epidermal differentiation and animal models for the study of human skin diseases

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Introduction
The epidermis comprises only a small fraction of the skin, and yet it has a vital function: it must keep micro-organisms out and essential body fluids in (for review, see [1, 2]). It must also survive the physical and chemical traumas of the environment. To manifest these functions, the epidermis must continuously rejuvenate itself and produce a resilient and impenetrable mass of stable proteins and lipids. In humans, there are about 10-20 layers of epidermal cells. Only the innermost, basal layer has the capacity for DNA synthesis and mitosis, and consequently, this layer contains the progenitor cells of the epidermis. Under an as yet unidentified trigger, a basal cell will begin its journey to the skin surface. In transit, it undergoes a series of morphological and biochemical changes culminating in the production of dead, enucleated squames, which are sloughed from the surface, and continually replaced by inner cells differentiating outward. In this way, the rejuvenation of the epidermis is achieved, and the progenitor cells are tucked safely under an armour of dead cells.

The protective function of the epidermis is manifested in part by the organization of cells within the epidermis. In basal and spinous layers, cells are tightly connected by calcium-activated proteinaceous membrane junctions, called desmosomes. In the upper layers, these junctions are weakened, and in their place, intercellular lipids serve as cell adhesion molecules. In this way, no micro-organisms or fluids can pass through the intercellular spaces of differentiated squames. An equally important factor involved in the protective role is the construction of an extensive cytoskeletal network. The major structural components of the epidermal cytoskeleton are keratins, a family of proteins which self-assemble into 10 nm filaments forming a network spanning from the desmosomes to the nuclear envelope. Keratins are to an epidermal cell what globin is to the red cell; they constitute ~85% of the total protein of the fully differentiated squame. All epidermal cells synthesize keratin, but basal cells have fewer and more disperse filaments than spinous cells, where bundles of keratin filaments begin to aggregate. In the granular layer, a keratin filament-associated protein called filaggrin is synthesized and seems to play a role in further bundling of keratin filaments into even larger aggregates, called macrofibrils [3]. It is likely that formation of macrofibrillar cables confers both rigidity and protection against proteolytic attack of keratin filaments.

In the final stages of the differentiative process, the dying cells become permeable, and intracellular calcium levels increase, thereby activating the enzyme transglutaminase. Transglutaminase catalyses formation of ε-(γ-glutamyl) lysine isopeptide bonds, which biochemically cross-link a group of proteins deposited beneath the plasma membrane [4]. This structure is called a cornified envelope, which serves as a container for the macrofibrils. As a cell finally reaches the outermost stratum corneum layer and is sloughed from the skin surface, it has lost all its metabolic capabilities, and is simply a cellular skeleton densely packed with keratin macrofibrils.

Epidermal keratin filaments
To survive on the surface of the skin, epidermal keratin filaments must have some very unusual properties that set them apart from simple epithelial keratins and other intermediate filaments. Based on amino acid sequence, all keratins can be subdivided into two groups: type I keratins are acidic (pK, 4.5-6.0), whereas type II keratins are basic (pK, 6.0-7.5) [5, 6]. Approximately 10000-20000 type I and type II keratin heterodimers are utilized in the formation of each 10 nm filament [7-9]. Recently, it has been shown that the intermolecular protein interactions that take place in the early stages of assembly of epidermal keratins into 10 nm filaments are among the most stable known in nature [7].

Basal cells express the type II keratin K5 (58 kDa) and the type I keratin K14 (50 kDa) [10], which can assemble into non-covalent heterodimers in vitro even in the presence of 9 M-urea and a reducing agent [7]. The 310 amino acid central α-helical domains of the two keratin polypeptides are

Abbreviations used: EBS, epidermolysis bullosa simplex; EGF, epidermal growth factor; TGF, transforming growth factor α.
intertwined in a coiled-coil fashion, aligned in parallel and in register, and are stabilized by both hydrophobic and ionic interactions (for reviews, see [6, 11–13]). Keratin dimers align in an antiparallel fashion to form heterotetramers [7, 14, 15]. Studies in vitro using bacterially expressed keratins have shown that heterotetramers are the major species formed in the presence of 6 M-urea and a reducing agent [7]. In 4 M-urea, lateral and end-to-end interactions occur among tetramers, and under physiological conditions, filaments assemble in vitro in the apparent absence of any auxiliary proteins or factors [7]. Hence, filament formation involves a hierarchy of complex interactions that are extraordinarily tight and remarkably stable. These interactions greatly enhance the stability of keratins, and coupled with filament bundling, probably enable keratins to be among the few survivors in the massive destruction phases which ensue in the latter stages of terminal differentiation. In the past few years, genetically engineered mutants of K5 and K14 have been utilized to begin to explore the sequences necessary for keratin filament assembly in vitro [16] and for integration and organization into a cytoskeletal network in vivo [17, 18]. In coming years, approaches such as these should help to elucidate the molecular basis for the extraordinary stability of epidermal cytoskeletons.

As an epidermal cell becomes post-mitotic and enters the first suprabasal layer, it initiates a series of transcriptional changes. Among these changes are cessation of K5 and K14 expression, and induction of K1 and K10 expression [19, 20]. The type II keratin K1 (67 kDa) and type I keratin K10 (56.5 kDa) are expressed in abundance only in terminally differentiating epidermal cells, and their synthesis proceeds throughout the four to eight spinous layers.

**Transcriptional regulation of the keratin genes**

A knowledge of the major transcription factors controlling keratinocyte-specific gene expression is important not only in the quest for understanding the molecular mechanisms underlying epidermal differentiation, but also for the targeting of genes to the skin. While little is presently known about keratinocyte transcription factors, there are a number of keratinocyte genes that have already been isolated and characterized, and which serve as a foundation for pursuing these factors. These include human genes encoding (i) the basal keratins K5 and K14 [21, 22]; (ii) the suprabasal keratins K1 [23], K10 [24], K6 [25] and K16 [26]; and (iii) the cornified envelope protein, involucrin [27]. Sequence comparisons have provided a few clues as to possible common regulatory elements involved in controlling keratinocyte-specific gene expression [28, 29]. Recently, a regulatory sequence in a *Xenopus* embryonic epidermal keratin gene, XK81A1, was shown to bind a factor, KTF-1, which is enriched in embryonic ectoderm nuclei [30]. This finding was interesting in light of our own studies demonstrating that a similar 10 bp sequence located 5’ from the TATA box of the human K14 gene, acted in conjunction with a distal element to control transcription of this gene [31]. This sequence is also the target for the binding of a factor, KER1, which is especially abundant in keratinocytes, and which is similar, if not identical, to the nuclear transcription factor AP2 [31, 32]. AP2 sites are also found in the up-stream regions of a number of other genes expressed exclusively in keratinocytes [31, 32]. In addition, AP2 was recently shown to be expressed predominantly in cells of epidermal and neural lineages [33]. However, since epidermal keratin genes are not expressed in neural cells, this provides additional evidence to suggest that AP2 might be necessary, but is not sufficient for keratinocyte-specific gene expression [32].

While the precise mechanisms controlling keratinocyte-specific gene expression remain to be elucidated, these studies have led to the identification of sequences sufficient to drive expression of genes in epidermal cells in culture and in transgenic mice in vivo [34–36]. The ability to target genes to the epidermis of transgenic mice has opened the door to the development of animal models for the study of human skin diseases.

**Transgenic mice expressing mutant human keratin genes**

Given the importance of keratin filaments in the epidermis, it is interesting that there are no known skin diseases whose aetiology resides in genetic defects in epidermal keratin genes. To explore the possible relation between keratin gene mutations, epidermal differentiation and genetic disease, we made transgenic mice harbouring a mutant human K14 keratin gene encoding a C-terminal truncated, K14 keratin [37]. Mice expressing this mutant keratin exhibited gross skin blistering upon mild trauma. Blistering was attributed to basal cell cytology which occurred as a consequence of marked perturbations in keratin filament formation and organization. Differentiating cells showed near-normal morphology, as evidenced by expression of keratinization proteins and formation of a stratum
The apparent recovery of outer layer cells was attributed to down-regulation of mutant keratin expression and induction of K1 and K10 as cells leave the basal layer. Collectively, the morphology, pathology and biochemistry of the transgenic mice and their cultured keratinocytes bore a remarkable resemblance to the genetic human skin disease, epidermolysis bullosa simplex (EBS) [38]. While additional studies will be necessary to determine whether patients with EBS have lesions in either their K5 or K14 genes, this animal model has provided the best evidence to date that the major phenotypic traits of EBS arise from disruption of keratin filament networks [37]. Whether cell lysis then arises because the mechanical integrity of the basal cells is compromised or, alternatively, because aggregates of keratin protein perturb the cellular physiology, remains to be determined.

Transgenic mice over-expressing transforming growth factor alpha (TGF-α)

Of the regulators involved in stimulation of keratinocyte growth, epidermal growth factor (EGF) and TGF-α have been the most extensively studied [39-41]. The targets of both growth factors are tyrosine kinase-activatable EGF receptors on the surface of basal epidermal cells [42]. TGF-α differs from EGF in that it is synthesized by keratinocytes both in vitro and in vivo [40]. Since epidermal cells can autoregulate their own growth via TGF-α production, it is perhaps not surprising that such control can go awry, leading to uncontrolled growth. Several reports have implicated an increase in EGF receptors with epidermal tumorigenesis [43, 44], and it was recently reported that psoriatic epidermis contains higher levels of TGF-α than normal skin [45, 46]. Thus, TGF-α and EGF receptors clearly play an important role in epidermal growth, and their intricate regulation seems to be crucial for maintaining the proper balance between growth and differentiation in epidermis.

To further explore the role of TGF-α in epidermal growth and differentiation, we used our human keratin K14 promoter to target expression of rat TGF-α cDNA to the stratified squamous epithelia of transgenic mice [47]. Unexpectedly, the only regions of epidermis especially responsive to TGF-α-over-expression were those which were normally thick and where hair follicle density was typically low. This included most if not all body skin from 2-day to 2-week-old mice and ear, foot pad, tail and scrotum skin in adult mice. In these regions, excess TGF-α resulted in thicker epidermis and more stunted hair growth. Epidermal thickening was attributed to both cell hypertrophy and to a proportional increase in the number of basal, spinous, granular and stratum corneum cells. During both post-natal development and epidermal differentiation, responsiveness to elevated TGF-α seemed to correlate with existing EGF receptor levels, and we saw no evidence for TGF-α-mediated control of EGF receptor expression. In adults, no squamous cell carcinomas were detected, but benign papillomas were common, developing primarily in regions of mechanical irritation or wounding. In addition, adult transgenic skin that was still both sensitive to TGF-α and subject to mild irritation displayed localized regions of leukocytic infiltration and granular layer loss, characteristics frequently seen in psoriasis in humans. These unusual regional and developmental effects of TGF-α suggest a natural role for the growth factor in (i) controlling epidermal thickness during development and differentiation; (ii) involvement in papilloma formation, presumably in conjunction with TGF-β; and (iii) involvement in psoriasis, in conjunction with some as yet unidentified secondary stimulus stemming from mild mechanical irritation/bacterial infection.

Summary

The examples shown here illustrate the power of gene targeting to the epidermis as a means of developing animal models for the study of human skin diseases. In this short review, I have focused on contributions which stem predominantly from my own laboratory [31, 32, 34, 37, 47]. However, other laboratories have also contributed heavily to the development of this technology [28-30, 35, 36]. The opportunities for these models are vast: there are a myriad of human skin diseases which have been characterized extensively at a biological level, but whose aetiology is presently unknown. A combined knowledge of (a) the biochemistry of epidermal differentiation; (b) epidermal-specific gene expression; and (c) transgenic mouse technology has provided the foundation for these and future studies in this area.

Note added in proof (received 2 October 1991)

Additional studies involving expression of mutant K14 genes in transgenic mice have provided a strong correlation between the severity of EBS phenotype and the degree to which keratin filament assembly is perturbed [48]. These studies suggest a genetic link among EBS subtypes, including Dowling Meara and Weber Cockayne, and they
indicate a function for keratin filaments in providing mechanical integrity to the columnar basal cells [48]. In addition, K14 point mutations have now been discovered in two different humans with spontaneous cases of Dowling Meara [49]. This finding illuminates the strong impact that transgenic mouse technology can have in elucidating the genetic basis for human skin diseases.

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