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THE KERATINOCYTE: INTER- AND EXTRACELLULAR EVENTS

0930–1015 D. R. GARROD	Intercellular junctions in normal epidermis
1015–1100 M. F. JONKMAN	Dysfunction of keratinocyte adhesion
1100–1145 D. M. PROWSE	Developmental signals in skin morphogenesis
1145	*Winners of fellowships*
1230	*Cocktail and Lunch*
1430–1500 Y. BARRANDON	Keratinocyte stem cells. Is stem plasticity a likely contribution?
1500–1530 T. M. MAGIN	Animal models of human skin disease
1530–1600 D. P. KELSELL	Connexin mutations in human disease *Pause*
1630–1700 L. BRUCKNER-TUDERMAN	Inherited and acquired diseases of basement membrane – potential for therapy
1700–1730 I. R. HART	Role of integrins in tumor invasion and metastasis

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Intercellular junctions in normal epidermis

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Desmosomal cadherins

Ruswick S K, O'Hare M J, Jones J L, Streuli C H, Garrod D R. Desmosomal adhesion regulates epithelial morphogenesis and cell positioning. Nat Cell Biol 2001: 3: 823–830.

Anti-adhesion peptides, desmocollin and desmoglein, corresponding to the cell-adhesion recognition sites of desmosomal cadherins completely but reversibly blocked alveolar morphogenesis of mouse mammary cells and disrupted positional cell sorting in aggregates of human mammary luminal and myoepithelial cells. Desmosomal adhesion appears to be as important for morphogenesis as the adherens junctions adhesion molecule, E-cadherin. Positioning of luminal and myoepithelial cells can be determined by cell–cell adhesion without a contribution from the extracellular matrix.

Windoffer R, Borchert-Stulträger A, Leuber R E. Desmosomes: interconnected calcium-dependent structures of remarkable stability with significant integral membrane protein turnover. J Cell Sci 2002: 115: 1717–1732.

Expression of GFP-Dsc2a in cultured cells was used to image desmosomes. Although desmosomes are stable, fluorescence recovery after photobleaching revealed that desmocollin within them turns over rapidly. Desmosomes also persist during cell division, and internalized desmosomal halves following low calcium treatment are not re-utilized for desmosome assembly.

Chidgey M A J, Brakebusch C, Gustafsson E, et al. Mice lacking desmocollin 1 show epidermal fragility accompanied by barrier defects and abnormal differentiation. J Cell Biol 2001: 155: 821–832.

Dsc1 is required for strong adhesion in the upper epidermis. Its absence causes flaky skin and a striking punctate epidermal barrier defect in newborn mice and ulcerating lesions in older mice. In addition, $Dsc1^{-/-}$ mice show epidermal hyperproliferation, abnormal expression of keratins 6 and 16, and hair follicle degeneration, suggesting that Dsc1 may play some role in regulating epidermal differentiation.

Merritt A J, Berika M, Zhai W, et al. Suprabasal desmoglein 3 expression in the epidermis of transgenic mice results in hyperproliferation and abnormal differentiation. Mol Cell Biol 2002: 22: 5846–5858.

Misexpression of Dsg3 in the upper epidermis by the keratin 1 promoter causes abnormal epidermal differentiation and hyperproliferation providing further evidence that desmosomal cadherins have a regulatory role in epidermis.

Elias P M, Matsuyoshi N, Wu H, et al. Desmoglein isoform distribution affects stratum corneum structure and function. J Cell Biol 2001: 153: 243–249.

Ectopic expression of Dsg3 in the upper epidermis was driven by the involucrin promoter. This induced an abnormal stratum corneum with gross scaling and a lamellar histology resembling that of the oral muscosa, causing excessive lethal transepidermal water loss and early lethality. Again, desmosomal abnormal cadherin expression effects epidermal differentiation.

Andl C D, Stanley J R. Central role of the plakoglobin-binding domain for desmoglein 3 incorporation into desmosomes. J Invest Dermatol 2001: 117: 1069–1074.

Demonstration that the 87-amino acid-long plakoglobin-binding domain in the cytoplasmic tail of Dsg3 is necessary to target this desmosomal cadherin to desmosomes.

Wan H, Stone M G, Simpson C, et al. Desmosomal proteins, including desmoglein 3, serve as novel negative markers for epidermal stem cell-containing populations of keratinocytes. J Cell Sci 2003: 116: 4239–4248.

Basal keratinocytes at the tips of nete ridges in human palm, a region where stem cells are believed to be located, showed low expression of desmoplakin. Isolated palm keratinocytes that adhered rapidly to collagen IV also showed reduced expression of desmosomal proteins. Cells with low surface expression of desmoglein 3 (Dsg3) exhibited high clonogenicity, colony-forming efficiency, and enhanced proliferative potential. A population containing putative epidermal stem cells could be sorted on the basis of β1-integrin-bright and Dsg3-dull fluorescence.

Kljuic A, Bazzi H, Sundberg J P, et al. Desmoglein 4 in hair follicle differentiation and epidermal adhesion: evidence from inherited hypotricosis and acquired pemphigus vulgaris. Cell 2003: 113: 249–260.

The newly discovered desmosomal cadherins, desmoglein 4 (Dsg4), is expressed in suprabasal epidermis and hair follicles. Mutations of the *Dsg4* gene are found in families with inherited hypotricosis and the laceolate hair mouse. Pemphigus vulgaris sera recognize the Dsg4 protein. lah^J/lah^J mice having a mutation in the *Dsg4* gene show hyperproliferation and abnormal differentiation in the epidermis, providing additional evidence that desmosomal cadherins regulate these processes.

He W, Cowin P, Stokes D L. Untangling desmosomal knots with electron tomography. Science 2003: 302: 109–113.

The intercellular organization of desmosomal cadherins was examined by electron tomography of plastic sections from neonatal mouse skin. This analysis suggests that desmosomal cadherins form discrete groups with individual molecules interacting at their tips. Such an arrangement is consistent with a flexible adhesive interface involving standard exchange between the amino terminal subdomains, suggested by X-ray cryotallography of classical cadherins.

Desmosomal plaque components

Vasioukhin V, Vauer C, Yin M, Fuchs E. Directed actin polymerization is the driving force for epithelial cell–cell adhesion. Cell 2000: 100: 209–219.

During the early stages of calcium-induced keratinocyte cell adhesion, filopodia of adjacent cells interdigitate, driven by actin polymerization and form a double row of adherens junctions, the adhesion

zipper. Adhesion is then stabilized by the formation of desmosomes between the lateral surfaces of the filopodia.

Gallicano G I, Bauer C, Fuchs E. Rescuing desmoplakin function in extra-embryonic ectoderm reveals the importance of this protein in embryonic heart, neuroepithelium, skin and vasculature. Development 2001: 128: 929–941.

Because homozygous desmoplakin mutant embryos did not survive beyond embryonic day 6.5, the authors rescued the extra-embryonic tissues until mid-gestation by aggregation of knockout embryos with tetraploid morulae. Investigation of the fused embryos demonstrated the importance of desmosomal adhesion in the heart muscle and skin (which contain desmosomes) and the microvasculature (which does not contain desmosomes, but possesses unusual intercellular juctions composed of desmoplakin, plakoglobin, and VE-cadherin).

Vasioukhin V, Bowers E, Vauer C, Degenstein L, Fuchs E. Desmoplakin is essential in epidermal sheet formation. Nat Cell Biol 2001: 3: 1076–1084.

Conditional knockout of desmoplakin in mouse epidermis compromises epidermal cell sheet formation. The desmosomes are non-adhesive because they lack a fully formed plaque and attachment to the keratin cytokeleton. Other desmosomal components including plakoglobin, plakophilin 1, Dsc1, Dsg1, and Dsg3 still localize to cell–cell borders. Adherens junctions are also absent indicating a mutual dependence of these junctions and desmosomes.

Dhitavat J, Cobbold C, Leslie N, Burge S, Hovnanian A. Impaired trafficking of the desmoplakins in cultured Darier's disease keratinocytes. J Invest Dermatol 2003: 121: 1349–1355.

Darier's disease is an autosomal dominant skin disorder involving loss of desmosomal adhesion. It is caused by mutations in the *ATP2A2* gene which encodes the sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA) isoform 2 pump, responsible for Ca^{2+} transport into the endoplasmic reticulum to maintain a low cytosolic Ca^{2+} concentration. Inhibition of SERCA with thapsigargin impairs trafficking of desmosomal components to the cell surface. In Darier's disease, keratinocytes trafficking of desmoplakin, but not other components is significantly inhibited. Furthermore, interaction between SERCA2 and desmoplakin is demonstrated.

Gaudry C A, Palka H L, Dusek R L, et al. Tyrosine-phosphorylated plakoglobin is associated with desmogleins but not desmoplakin after epidermal growth factor receptor activation. J Biol Chem 2001: 276: 24871–24880.

Activated epidermal growth receptor tyrosine phosphorylates plakoglobin. The phosphorylated plakoglobin associates with Dsg2, but not with desmoplakin, and is predominantly situated in a membrane-associated Triton-X 100-soluble pool, disassociated from desmoplakin and intermediate filaments. Thus tyrosine phosphorylation of plakoglobin may regulate desmosomal adhesion.

Caldelari R, de Bruin A, Baumann D, et al. A central role for the armadillo protein plakoglobin in the autoimmune disease pemphigus vulgaris. J Cell Biol 2001: 153: 823–834.

Plakoglobin $(PG)^{+/+}$ keratinocytes respond to pemphigus vulgaris immunoglobulin G with keratin retraction and loss of adhesion but $PG^{-/-}$ keratinocytes do not. Re-introduction of PG to the $PG^{-/-}$ cells restores the response. This is the first evidence that PG plays a central role in mediating acantholysis in pemphigus vulgaris.

Miravet S, Piedra J, Castano J, et al. Tyrosine phosphorylation of plakoglobin causes contrary effects on its association with desmosomes and adherens junction components and modulates β-catenin-mediated transcription. Mol Cell Biol 2003: 23: 7391–7402.

Plakoglobin (PG), an armadillo family protein, is a component of both desmosomes and adherens junctions. The tyrosine kinase Src phosphorylates Tyr643 of PG decreasing its interaction with E-cadherin and catenin but increasing its interaction with desmoplakin. By contrast, phosphorylation of Tyr549 increases the interaction between PG and E-cadherin and α-catenin. Thus, tyrosine kinases may have different effects on desmosomes and adherens junctions. Increased binding of PG to adherens junction components by Tyr549 phosphorylation can also upregulate β-catenin/Tcf-4 transcriptional activity.

Chen X, Bonné S, Hatzfeld M, van Roy F, Green K J. Protein binding and functional characterization of plakophillin 2: evidence for its diverse roles in desmosomes and β-catenin signalling. J Biol Chem 2002: 277: 10512–10522.

The binding partners of the most widely expressed plakophillin, PP2, were identified. It has more binding partners than PP1, including desmoplakin, plakoglobin, Dsg1 and Dsg2, and Dsc1a and Dsc2a. Evidence is also provided that PP2 may regulate β-catenin signaling.

Bornslaeger E A, Gosel L M, Corcoran C M, et al. Plakophillin 1 interferes with plakoglobin binding desmoplakin, yet together with plakoglobin promotes clustering of desmosomal plaque complexes at cell–cell borders. J Cell Sci 2001: 114: 727–738.

Both plakophilin 1 (PP1) and plakoglobin bind to desmoplakin, but PP1 binds preferentially and excludes plakoglobin binding. Desmoplakin (DP) and plakoglobin form a complex with the cytoplasmic domain of Dsg1, but full-length DP also binds to Dsg1 in the absence of plakoglobin. Both plakoglobin and PP1 are required in association with DP and Dsg1 for the formation of structures resembling desmosomal plaques.

South AP, Wan H, Stone M G, et al. Lack of plakophilin 1 increases keratinocyte migration and reduced desmosome stability. J Cell Sci 2003: 116: 3303–3314.

Keratinocytes from patients with the autosomal recessive gonodermatosis skin fragility–ectodermal syndrome (OMIM 604536) lack plakophilin 1 (PP1) and possess few, poorly formed desmosomes. Re-introduction of PP1 enhanced desmosomal protein content and retarded cell migration. PP1-deficient keratinocytes in confluent cell sheets had fewer calcium-independent desmosomes than cells expressing PP1. The results suggest that PP1 has a key role in regulating desmosome assembly and adhesiveness and cell migration.

Wallis S, Lloyd S, Wise I, Ireland G, Fleming T P, Garrod D R. The α-isoform of protein kinase α-C is involved in signalling the response of desmosomes to wounding in cultured epithelial cells. Mol Biol Cell 2000: 11: 1077–1092.

Desmosomal adhesion develops from a calcium-dependent state to a calcium-independent state in confluent epithelial cell sheets and reversion to calcium dependence occurs on wounding. The change from calcium independence to calcium dependence is accompanied by activation of protein kinase C (PKC) and localization of PKCα to the cell periphery. Antisense depletion of PKCα promotes calcium independence of desmosomes. Desmosomes in mouse tissues including epidermis exhibit predominantly, or exclusively, calcium-independent adhesion.

Commented bibliographies

Dysfunction of keratinocyte adhesion

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Introduction

For normal skin function, epidermal keratinocytes need to maintain close proximity with each other. For that purpose, keratinocytes are equipped with intercellular junctions, such as desmosomes, adherens junctions, and tight junctions, that have an adhesive and signaling function. Inactivation of junctional components by mutation, autoimmune antibodies, and bacterial toxins breaches the structural integrity of embryos and adult tissues and affects proper cell morphogenesis and patterning. Recent discoveries have led to the understanding that dysfunction of keratinocyte adhesion not only may lead to bullous skin disease, but also may cause alopecia, keratoderma, nail deformaties, or dry wrinkled skin.

Selected papers in order of appearance

Bierkamp C, McLaughlin K J, Schwarz H, Huber O, Kemler R. Embryonic heart and skin defects in mice lacking plakoglobin. Dev Biol 1996: 180: 780–785.

First demonstration that defects in the armadillo protein plakoglobin cause structural defects in skin and heart and can result in embryonic lethality as a consequence of ruptured cardiac ventricles.

McGrath J A, McMillan J R, Shemanko C S, et al. Mutations in the plakophilin 1 gene result in ectodermal dysplasia/skin fragility syndrome. Nat Genet 1997: 17: 240–244.

The paper was the first to demonstrate that an inherited mutation of a desmosomal component causes dysadhesion of keratinocytes in humans. In retrospect, the described phenotype with alopecia and teeth abnormalities in addition to fragile skin preceeded the later concept that desmosomes not only have adhesive properties and but also play a crucial role in the formation of complex tissues such as hair and nails.

Rickman L, Simrak D, Stevens H P, et al. N-terminal deletion in a desmosomal cadherin causes the autosomal dominant skin disease striate palmoplantar keratoderma. Hum Mol Genet 1999: 8: 971–976.

Demonstration that mutations in the desmoglein 1 gene cause striate palmoplantar keratoderma. This study first supports the idea that desmosomal cadherins have crucial roles in regulating morphogenesis of complex epithelia.

Sakuntabhai A, Ruiz-Perez V, Carter S, et al. Mutations in ATP2A2, encoding a Ca^{2+} pump, cause Darier disease. Nat Genet 1999: 21: 271–277.

First demonstration that links mutations in an intracellular Ca pump with the loss of adhesion between epidermal cells and abnormal keratinization.

Amagai M, Matsuyoshi N, Wang Z H, Andl C, Stanley J R. Toxin in bullous impetigo and staphylococcal scalded-skin syndrome targets desmoglein 1. Nat Med 2000: 6: 1275–1277.

The paper was the first to demonstrate that the exfoliative toxins produced by *Staphylococcus aureus* can cleave desmoglein 1 and cause blisters in the superficial epidermis of mice that are similar to the blisters in patients with pemphigus foliaceus.

McKoy G, Protonotarios N, Crosby A, et al. Identification of a deletion in plakoglobin in arrhythmogenic right ventricular cardiomyopathy with palmoplantar keratoderma and woolly hair (Naxos disease). Lancet 2000: 355: 2119–2124.

Demonstration that mutations in plakoglobin are linked to heart and skin defects in men. This study extends the idea that desmosomal cadherins have crucial roles in regulating morphogenesis of complex tissues such as palmar skin and hair formation.

Sudbrak R, Brown J, Dobson-Stone C, et al. Hailey–Hailey disease is caused by mutations in ATP2C1 encoding a novel Ca(2+) pump. Hum Mol Genet 2000: 12: 9:1131–1140.

Demonstration that mutations in an intracellular Ca pump are linked with the loss of adhesion between epidermal cells in benign familial pemphigus.

Caldelari R, de Bruin A, Baumann D, et al. A central role for the armadillo protein plakoglobin in the autoimmune disease pemphigus vulgaris. J Cell Biol 2001: 153: 823–834.

Demonstrates an essential role for plakoglobin in mediating cytoplasmic responses to pemphigus antibodies that bind to desmoglein 3 at the cell surface.

Vasioukhin V, Bowers E, Bauer C, Degenstein L, Fuchs E. Desmoplakin is essential in epidermal sheet formation. Nat Cell Biol 2001: 3: 1076–1085.

Showed an essential role for desmoplakin in the formation of epithelial sheets and was the first to demonstrate that a lack of desmoplakin can influence adherens junction formation.

Furuse M, Hata M, Furuse K, et al. Claudin-based tight junctions are crucial for the mammalian epidermal barrier: a lesson from claudin-1-deficient mice. J Cell Biol 2002: 156: 1099–1111.

The paper compels us to consider that functional tight junctions in stratified mammalian epithelia are indeed a necessary part of the permeability barrier of the skin. Adhesion molecules, claudins, are responsible for the barrier function. The claudin-1 null mice displayed a wrinkled skin with increased transepidermal water vapor loss leading to death within 1 day.

Kljuic A, Bazzi H, Sundberg J P, et al. Desmoglein 4 in hair follicle differentiation and epidermal adhesion: evidence from inherited hypotrichosis and acquired pemphigus vulgaris. Cell 2003: 113: 249–260.

Demonstration that mutations in a novel desmosomal cadherin isoform are linked to hair follicle defects in mice and men. This study extends the idea that desmosomal cadherins have crucial roles in regulating morphogenesis of complex epithelia such as the formation or hair.

Nguyen V T, Chernyavsky A I, Arredondo J, et al. Synergistic control of keratinocyte adhesion through muscarinic and nicotinic acetylcholine receptor subtypes. Exp Cell Res 2004: 294: 534–549.

This study elucidated the roles of non-desmosomal acetylcholine receptors in physiologic control of keratinocyte adhesion, most probably by modulating cadherin and catenin levels and activities.

Nguyen V T, Arredondo J, Chernyavsky A I, Pittelkow M R, Kitajima Y, Grando SA. Pemphigus vulgaris acantholysis ameliorated by cholinergic agonists. Arch Dermatol 2004: 140: 327–334.

First demonstration in mice and men that the cholinergic agonists, such as carbachol and pyridostigmine bromide, may improve keratinocyte adhesion in pemphigus.

Getsios S, Huen A C, Green K J. Working out the strength and flexibility of desmosomes. Nat Rev Mol Cell Biol 2004: 5: 271–281.

Excellent review guiding the reader into the latest concepts of cell adhesion and tissue morphogenesis regulated by desmosomes.

Lorch J H, Klessner J, Park J K, et al. Epidermal growth factor receptor inhibition promotes desmosome assembly and strengthens intercellular adhesion in squamous cell carcinoma cells. J Biol Chem 2004 (online prepublication).

This study demonstrates that inhibition or blocking of the epidermal growth factor receptor promotes desmosome assembly in oral squamous cell carcinoma cells, resulting in increased cell–cell adhesion.

Developmental signals in skin morphogenesis

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The epidermis, with its appendage the hair follicle, is a fascinating system to study the mechanisms controlling tissue formation and homeostasis. It is a self-renewing stratified squamous epithelium that forms the outermost layer of the skin and an essential permeability barrier, preventing moisture escape and the entry of harmful substances. Failure to form this barrier, due to immaturity of the skin in premature infants or mutation in the case of inherited disorders, can be lethal. Terminally differentiated keratinocytes, lipids, and intercellular junctions are all essential for barrier formation. The terminal differentiation program involves mitotically active basal keratinocytes detaching from the basement membrane and migrating upward toward the skin surface, from which they are shed as cornified squames. In humans, barrier function develops at 34 weeks, while in mice it occurs between E16.5 and E17.5 as the granular layer and stratum corneum form. The hair follicle is also generated during embryogenesis though a series of signals sent between the keratinocytes and the underlying mesenchymally derived dermal cells. These signals cause cell fate changes in both populations, which ultimately results in the differentiation of the hair shaft, root sheaths, and dermal papilla. The postnatal hair subsequently undergoes successive cycles of hair growth and regression. This ability of the epidermis to self-renew is dependent on the maintenance of keratinocyte stem cells. Much of our understanding of epithelial development and self-renewal has come from studying inherited human disorders and mouse mutants. Several candidate molecules have been shown to regulate epidermal development including Wnt, p63, myc, and the hedgehog family of signaling molecules. This commented bibliography lists recent papers that have contributed to our understanding of the regulatory pathways involved in the process of epidermal development.

Bibliography

Fuchs E, Raghavan S. Getting under the skin of epidermal morphogenesis. Nat Rev Genet 2002: 3 (3): 199–209.

Review describing factors regulating epidermal differentiation and self-renewal.

Segre J. Complex redundancy to build a simple epidermal permeability barrier. Curr Opin Cell Biol 2003: 15 (6): 776–782.

A review of permeability barrier formation in mammalian epidermis, which also describes redundancy and compensatory mechanisms in the system.

Hardman M J, Sisi P, Banbury D N, Byrne C. Patterned acquisition of skin barrier function during development. Development 1998: 125 (8): 1541–1552.

Demonstration of that barrier formation during epidermal development is acquired in a regulated manner. The barrier was observed to occur at specific epidermal sites, then spreads around the embryo as a moving front, accompanied by multiple changes in the outer, stratum corneum-precursor cells.

Jaubert J, Cheng J, Segre J A. Ectopic expression of kruppel like factor 4 (Klf4) accelerates formation of the epidermal permeability barrier. Development 2003: 130 (12): 2767–2777.

The authors have previously shown that the transcription factor Klf4 is required for barrier function in the skin. In this report, they show that keratin 5 promoter-driven ectopic Klf4 expression causes competent barrier formation to occur 1 day earlier.

Chidgey M, Brakebusch C, Gustafsson E, et al. Mice lacking desmocollin 1 show epidermal fragility accompanied by barrier defects and abnormal differentiation. J Cell Biol 2001: 155 (5): 821–832.

The targeted disruption of the desmosomal cadherin desmocollin 1 (Dsc1) causes flaky skin and a striking punctate epidermal barrier defect. This shows that Dsc1 is required for strong adhesion and barrier maintenance in epidermis and contributes to epidermal differentiation.

Frye M, Gardner C, Li E R, Arnold I, Watt F M. Evidence that Myc activation depletes the epidermal stem cell compartment by modulating adhesive interactions with the local microenvironment. Development 2003: 130 (12): 2793–2808.

Expression of c-Myc in epidermal stem cells was previously reported by Waikel et al. and Arnold and Watt in 2001 to deplete the epidermal stem cell compartment by driving keratinocytes from the stem to the transit-amplifying compartment. Myc also stimulated proliferation and differentiation along the epidermal and sebaceous lineages at the expense of the hair lineage. This investigation used microarray analysis to show that Myc activation has profound affects on the adhesive and motile behavior of keratinocytes. This reduced adhesiveness is proposed to stimulate exit of epidermal cells from the stem cell compartment and may also affect the ability of keratinocytes to migrate along the outer root sheath, accounting for the failure of hair differentiation.

Koster M I, Kim S, Mills A A, DeMayo F J, Roop D R. p63 is the molecular switch for initiation of an epithelial stratification program. Genes Dev 2004: 18 (2): 126–131.

The laboratories of Bradley and McKeon in 1999 independently reported that mice lacking p63 have multiple defects that include epidermal and skeletal abnormalities. These mice do not have a recognizable epidermis but possess a thin single-cell layer covering the body, which does not form an effective barrier to prevent dehydration, and the mice die within hours of birth. This paper demonstrates that p63 isoforms regulate the initiation of epithelial stratification and suggests that p63 plays a dual role: initiating epithelial stratification during development and maintaining proliferative potential of basal keratinocytes in mature epidermis.

Sil A K, Maeda S, Sano Y, Roop D R, Karin M. IkappaB kinase-alpha acts in the epidermis to control skeletal and craniofacial morphogenesis. Nature 2004: 428 (6983): 660–664.

Ikappa B ($I_{\kappa}B$) kinase α (IKKα; also called IKK1)-deficient mice surprisingly exhibit perinatal lethality and exhibit a wide range of developmental defects including impaired limb outgrowth and abnormal epithelial development. The IKKα kinase activity is required for lymphoid organogenesis and mammary gland development, whereas a kinase-independent activity is required for epidermal keratinocyte differentiation. IKKα was previously found by this group to control production of a secreted factor that induces keratinocyte differentiation. This paper shows that IKKα in the epidermis is also an important regulator of the mesenchyme. The restoration of epidermal differentiation in IKKα-deficient mice by keratinocyte-specific IKKα

expression prevented the abnormal skeletal and craniofacial morphogenesis normally observed in these mice, an effect associated with the repression of fibroblast growth factor (FGF) family expression.

Randall V A, Sundberg J P, Philpott M P. Animal and in vitro models for the study of hair follicles. J Investig Dermatol Symp Proc 2003: 8 (1): 39–45.

A recent examination of the *in vitro* and *in vivo* models developed to study hair follicles. The advantages and disadvantages of cell culture, organ culture of isolated follicles, and the study of natural or genetically manipulated animals are considered.

Oshima H, Rochat A, Kedzia C, Kobayashi K, Barrandon Y. Morphogenesis and renewal of hair follicles from adult multipotent stem cells. Cell 2001: 104 (2): 233–245.

This paper demonstrates that the upper region of the outer root sheath of vibrissal follicle of adult mice contains multipotent stem cells, which respond to morphogenetic signals to generate multiple hair follicles, sebaceous glands, and epidermis. These stem cells are therefore able to generate all the lineages of the hairy skin, and this requires a precise control of stem cell trafficking. Importantly, clonogenic keratinocytes in culture were also found to be closely related, if not identical, to the multipotent stem cells.

Jamora C, DasGupta R, Kocieniewski P, Fuchs E. Links between signal transduction, transcription and adhesion in epithelial bud development. Nature 2003: 422 (6929): 272–273.

Epithelial bud development depends on epithelial mesenchymal interactions and plays an important role in the morphogenesis of several organs including the hair follicle. This research shows that remodeling of cell-adhesion junctions permits follicle formation and that this process is regulated by the Wnt and BMP extracellular signaling pathways.

Suzuki A, Itami S, Ohishi M, et al. Keratinocyte-specific Pten deficiency results in epidermal hyperplasia, accelerated hair follicle morphogenesis and tumor formation. Cancer Res 2003: 63 (3): 674–681.

An interesting paper demonstrating the effect of disrupting normal growth controls in the skin. PTEN is a tumor suppressor gene mutated in many human cancers, and a keratinocyte-specific null mutation of Pten in mice was generated using the Cre-loxP system [k5Pten(flox/flox) mice]. Skin morphogenesis was accelerated and the k5Pten(flox/flox) mice exhibited wrinkled skin because of epidermal hyperplasia and hyperkeratosis and ruffled, shaggy, and curly hair.

Peng X D, Xu P Z, Chen M L, et al. Dwarfism, impaired skin development, skeletal muscle atrophy, delayed bone development, and impeded adipogenesis in mice lacking Akt1 and Akt2. Genes Dev 2003 17 (11):1352–1365.

This paper reveals that the serine/threonine kinase Akt/PKB (which is a downstream effector of PI3K and acts as a transducer of multiple functions including cell growth, cell survival, and metabolic actions of insulin) plays a significant role in regulating epidermal proliferation and differentiation. Three isoforms of Akt (Akt1–3) have been reported in mammalian cells. Previous studies which generated Akt1 or 2 null mice did not reveal an epidermal phenotype. The double-knockout (DKO) mice reported here lack both Akt1 and Akt2 genes and exhibit severe growth deficiency, shiny skin (indicating a barrier defect), and lethality shortly after birth. The DKO mice displayed impaired skin development showing reduced interfollicular proliferation and abnormal differentiation, and reduced hair bud development.

Janes S M, Ofstad T A, Campbell D H, Watt F M, Prowse D M. Transient activation of FOXN1 in keratinocytes induces a transcriptional programme that promotes terminal differentiation; contrasting roles of FOXN1 and Akt. J Cell Sci 2004 (in press).

The forkhead transcription factor FOXN1 is required for normal cutaneous and thymic epithelial development. Mutations in *FOXN1* give rise to the nude phenotype in mice, rats, and man. Transient activation of FOXN1 in proliferating primary human epidermal keratinocytes induced terminal differentiation. Genes promoting growth arrest, survival, and differentiation were induced by FOXN1 including the kinase Akt. Interestingly, when activated in reconstituted epidermis, FOXN1 promoted early stages of terminal differentiation, whereas Akt activation was sufficient to induce late stages including formation of the cornified layers. These results establish a role for FOXN1 in the initiation of terminal differentiation and implicate Akt in subsequent events.

Gat U, DasGupta R, Degenstein L, Fuchs E. De novo hair follicle morphogenesis and hair tumors in mice expressing a truncated beta-catenin in skin. Cell 1998: 95 (5): 605–614.

Demonstration that stabilized β-catenin expressed in the basal layer of the epidermis from E15 with a keratin promoter causes *de novo* hair follicle formation. The new follicles show induction of Lef-1, but they are also disoriented and defective in sonic hedgehog polarization. Additionally, transgenic hair follicle proliferation can continue unchecked, causing hair follicle tumors. This paper shows that β-catenin is a key player in hair development and implicates aberrant β-catenin activation in hair tumor formation.

Huelsken J, Vogel R, Erdmann B, Cotsarelis G, Birchmeier W. β-Catenin controls hair follicle morphogenesis and stem cell differentiation in the skin. Cell 2001: 105 (4): 533–545.

β-Catenin is an essential molecule in Wnt/wingless signaling, and this group have previously shown that β-catenin-deficient mice show embryonic lethality at E5.5. This paper describes a keratinocyte-specific null mutation of β-catenin in mice, generated using the Cre-loxP system, that shows that β-catenin mutation during embryogenesis blocks formation of hair follicle placodes. If β-catenin deletion occurred after hair follicles formed, hair was completely lost after the first hair cycle. During placode formation, β-catenin was found to be downstream of tabby/downless and upstream of bmp and shh. This paper demonstrates that β-catenin plays a major role in regulating stem cell fate: β-catenin signaling promotes the hair follicle lineage, while keratinocyte stem cells adopt an interfollicular fate in the absence of β-catenin.

Andl T, Reddy S T, Gaddapara T, Millar S E. WNT signals are required for the initiation of hair follicle development. Dev Cell 2002: 2 (5): 643–653.

This paper demonstrates the importance of Wnt signals for hair follicle development. In transgenic mice, an ectopically expressed diffusible inhibitor of Wnt action, Dickkopf 1, was shown to inhibit hair follicle placode formation prior to morphological or molecular signs of differentiation, and also blocked tooth and mammary gland development before the bud stage. This indicates that activation of Wnt signaling in the skin precedes, and is required for, localized expression of regulatory genes and initiation of hair follicle placode formation.

DasGupta R, Fuchs E. Multiple roles for activated LEF/TCF transcription complexes during hair follicle development and differentiation. Development 1999: 126 (20): 4557–4568.

This paper demonstrates the value of utilizing a TOPGAL reporter to define activated Lef/Tcf transcription factor complexes in hair follicle morphogenesis and differentiation. Interestingly, activated Lef/Tcf complexes were observed at distinct times in hair development and cycling when changes in cell fate and differentiation commitments take place. Tcf3 was expressed in the follicle stem cells in the bulge, and TOPGAL was stimulated in this compartment at the start of the hair cycle in a manner that appeared to be dependent on stabilization of β-catenin. This shows the existence of positive and negative regulators of Lef/Tcf factors in the skin and suggests they are necessary but not sufficient for TOPGAL activation.

Niemann C, Unden A B, Lyle S, Zouboulis C H C, Toftgard R, Watt F M. Indian hedgehog and beta-catenin signaling: role in the sebaceous lineage of normal and neoplastic mammalian epidermis. Proc Natl Acad Sci USA 2003: 100: 11873–11880.

Overexpression of N-terminally truncated Lef1 (ΔNLef1), which blocks β-catenin signaling, results in hair loss, development of dermal

cysts, and spontaneous sebaceous tumors. This paper further characterizes the sebaceous tumors in mice and humans and finds overexpression of the receptor PTCH and the ligand Indian hedgehog (IHH) but not Sonic hedgehog. A role for IHH in sebaceous neoplasia was supported by the observation that ΔNLef1 was found to upregulate IHH and stimulate proliferation of cultured undifferentiated sebocytes.

Celso C L, Prowse D M, Watt F M. Transient activation of β-catenin signalling in adult mouse epidermis is sufficient to induce new hair follicles but continuous activation is required to maintain hair follicle tumours. Development 2004: 131 (8): 1787–1799.

When β-catenin signaling is disturbed from mid-gestation onward lineage commitments is profoundly altered in postnatal mouse epidermis. This paper shows that adult epidermis also has the capacity for β-catenin-induced lineage conversion without prior embryonic priming. An activatable N-terminally truncated, stabilized β-catenin estrogen receptor fusion was expressed in the epidermis of transgenic mice. β-catenin signaling was observed to trigger the active growth phase of the hair cycle (confirming a report by Van Mater et al. 2003) and *de novo* follicle formation in adult epidermis. Sebocyte differentiation was also affected. This shows that interfollicular epidermis and sebaceous glands retain the ability to be reprogrammed in adult life. Interestingly, long-term treatment also caused conversion of hair follicles to benign tumors resembling trichofolliculomas which regressed upon cessation of β-catenin activation.

Animal models of human skin disease

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Commentary

The epidermis is a stratified epithelium which continuously regenerates from stem cells. The keratin intermediate filament cytoskeleton connects with adhesive junctions and the cornified envelope and is largely responsible for skin integrity. Many of these proteins are encoded by gene families which are differentially expressed during epidermal differentiation and regeneration. Together with human genetic studies, mouse models have been instrumental to identify disease-causing genes and have provided some unexpected and exciting insights. Loss of function studies has shown that epidermal keratins, plakins, and plakophilins as well as integrins are essential to maintain the integrity of the basal epidermis. In line, mutations in corresponding human genes underlie severe skin pathology. Genetic ablation of suprabasal keratins and other structural proteins in the suprabasal epidermis, on the other hand, have a remarkably limited influence on skin integrity. This is also true for the ablation of cornified envelope genes, at least in the mouse, either pointing to redundancy or pointing to the need to re-evaluate our hypothesis on structure–function relationships. The latter is supported by the notion that deletion of claudin-1 disrupts the epidermal water barrier without affecting the structure and composition of the lipid envelope. Also, point mutations in several genes, including keratins, produce severe phenotypes while the loss of function mutations does not. Therefore, in order to understand mechanisms leading to disease and to develop successful therapies, it will be essential to understand why epidermal protein networks are encoded by such complex gene families and how the protein networks are formed and regulated.

References

Furuse M, Hata M, Furuse K, et al. Claudin-based tight junctions are crucial for the mammalian epidermal barrier: a lesson from claudin-1-deficient mice. J Cell Biol 2002: 156: 1099–1111.

Describes the previously unnoted presence of tight junctions in epidermis and shows that the loss of claudin-1 disrupts the epidermal barrier.

Vasioukhin V, Bowers E, Bauer C, Degenstein L, Fuchs E. Desmoplakin is essential in epidermal sheet formation. Nat Cell Biol 2001: 3: 1076–1085.

Demonstrates that desmoplakin is essential for the linkage of epidermal keratins to desmosomes and for epithelial integrity.

Vasioukhin V, Bauer C, Degenstein L, Wise B, Fuchs E. Hyperproliferation and defects in epithelial polarity upon conditional ablation of alpha-catenin in skin. Cell 2001: 104: 605–617.

Provides evidence that loss of alpha-catenin has profound effects on keratinocyte differentiation *in vivo* and *in vitro*.

Vassar R, Coulombe P A, Degenstein L, Albers K, Fuchs E. Mutant keratin expression in transgenic mice causes marked abnormalities resembling a human genetic skin disease. Cell 1991: 64: 365–380.

The first demonstration that an intermediate filament mutation causes tissue fragility syndromes.

Cao T, Longley M A, Wang X J, Roop D R. An inducible mouse model for epidermolysis bullosa simplex: implications for gene therapy. J Cell Biol 2001: 152: 651–656.

The first inducible model for epidermolysis bullosa simplex (EBS), providing evidence for lateral migration of stem cells and pointing out routes to EBS therapies.

Dowling J, Yu Q C, Fuchs E. Beta4 integrin is required for hemidesmosome formation, cell adhesion and cell survival. J Cell Biol 1996: 134: 559–572.

Demonstrates the need for hemidesmosomal integrins to maintain epidermal integrity and serves as model for junctional EB (Epidermolysis Bullosa).

Porter R M, Leitgeb S, Melton D W, Swensson O, Eady R A, Magin T M. Gene targeting at the mouse cytokeratin 10 locus: severe skin fragility and changes of cytokeratin expression in the epidermis. J Cell Biol 1996: 132: 925–936.

The first mouse model for EHK (Epidermolysis Hyperkeratosis). In conjunction with the paper of Reichelt and Magin, it demonstrates that the gain of toxic function can be far more severe than the loss of function mutations.

Peters B, Kirfel J, Bussow H, Vidal M, Magin T M. Complete cytolysis and neonatal lethality in keratin 5 knockout mice reveal its fundamental role in skin integrity and in epidermolysis bullosa simplex. Mol Biol Cell 2001: 12: 1775–1789.

The most severe pathology in epidermis of all keratin knockouts, resulting from the complete absence of filaments in basal epidermis.

Reichelt J, Magin T M. Hyperproliferation, induction of c-Myc and 14-3-3sigma, but no cell fragility in keratin-10-null mice. J Cell Sci 2002: 115: 2639–2650.

Provides evidence for the role of keratins as mechanotransducers. Loss of K10 leads to increased proliferation in another epidermal compartment.

McGowan K M, Tong X, Colucci-Guyon E, Langa F, Babinet C, Coulombe P A. Keratin 17 null mice exhibit age- and strain-dependent alopecia. Genes Dev 2002: 16: 1412–1422.

Demonstrates that K17 is important for hair formation and integrity, as alopecia develops in its absence.

Wong P, Colucci-Guyon E, Takahashi K, Gu C, Babinet C, Coulombe P A. Introducing a null mutation in the mouse K6alpha and K6beta genes reveals their essential structural role in the oral mucosa. J Cell Biol 2000: 150: 921–928.

An unexpected observation suggesting that the major role of K6 isoforms may be to act as reinforcement keratins and not so much in wound healing.

DiPersio C M, van der N R, Georges-Labouesse E, Kreidberg J A, Sonnenberg A, Hynes R O. alpha3beta1 and alpha6beta4 integrin receptors for laminin-5 are not essential for epidermal morphogenesis

and homeostasis during skin development. J Cell Sci 2000: 113 (Pt 17): 3051–3062.

Shows that epidermal stratification takes place in the absence of major integrins.

Koch P J, de Viragh P A, Scharer E, et al. Lessons from loricrin-deficient mice: compensatory mechanisms maintaining skin barrier function in the absence of a major cornified envelope protein. J Cell Biol 2000: 151: 389–400.

In conjunction with paper of Djian et al., this work shows that cornified envelope formation has many backup proteins and does not require loricrin.

Djian P, Easley K, Green H. Targeted ablation of the murine involucrin gene. J Cell Biol 2000: 151: 381–388.

See paper Koch et al.

Maatta A, DiColandrea T, Groot K, Watt F M. Gene targeting of envoplakin, a cytoskeletal linker protein and precursor of the epidermal cornified envelope. Mol Cell Biol 2001: 21: 7047–7053.

Supports the redundancy of cornified envelope proteins and shows that in newborn mice, its formation is slightly delayed but not impaired.

Herrmann H, Hesse M, Reichenzeller M, Aebi U, Magin T M. Functional complexity of intermediate filament cytoskeletons: from structure to assembly to gene ablation. Int Rev Cytol 2003: 223: 83–175.

A concise review.

Connexin mutations in human disease

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Introduction

Gap junctions composed of connexins provide a mechanism of synchronized cellular response facilitating the metabolic and electronic functions of the cell by the direct intercellular transfer of ions and small molecules. Germline mutations in different human connexins, and indeed within the same connexin protein, can produce an array of phenotypes associated with the human ectodermal epithelium including hearing loss, neuropathy, hair growth abnormalities, and hyperkeratosis. These genetic studies also suggest that different mutations within a specific connexin have distinct effects on epidermal differentiation and the sensory epithelia of the inner ear. The association of hyperproliferative skin disease with connexin mutations supports an important function for these gap junction proteins in epidermal differentiation. The keratinocyte and its programmed cellular differentiation is proving to be an excellent model to further our understanding of connexin biology in other complex but less accessible epithelial and non-epithelial tissues including the inner ear.

References

Bergoffen J, Scherer S S, Wang M, et al. Connexin mutations in X-linked Charcot–Marie–Tooth disease. Science 1993: 262: 2039–2041.

Connexin 32 was the first disease-associated connexin and is mutated in the X-linked form of Charcot–Marie–Tooth disease. This progressive neuropathy results from myelin disruption and axonal degeneration of peripheral nerves. It is proposed that Cx32 mutations impair the diffusion of metabolites through a type of gap junction termed a 'reflexive gap junction' that is found between the Schwann cell body and its distal processes.

Kelsell D P, Dunlop J, Stevens H P, et al. Connexin 26 mutations in hereditary non-syndromic sensorineural deafness. Nature 1997: 387: 80–83.

The first paper describing recessive mutations in the coding region of GJB2 encoding Cx26 associated with non-syndromic hearing loss (NSHL). Numerous subsequent studies have shown that GJB2 mutation account for a significant proportion of NSHL worldwide. Within different ethnic groups, there are specific common founder mutations that account for the majority of GJB2-related hearing loss, for example, 35delG, 235delC, and R143W in the European, Japanese, and African populations, respectively.

Maestrini E, Korge B P, Ocaña-Sierra J, et al. A missense mutation in connexin26, D66H, causes mutilating keratoderma with sensorineural deafness (Vohwinkel's syndrome) in three unrelated families. Hum Mol Genet 1999: 8:1237–1243.

This paper was the first to associate connexin mutations with a disorder of epidermal keratinization. The authors describe a specific Cx26 mutation that underlies Vohwinkel's syndrome. This disease is characterized by a honeycomb pattern of keratoderma with starfish-like keratoses on the knuckles and mild-moderate sensorineural hearing loss. Other studies have now shown other Cx26 mutations linked to keratitis–ichthyosis–deafness syndrome, Cx31 mutations associated with the skin disease erythrokeratoderma variabilis, and Cx30 mutations with the ectodermal dysplasia, Clouston's syndrome.

Di W L, Monypenny J, Common J E, et al. Defective trafficking and cell death is characteristic of skin disease-associated connexin 31 mutations. Hum Mol Genet 2002: 11:2005–2014.

EGFP (Enhanced Green Fluorescent Protein)-tagged wildtype or mutant connexin Cx31 fusion proteins were used to study mutant connexins with regard to junction assembly and function in keratinocytes. Following transfection, wildtype connexin proteins are able to traffick to the membrane and form functional gap junction plaques indicated by dye transfer. The skin disease-associated mutant proteins display limited trafficking with primarily a cytoplasmic localization. Another striking cellular phenotype was that skin disease-associated mutant Cx31 induced keratinocyte cell death, but the expression of hearing loss and/or neuropathy-associated Cx31 mutations did not.

Cohen-Salmon M, Ott T, Michel V, et al. Targeted ablation of connexin26 in the inner ear epithelial gap junction network causes hearing impairment and cell death. Curr Biol 2002:12:1106–1111.

The hearing loss phenotype seen in human Cx26 'knockouts' have been replicated from gene-targeting studies in the mouse. These transgenic mice displayed cell death in the inner ear leading to degeneration of the cochlear sensory epithelium. This cell death phenotype is analogous to the keratinocyte cell death observed with skin disease-associated connexin mutations.

Di W L, Rugg E L, Leigh I M, Kelsell D P. Multiple epidermal connexins are expressed in different keratinocyte subpopulations including connexin 31. J Invest Dermatol 2001: 117: 958–964.

This study demonstrated that there are, at least, 10 connexin proteins expressed in the human epidermis with the junctional composition and cellular localization changing as keratinocytes differentiate.

Goldberg G S, Lampe P D, Nicholson B J. Selective transfer of endogenous metabolites through gap junctions composed of different connexins. Nat Cell Biol 1999: 1: 457–459.

The complex pattern of connexin expression in many tissue types including the epidermis suggests that there may be distinct cellular roles for individual or subgroups of connexins. This study demonstrated that intercellular channels have distinct permeabilities and selectivities for different molecules such as ATP, AMP, and IP3 depending on their connexin composition.

Quist A P, Rhee S K, Lin H, Lal R. Physiological role of gap-junctional hemichannels. Extracellular calcium-dependent isosmotic volume regulation. J Cell Biol 2000: 148: 1063–1074.

This paper suggests an additional role for connexins other than intercellular communication. Open connexons (hemichannels) at the non-junctional plasma membrane may play a role in paracrine signaling. These hemichannels can function within the plasma membrane of mammalian cells to regulate cell volume and that the channels are gated within the physiological range of extracellular calcium ion concentration.

Marziano N K, Casalotti S O, Portelli A E, Becker D L, Forge A. Mutations in the gene for connexin 26 (GJB2) that cause hearing loss have a dominant negative effect on connexin 30. Hum Mol Genet 2003: 12: 805–812.

These studies in HeLa cells showed that overexpressing either WT-Cx26 or WT-Cx30 could overcome the inherent trafficking defect of skin disease-associated mutant Cx26 proteins but these channels had impaired dye permeability.

Bakirtzis G, Choudhry R, Aasen T, et al. Targeted epidermal expression of mutant Connexin 26(D66H) mimics true Vohwinkel's syndrome and provides a model for the pathogenesis of dominant connexin disorders. Hum Mol Genet 2003: 12: 1737–1744.

In addition to epidermal phenotypes associated with the human skin disease Vohwinkel's syndrome, keratinocytes from these transgenic mice overexpressing the D66H-Cx26 mutation driven off the keratin 10 promoter displayed increased cell death. This phenotype replicates the cell death phenotype seen from previous *in vitro* studies.

Role of integrins in tumor invasion and metastasis

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Polarized cell movement and extracellular matrix degradation are important components of tumor cell invasion, and both activities are regulated by members of the integrin family of transmembrane receptors.

The integrin αvβ6 is a fibronectin tenascin and latency-associated peptide (LAP) of TGFB receptor, which is not detectable on normal epithelium but is neo-expressed in a range of carcinomas including oral epithelial dysplasia and oral squamous cell carcinoma (SCC), suggesting it has a role in promoting malignant behavior and tumor progression. We have used transfection and retroviral infection to create a panel of SCC cell lines expressing various levels of αvβ6 to examine this possibility. We found that increased expression of αvβ6 in malignant keratinocytes upregulates matrix metalloproteinase-9 (MMP-9) and MMP-2 expression and promotes invasion in an MMP-9-dependent manner.

Amino acids with the sequence EKQKVDLSTDC, which are the C-terminal residues of the integrin β6 subunit, were shown to promote αvβ6-dependent invasion in an MMP-9-dependent fashion. This same peptide sequence, when expressed at the cytoplasmic end of the β3-integrin subunit, was able to enhance αvβ3-mediated invasive and enzymatic activity of tumor cells in an MMP-2-dependent fashion. Our results show that these 11 amino acids, when expressed at the C terminus of the β subunit, are responsible for regulating the activity of invasion-promoting degradative enzymes, whereas the specific MMP involved in this cellular behavior was dependent on the context of the remainder of the β-integrin subunit.

It appears that integrins are moved from the invading to the retracting edge of motile cells, and this suggests that an understanding of the control of integrin internalization and endocytic recycling might permit interference with cellular motility if target molecules involved in this process can be identified.

In an effort to identify interactions between β6 and putative intracellular signaling molecules, we have used the β6-integrin cytoplasmic domain as bait in a yeast two-hybrid screen of a human keratinocyte cDNA library. We found an interaction with the widely (possibly ubiquitously) expressed 34 kDa protein HS1-associated protein X-1 (Hax-1). This protein appears to be involved in recycling of cell surface-located proteins and when Hax-1 was reduced in a variety of cell lines, by transfection with siRNA, transfected cells showed no alteration in morphology, growth, or adhesion to a variety of substrates but a marked reduction in cell migration toward LAP, an αvβ6-specific ligand. This suggests that recycling of β6 is a necessary component of αvβ6-mediated migration.

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