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15 March 2005

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Cell, Vol. 69, 899–902, June 12, 1992, Copyright © 1992 by Cell Press

Minireview

Of Mice and Men: Genetic Skin Diseases of Keratin

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The epidermis provides a protective interface between the body and the chemical and physical traumas of the environment. Its armor is an extensive cytoskeletal network of 10 nm intermediate filaments (IFs) composed of keratins. Two sequence types of keratins (40–70 kd) coassemble into parallel, coiled-coil heterodimers, which then stably align as antiparallel tetramers. Ten nanometer filaments are composed of four intertwined protofibrils, each with a diameter that can encompass two tetramers and a length that can encompass more than 100 tetramers. While the hierarchy of higher order interactions involved in forming 10 nm filaments remains to be unraveled, the assembly process can take place in vitro in the absence of any auxiliary factors (reviewed in Conway and Parry, 1988).

During epidermal development, embryonic basal cells are the first to express the type I keratin K14 and the type II keratin K5 (Dale et al., 1985, and references therein). Expression of this pair is elevated concomitant with the commitment of an embryonic basal cell to an epidermal cell fate; another pair of keratins, K1 and K10, is expressed in suprabasal cells concomitant with stratification in the developing epidermis. In the adult, as basal epidermal cells differentiate and move outward toward the skin surface, they down-regulate K5 and K14 and switch on K1 and K10 expression. In the fully differentiated squame, these keratins constitute ~85% of total cellular protein. Thus, keratins are to an epidermal cell what globins are to an erythrocyte.

Are there defects in keratin genes giving rise to genetic skin diseases, as there are defects in globin genes that cause sickle cell anemia and thalassemias? One way to address this question was to alter keratin sequences (such as the human K14 and K5 genes) and evaluate the functional consequences of these defects with respect to IF network formation in transfected cultured keratinocytes, and 10 nm filament assembly in vitro (e.g., Coulombe et al., 1990). Coupled with similar approaches using other IF proteins (see Lu and Lane, 1990; Hatzfeld and Weber, 1991; Bader et al., 1991, and references therein), and computer modeling analyses (Conway and Parry, 1988), the >300 aa α-helical rod domain has been identified as essential for proper coiled-coil formation and IF structure.

Within the rod domain, the highly conserved end domains are less able to tolerate perturbations than the central portions of the rod (Coulombe et al., 1990; Hatzfeld and Weber, 1991; Heald and McKeon, 1990; Letai et al., 1992). The flanking head and tail domains may contribute to filament stabilization (Hatzfeld and Weber, 1990; Lu and Lane, 1990) and/or the dynamics of the assembly–disassembly process (Heald and McKeon, 1990; Kouklis et al., 1991). In addition, some of the head and tail protrude along the surface of IFs and are likely to interact with other organelles and proteins, a feature that may also be crucial for the spatial architecture of an IF network (e.g., Steinert et al., 1983; Georgatos and Blobel, 1987; Bader et al., 1991).

Many IF mutants act in a dominant-negative fashion, combining with wild-type proteins to perturb IF assembly. These mutants often produce shorter filaments than wild type; the severity of the effect depends upon the mutation and its level of expression. Such mutants in epidermal keratin genes expressed in the skin of transgenic mice enabled an in vivo search for a link between keratin mutations and genetic skin disease. Mice expressing mutant human K14 genes (Coulombe et al., 1991a; Vassar et al., 1991), exhibit the morphological and biochemical features of epidermolysis bullosa simplex (EBS), an autosomal dominant human skin disease in which the epidermis blisters upon mild physical trauma (Anton-Lamprecht, 1983). The severity and extent of the disease in mice correlate with the degree to which the mutations expressed disrupt filament assembly. Coupled with the pioneering electron microscopy studies on human EBS of Anton-Lamprecht and colleagues (Haneke and Lamprecht, 1982; Anton-Lamprecht, 1983, and references therein), these studies provide a clue to the genetic basis for a sometimes life-threatening human skin disease that affects ~1:50,000 in the population.

Analyses of the K5 and K14 genes from human EBS patients have now revealed point mutations in the severest form of the disease, Dowling–Meara, and in the Koebner form. The mutation in a family of patients with the disease has mapped to chromosome 17 (Bonifas et al., 1991), where the K14 gene is located. In this family, only affected members carry a point mutation in the K14 gene. Furthermore, two spontaneous cases of Dowling–Meara EBS contain K14 point mutations not carried by the parents (Coulombe et al., 1991b). Interestingly, both mutations are at CpG dinucleotides and involve C→T transitions, which is suggestive of methylation of cytosine, followed by deamination and mutation (Cooper and Youssoufian, 1988). Finally, affected members of a large family with Dowling–Meara EBS have a point mutation in the K5 gene (Lane et al., 1992). Two families with EBS (Bonifas et al., 1991; Ryynanen et al., 1991) have been mapped to chromosome 12, where the K5 gene is located.

All the EBS point mutations identified are in the rod domain of either the K14 or K5 protein. This is consistent with the dominant-negative behavior of most rod domain mutations and with the autosomal dominant nature of most EBS families. The three Dowling–Meara mutations are in the highly conserved amino or carboxyl ends of the rod domain (Figure 1). The Koebner EBS family (Bonifas et al.,

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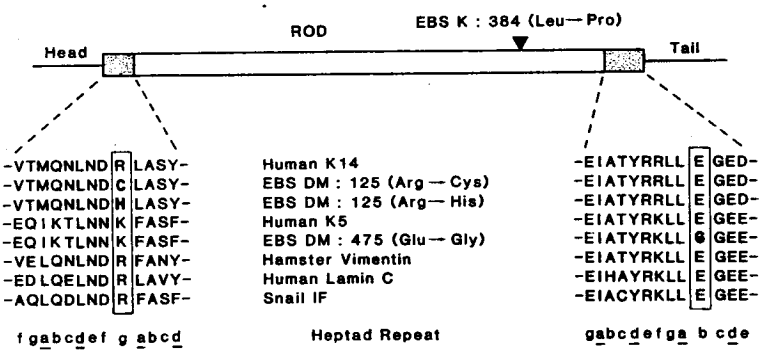

Figure 1. Location of Human EBS Mutations Relative to Keratin Structure

The schematic structure shown is characteristic of all IF proteins. Open box, the central rod domain, predicted to be largely α helical; stippled boxes, highly conserved end domains of the rod; EBS DM, Dowling–Meara EBS mutations. The Koebner EBS mutation in K14 (EBS K) is located outside the end domains (arrowhead). The initiation methionine is counted as the first amino acid of K14 and K5.

1991) has a mutation in a less conserved region of the rod domain. While it is the only EBS proline mutation identified, proline mutations in the central portion of the K14 rod are often less deleterious to filament formation in vitro than more subtle mutations in the rod ends (Letai et al., 1992). Thus, a good correlation exists between the sites of the natural mutations, the degree to which filament assembly is likely to be perturbed, and the severity of the human disease. This correlation has been verified experimentally, using site-directed mutagenesis of the wild-type K14 to engineer the precise EBS point mutation (Coulombe et al., 1991b). Keratinocytes transfected with this point mutant have a perturbed keratin network similar to that of the EBS patient's epidermal cells, and filaments assembled in vitro are shorter than normal, as are those assembled from the patient's keratins. Collectively, these studies demonstrate that the functional basis of at least some EBS cases resides in defects in K5 and K14.

What does this knowledge tell us about the function of keratin filaments? A clue stems from investigating the blistering process, which arises from basal cell cytolysis upon mechanical trauma (Figures 2A and 2B; Haneke and Anton-Lamprecht, 1982). According to criteria established for classification (Fine et al., 1991), Dowling–Meara EBS is typified by clumps of keratin filament aggregates that are prevalent throughout the basal cell cytoplasm, whereas filaments in Koebner and Weber–Cockayne EBS merely seem disorganized (see also Haneke and Anton-Lamprecht, 1982; Anton-Lamprecht, 1983). Interestingly, even in the absence of tonofilament clumping, columnar EBS cells break in a defined zone roughly midway between the nucleus and the hemidesmosomes (Figure 2D). In this zone, mutants affecting filament length might be expected to weaken the IF network, which must span a greater cytoplasmic distance from the nucleus to the hemidesmosomes than from the nucleus to the desmosomes (see Figure 2D).

Interestingly, when transgenic and human EBS cells flatten, either in tissue culture or during the wound healing process that follows a blister, cytolysis is not appreciable (Anton-Lamprecht, 1983; Coulombe et al., 1991a). Conditional cytolysis may be explained if an EBS cell, when columnar in the context of its tissue but not when flattened, is fragile without a proper keratin filament network. While tonofilament clumping may exacerbate cell degeneration in the more severe cases, these findings imply that an important function of keratin filaments is to provide mechanical integrity to a keratinocyte.

Other studies point to a general role of IFs in imparting mechanical strength to cells and tissues. Thus, overexpression of a type II hair keratin gene can generate brittle hair in transgenic mice (Powell and Rogers, 1990). In this case, expressing the natural type II keratin gene in gross excess relative to its type I partner leads to protein aggregation that interferes with normal IF organization. In another study, a Xenopus oocyte extract depleted of its single lamin (L_{III}) can still produce a nuclear envelope encapsulating chromatin, but the nuclei are fragile and fail to synthesize DNA (Newport et al., 1990). Finally, rheologic methods have revealed that IFs resist breakage and may even become stronger under stresses that would rupture other cytoskeletal networks (Janmey et al., 1991).

However, cytoplasmic IFs are clearly not needed under all circumstances. Several cell lines grow and divide normally in the absence of any cytoplasmic IF network. Furthermore, embryonic stem cells harboring two nonfunctional simple epithelial keratin (K8) alleles can differentiate normally into yolk sac–like embryoid bodies (Baribault and Oshima, 1991). During embryogenesis in Xenopus, wild-type vimentin can be overexpressed up to 10-fold with no detectable morphological abnormalities (Christian et al., 1990). Finally, desmin–vimentin IF networks may be dispensable for some aspects of myogenesis (Schultheiss et al., 1991).

Future studies will assess whether mutations in other IF genes give rise to other genetic diseases. However, at least in epidermis, both pairs of keratins appear to play a crucial role in manifesting the protective function of the skin (Fuchs et al., 1992). Expression of a modified truncated human keratin 10 gene in transgenic mice gives rise to skin with the morphological and biochemical characteristics of a different human genetic skin disease, epidermolytic hyperkeratosis (EH). As in K5 and K14 mutations, this mutant keratin interferes with proper filament network formation and leads to cell degeneration, but in this case, the phenotype is manifested in the suprabasal layers of the epidermis (Figure 2C). As epidermal cells differentiate, K1 and K10 protein levels increase, and K14 and K5 protein levels decrease (Eichner et al., 1984, and references therein). Therefore, as differentiation proceeds, an increasing gradient of mutant:wild-type keratin is established, yielding epidermal layers with progressively

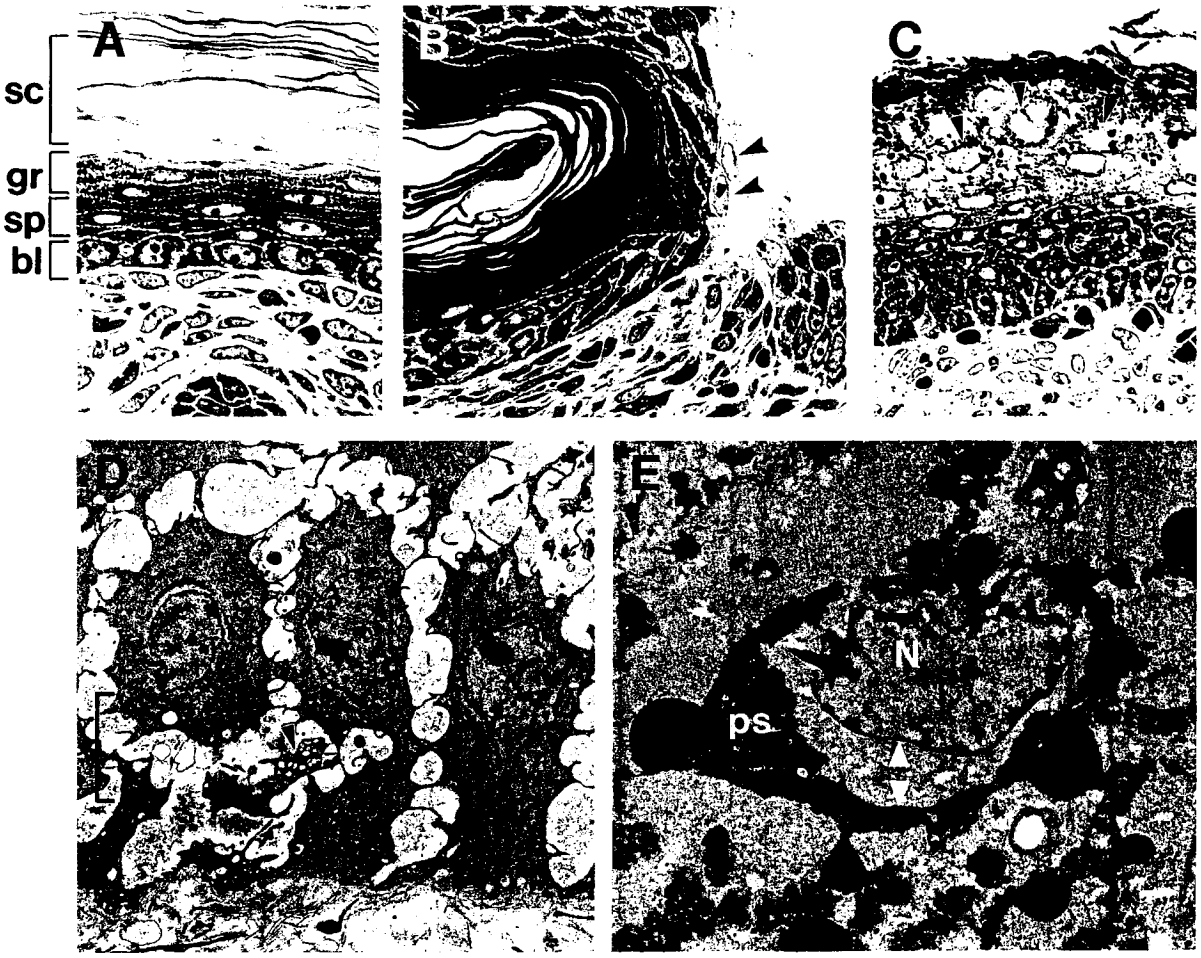

Figure 2. Cell Cytolysis, Altered Keratin Filament Networks, and Altered Nuclear Structure in Transgenic Mice Expressing Mutant Keratin Genes

(A–C) Toluidine blue staining of semithin sections of paw skin from neonatal control (A), transgenic mouse expressing a mutant K14 (B), and transgenic mouse expressing a mutant K10 (C). Arrowheads in (B) denote remnants of lysed basal cells still attached to the basement membrane, typical of human EBS, while arrowheads in (C) denote cell degeneration in the upper spinous and granular layers, typical of human EH. (A and C) courtesy of Dr. Q.-C. Yu. sc, stratum corneum; gr, granular layer; sp, spinous layers; bl, basal layer.

(D) Electron micrograph showing three columnar-shaped basal cells of skin from a transgenic mouse expressing a mild K14 mutant. (Arrowhead denotes vesicles and debris indicative of cell degeneration). This mutant did not cause tonofilament clumping in basal cells but did cause filament disorganization with seemingly fewer/shorter filaments than normal. Plane of rupture lies between the hemidesmosomes and the nucleus (bracket).

(E) Spinous cell from a transgenic mouse expressing a modified truncated K10 mutant. Note halo of clear cytoplasm around the nucleus (opposing arrowheads) surrounded by a perinuclear shell (ps) of tonofilament aggregates, as well as distorted shape of the nucleus (N).

Bar in (A) represents 17 μm for (A), 13 μm for (B), 15 μm for (C), 2.7 μm for (D), and 1 μm for (E).

greater levels of filament disorganization and cell degeneration.

The correlation between EH phenotype and a mutant K10 gene has offered new insights into the biology of the epidermis and additional functions for IFs. In spinous cells of the skin from both EH and K10 mutant mice, shells of keratin filament aggregates often surround the nuclei, sometimes leaving perinuclear gaps of as much as 100 nm or more of free cytoplasmic space (Figure 2E). The binucleate appearance of some of these nuclei is also seen in basal cells of EGS skin (Wilgram and Caulfield, 1966; Anton-Lamprecht and Schnyder, 1974; Fuchs et al., 1992). In addition, keratohyalin granules and nuclei are often distorted, as is the general shape of the upper spinous and granular cells.

Aggregation of keratin protein could elicit these changes in cellular physiology, leading to a perturbation of intracellular organization and cytoarchitecture. Alternatively, formation of perinuclear shells (Figure 2E) might prevent proper cell division, either as basal cells replicate (EBS) or when cells undergo their final mitotic division as they commit to differentiate (EH). Finally, it is also possible that the normal keratin network provides a structural scaffold for the spatial organization and structure of intracellular organelles, and that this can be compromised by keratin mutations. While additional experiments will be necessary to distinguish between these possibilities, it may be relevant that cytoplasmic IF networks are often intricately associated not only with the nuclear envelope, but also with other cytoplasmic constituents (reviewed in Klymkowsky

et al., 1989). Finally, given that IF networks in various cell types are configured differently, differentially expressed IF mutant genes may likely exert somewhat different effects, depending upon their target tissues. While some indications of this have been seen already, many more studies will be necessary before definitive conclusions can be drawn.

In summary, an understanding of the biology of keratin has been essential in elucidating the genetic basis for one, and likely another, human genetic skin disease. Similarly, a knowledge of the differential expression, structure, and intracellular–extracellular interactions of other skin proteins should provide valuable information as to the types of diseases that may arise when their functions are compromised. As in studies on EBS, chromosomal linkage analyses of families with skin diseases should be further facilitated by a knowledge of the chromosomal locations of genes encoding structural proteins of the skin. Finally, the strategy of using transgenic animals to assess protein function should continue to provide candidates for the possible genetic bases of additional skin diseases whose etiologies are presently unknown. As the example of EBS has pointedly illustrated, the coupling of classical genetics with transgenic approaches provides a powerful way to decipher the genetic basis of disease and ultimately to develop improved diagnosis and treatment. Conversely, deducing the genetic basis of a human skin disease allows the use of dermatological data in assessing protein function.

In closing, given that intermediate filaments are ubiquitous in higher eukaryotic cells and constitute a family of at least 40–60 genes, the field of IFs now enters a new phase, in which an understanding of keratin-related diseases should be helpful in exploring other diseases that may involve defects in either IFs, IF-associated, or IF-affecting proteins. For example, the parallels between EBS, EH, and Alzheimer's disease are intriguing, in that cell degeneration and disorganization of IFs are hallmarks of all three diseases. While Alzheimer's disease may be a defect in β amyloid rather than neurofilament proteins, the studies on keratin disorders suggest that the formation of neurofibrillary tangles may contribute to overall cell degeneration. As science and medicine continue to meet, biology and genetics will become increasingly intertwined, challenging traditional approaches and producing tantalizing new insights into human biology and disease.

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