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The Role of Keratins in Epidermal Development and Homeostasis—Going Beyond the Obvious

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Intermediate Filaments—The Basics

(Cyto)-Keratins are structural proteins of epithelial cells that can be divided into two subgroups, type 1 and type 2 keratins, based on biochemical properties such as molecular weight and isoelectric point. *In vitro* and *in vivo*, type 1 and type 2 keratins polymerize to form hetero-polymeric intermediate filaments (IF). IF are abundant in stratified epithelia, in particular the suprabasal layers of the epidermis. They connect to desmosomes (Cheng and Koch, 2004) at cell–cell junctions, and to hemidesmosomes (Nievers *et al*, 1999) at the interface of epidermis and dermis, establishing a three-dimensional network of fibrous proteins that cross the epidermis. It is widely accepted that IF are indispensable for the normal development and maintenance of stratified epithelia. Furthermore, animal models have established a cause–effect relationship between mutations in epidermal keratin and cell fragility disorders. Often, keratin missense mutations lead to a collapse of the IF network that causes cytolysis in response to even minor mechanical stress. In humans, f.i., mutations in the epidermal keratins K14 can lead to epidermolysis bullosa simplex, whereas mutations in K10 can lead to epidermolysis hyperkeratosis (for a more detailed discussion of the various keratin disorders, see Irvine and McLean, 1999).

Is there more to keratins than just providing mechanical resilience to stratified epithelia?

Several observations suggest that keratins might be more than just structural reinforcement; first, mammals maintain a large set of keratin genes. A recent survey of publicly available human genome sequences identified 27 type 1 and 27 type 2 keratin genes, clustered on chromosomes 17 and 12, respectively (Hesse *et al*, 2004). Secondly, these genes show cell type- and differentiation pathway-specific expression patterns.

Both observations suggest that individual keratins might have cell type-specific functions. The classical example of skin keratin pairs with tightly controlled expression patterns are K5/K14 and K1/K10. K5/K14 are expressed in the basal layer of the epidermis that contains epidermal stem cells and transient amplifying (TA) cells. K1/K10, on the other hand, are synthesized only in post-mitotic keratinocytes and are, consequently, found in the suprabasal layers of the epidermis.

Transgenic mouse experiments by Pierre Coulombe's group have shown that loss of an epidermal keratin (K14) cannot be completely compensated by the ectopic expression of other keratins (Hutton *et al*, 1998; Paladini and Coulombe, 1999), supporting the view that each keratin pair has evolved to specifically meet the needs of a particular cell type.

Cell Proliferation and Susceptibility to Tumor Formation—Keratinocyte Properties Affected by Keratin 10?

In a series of publications, the Jorcano laboratory has proposed that keratins might affect keratinocyte proliferations, and the susceptibility of these cells to tumor formation (reviewed in Paramio and Jorcano, 2002). These experiments focused on keratin 10, which is normally expressed only in post-mitotic keratinocytes. The Jorcano group used keratinocyte transfection assays (Paramio *et al*, 1997, 1999; Paramio and Jorcano, 2001) and transgenic mice that expressed K10 under the control of a bovine K5 promoter in the basal layer of the epidermis in their studies (bK5.K10, Santos *et al*, 2002a, b; 2003). Their results suggested that ectopic expression of K10 prevents cell cycle progression of basal keratinocytes. Using keratinocyte transfection assays, they also showed that K16 expression can rescue K10-mediated cell cycle arrest (Paramio *et al*, 1999). This fits very well with the expression patterns of these two keratins in normal and wounded skin, respectively. K16 is usually not expressed in the interfollicular epidermis. Upon wounding, K10 expression is downregulated and K16 upregulated, which has been interpreted as a means to allow proliferation and migration of keratinocytes necessary to repair cutaneous wounds.

By using a combined genetic and chemical carcinogenesis protocol, Jorcano's group showed that bK5.K10 transgenic mice were less susceptible to tumor formation, i.e. developed fewer and smaller tumors later than controls, and showed less conversion of papillomas to squamous cell carcinomas (SSC, Santos *et al*, 2002b). All of these observations are in accordance with the well known changes in keratin expression patterns observed when benign papillomas convert to SSC and spindle cell carcinomas (e.g., Nischt *et al*, 1988; Roop *et al*, 1988; Wang *et al*, 1998). K10

Abbreviation: IF, intermediate filament

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is one of the first marker proteins that is lost during malignant conversion.

The studies summarized above suggest a sequence of events in which K5/14-expressing TA cells induce expression of K10, which then inhibits cell cycle progression and commits the cell to terminal differentiation.

Nevertheless, a study published by Reichelt and colleagues in this issue of the JID suggests that it might not be as simple as that. Instead of using ectopic expression of K10 in cells that do not synthesize this protein *in vivo*, they analyzed mice with a K10 null mutation (Reichelt *et al*, 2001; Reichelt and Magin, 2002). In these mice, basal keratinocytes express the normal complement of keratins (K5/K14), and this IF network appears to partially compensate for a loss of the suprabasal K1/K10 cytoskeleton. Keratinocyte hyperproliferation was observed in these mice, however, it was restricted to the basal cell layer.

Although K10 expression in basal keratinocytes might suppress proliferation, loss of K10 expression does not appear to be sufficient to allow suprabasal cells to re-enter the cell cycle. What about tumor susceptibility? Reichelt *et al* (2004) report that the K10 null mice do not show spontaneous tumor formation. To further analyze tumor susceptibility, these authors used a well-established two-stage chemical carcinogenesis protocol on their mice. The surprising finding was that the K10 null mice developed less papillomas per mouse than wild type controls. Furthermore, there was no significant difference conversion of papillomas to SSC. Reichelt and colleagues also provided an explanation for the reduced number of tumors in K10 null mice: The turnover of keratinocytes is increased in these mice, i.e., basal keratinocyte reach the skin surface and are shed into the environment much faster than wild-type cells. The authors suggest that the reduced transition time might reduce the pool of cells that accumulate enough genetic damage to convert to tumor cells.

Where Do We Stand?

The work outlined above highlights the fact that the type of IF cytoskeleton assembled in an epithelial cell can influence basic cell properties such as the ability to divide. The observed increase in the turnover of the epidermis raises the question of whether keratinocyte migration and/or cell detachment from the basement membrane are increased in the K10 null epidermis.

Skin cancer most likely develops as a result of genetic alterations that accumulate in epidermal stem cells. If Reichelt *et al*, are correct in their assumption that increased epidermal turnover is responsible for the reduced tumor incidence, one would have to assume that K10 null mice show accelerated stem cell depletion. As a consequence of this, one would expect to see the development of spontaneous phenotypes such as skin erosions that fail to heal, as was observed in another mouse model which exhibited an accelerated depletion of stem cells due to deregulated expression of c-Myc (Waikel *et al*, 2001). Interestingly, Reichelt and colleagues previously observed overexpression of c-Myc in the basal layer of K10 null mice (Reichelt and Magin, 2002), but to our knowledge they have not observed phenotypes with aging that would be associated with stem cell depletion.

The work by Reichelt and colleagues clearly shows that K10 is not a tumor suppressor gene, as suggested before. Nevertheless, the finding that loss of the normal K1/K10 IF network affects transit time and cell proliferation (although in trans) needs to be followed up and promises to provide new insights into keratinocyte differentiation.

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