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CASE REPORT

Exon 87 skipping of the *COL7A1* gene in dominant dystrophic epidermolysis bullosa

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ABSTRACT

Dystrophic epidermolysis bullosa (DEB) is a rare, inherited, blistering disorder resulting from mutations in the *COL7A1* gene, which encodes the anchoring fibrils, type VII collagen. We herein describe a further Japanese girl diagnosed with dominant DEB (DDEB). She had blisters sporadically and erosions healed with mild scarring and milia on the knees and pretibial regions. Severe pruritus was present at this time. Direct nucleotide sequencing of genomic DNA disclosed a heterozygous same splice-site mutation c.6900G>A in the *COL7A1*, which causes in-frame exon 87 skipping. So far, five different *COL7A1* mutations leading to exon 87 skipping have been identified in rare forms of DEB: four DDEB pruriginosa and one pretibial DDEB. Therefore, a recent study suggested that exon 87 skipping in *COL7A1* was related to the phenotype of DDEB pruriginosa. When she was 18 years old, however, the blister formation and pruritus markedly decreased. Therefore, her clinical symptoms were consistent to very mild DDEB but not to DDEB pruriginosa. Taken together, in-frame exon 87 skipping through c.6900G>A mutation may account for the mild skin features, rather than DDEB pruriginosa, in the present case.

Key words: *COL7A1*, dystrophic epidermolysis bullosa, genodermatosis, genotype–phenotype correlation, pruriginosa.

INTRODUCTION

Dystrophic epidermolysis bullosa (DEB) is a rare inherited blistering disorder resulting from mutations in the *COL7A1* gene, which encodes type VII collagen, the major component of anchoring fibrils at the dermal–epidermal junction. Over 300 pathogenic mutations have been described within *COL7A1* in various clinical forms of DEB. So far, five different *COL7A1* mutations leading to exon 87 skipping have been identified in rare forms of DEB: four dominant DEB (DDEB) pruriginosa and one pretibial DDEB.[1–4] Therefore, a recent study suggested that exon 87 skipping in *COL7A1* was related to the phenotype of DDEB pruriginosa.[5] We herein describe a further DDEB patient with the same splice-site mutation c.6900G>A, but the clinical appearance of the patient was quite different from that of DDEB pruriginosa.

CASE REPORT

The patient was a Japanese girl who was the offspring of healthy unrelated parents. She presented with a history of trauma-induced skin blistering and erosions mainly on the extremities at the age of

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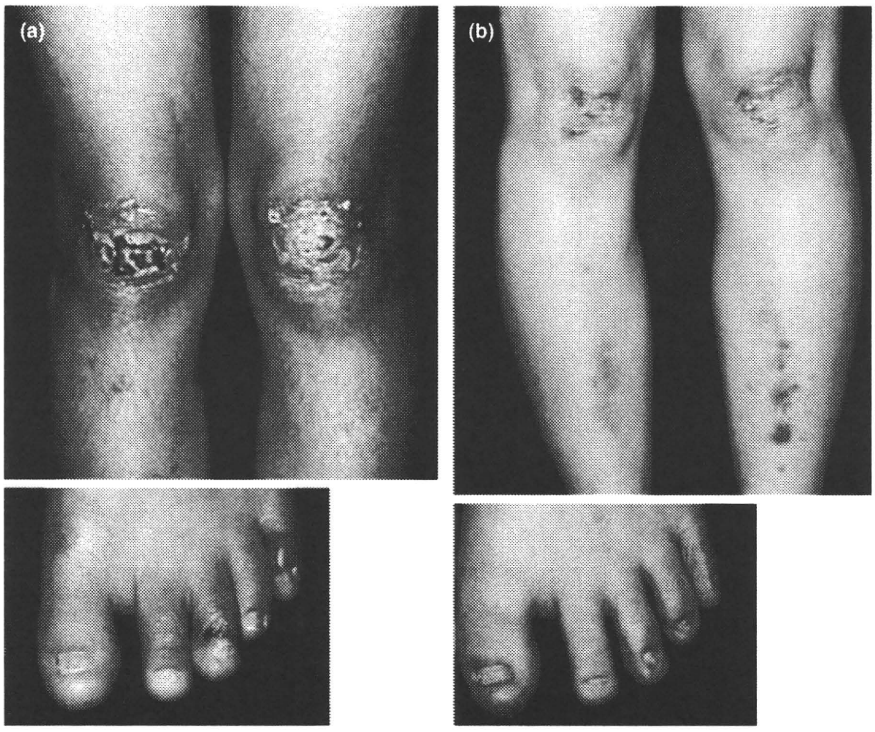

Figure 1. Clinical features of the patient. (a) Erosions healed with mild scarring on the knee, and blisters and nail dystrophy were seen on the toes at 8 years of age. (b) Symptoms were markedly improved by 18 years of age.

1 month. She also had nail dystrophy. She was referred to our hospital when she was 8 years old. There were scattered blistering and erosions healed with mild scarring and milia on the knees and pretibial regions (Fig. 1a). Severe pruritus was initially present. However, blister formation and pruritus markedly decreased by 18 years of age (Fig. 1b). The clinical manifestations of this patient were consistent to very mild DDEB but not to DDEB pruriginosa. Direct nucleotide sequencing of genomic DNA from the patient disclosed a heterozygous G to A transition at nucleotide c.6900 that does not lead to an amino acid change in p.Gln2300 residue (Fig. 2a). Reverse transcription polymerase chain reaction (RT–PCR) across the site of the c.6900G>A transition identified two bands of 475 and 544 bp in size, compared to a single 544 bp band in the normal control (Fig. 2b). Subcloning and direct sequencing disclosed that the 475-bp band was a mutant transcript with in-frame exon 87 skipping (Fig. 2c). In contrast to the previous report by Saito *et al.*,[5] we further detected two additional aberrant transcripts with inclusion of the entire intron 86 or 87 as a new exon, leading to premature termination codon 27 or 120 bp downstream, respectively (Fig. 2c). The 544-bp band was the normal wild-type transcript.

DISCUSSION

As Saito *et al.* described,[5] it seems to be probable that exon 87 skipping is related to the phenotype of DDEB pruriginosa. However, the present case was clinically quite different from DDEB pruriginosa, although she was shown to have exon 87 skipping by RT–PCR analysis. Inter- and intrafamilial variability from the same *COL7A1* mutations has been previously described in the published work. For example, the most common *COL7A1* mutation p.Gly2043Arg has been identified in a different phenotype of DDEB.[6] It is plausible that modifying genes, epigenetic or environmental factors might influence the phenotypic variations in DEB. Therefore, genetic counseling in such cases is fraught with difficulty. It is important that families are made aware of the

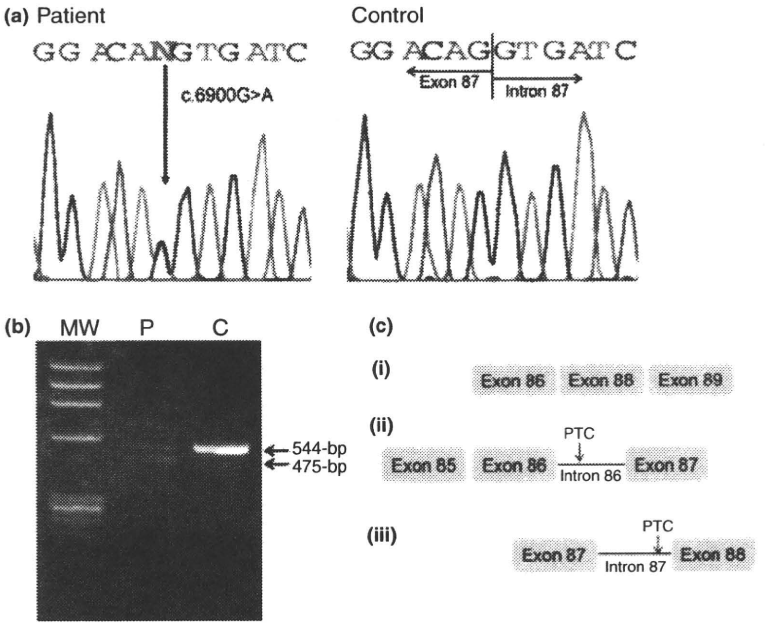

Figure 2. Molecular basis of dominant dystrophic epidermolysis bullosa (DEB) in the present patient (a) Direct nucleotide sequencing of genomic DNA from the patient disclosed a heterozygous G>A transition at nucleotide c.6900. (b) Reverse transcription polymerase chain reaction (RT–PCR) across the site of the c.6900G>A transition. In the amplified cDNA from control (lane C), a single band of 544 bp was present. By contrast, in the amplified cDNA from the patient (lane P), two different bands of 544 and 475 bp were identified. (c) Subcloning and direct sequencing revealed the band of 475 bp is a mutant transcript with in-frame exon 87 skipping. Two aberrant mutant transcripts with inclusion of the entire intron 86 or 87 were also identified, although the bands were not visible on the agarose gel, shown in (b). PTC, premature termination codon.

clinical diversity in DEB and are offered appropriate counseling.

The RT–PCR analysis in this study revealed two aberrant mutant transcripts with inclusion of the entire intron 86 or 87 as a new exon, as well as exon 87 skipping. However, the RT–PCR bands of these two mutant transcripts were not visible on the agarose gel, suggesting low mRNA expression of these mutants in the patient's skin. Taken together, in-frame exon 87 skipping through c.6900G>A mutation may account for the mild skin features, rather than DDEB pruriginosa, in the present case.

ACKNOWLEDGMENTS

We are grateful to Miss Ayumi Suzuki and Miss Takako Ishikawa for technical assistance, and Miss Akiko Tanaka and Mrs Yasuko Nakayama for secretarial work. We thank the patients for their participation. This work was supported by Grants-in-Aid for Scientific Research and Strategic Research Basis Formation Supporting Project from the Ministry of Education, Culture, Sports, Science and Technology of Japan, by Health and Labor Sciences Research Grants and grants for Research on Intractable Diseases from the Ministry of Health, Labor and Welfare of Japan. This work was also supported by grants from the Uehara Memorial Foundation, the Nakatomi Foundation, the Kaibara Medical Foundation, the Japan Lydia O'leary Memorial Foundation, and the Japanese Dermatological Association (Shiseido Award).

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LETTER TO THE EDITOR

Immunoglobulin A anti-BP230 autoantibodies in linear immunoglobulin A/immunoglobulin G bullous dermatosis

Dear Editor,

Linear immunoglobulin (Ig)A bullous dermatosis (LABD) is an autoimmune subepidermal bullous disease characterized by linear IgA deposits at the epidermal basement membrane zone (BMZ).[1] Immunoblot analyses have shown a variety of corresponding antigens with molecular weights of mainly 97, 120, 180 and 290 kDa.[2,3] Most LABD sera react with the epidermal side (lamina lucida type), and a few LABD sera react with the dermal side (sublamina densa type) on NaCl split skin.[4] Most patients of lamina lucida type show IgA autoantibodies against the 97 or 120 kDa LAD-1, which is a degradation product of the extracellular domain of BP180.[3,5] Some cases demonstrate linear deposits of IgG and IgA, called linear IgA/IgG bullous dermatosis (LAGBD).[4] In a review of Japanese 213 LAD cases reported in 1975–2006, deposits of IgG in addition to IgA were detected more frequently in adult cases than in infantile cases (ratios of IgA/IgG type to IgA type 26.1% [>60 years] and 7.9% [<16 years], respectively).[4] We report here a case of LAGBD in which immunoblotting using epidermal extract as substrate demonstrated IgA anti-BP230 antibodies.

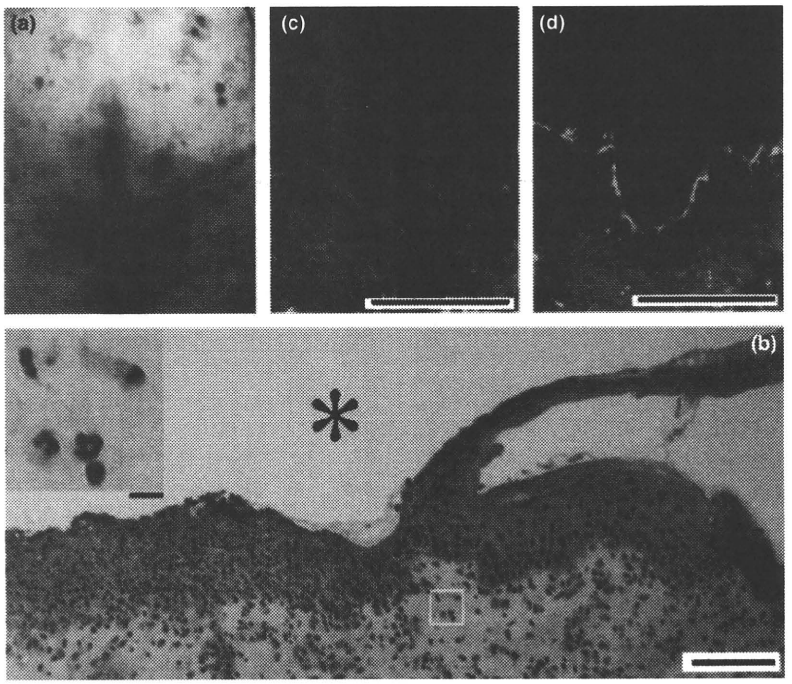

Figure 1. (a) Multiple pustules, vesicles and erosions with erythema on the buttock and thighs. (b) Subepidermal blistering (*) with infiltration of lymphocytes and neutrophils (inset). Linear immunoglobulin (Ig)G (c) and IgA (d) deposits at the basement membrane zone. (Bar, 100 μm; inset, 10 μm.)

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A 74-year-old Japanese man was referred to us with a half-year history of pruritic blistering and erosive lesions on the trunk. He had been treated with topical and systemic steroids. He had a history of malignant B-cell lymphoma with complete remission, myocardial infarction and mitral valve prolapse. He did not take vancomycin. There was neither particular family history nor other medical problems. Physical examination demonstrated multiple pustules, vesicles and erosions with erythema on the trunk and proximal lower extremities (Fig. 1a). Mucous membranes were not involved. A skin biopsy from a vesicle showed subepidermal blistering, infiltration of neutrophils and lymphocytes in the dermis (Fig. 1b). Direct immunofluorescence showed linear IgA, IgG and C3 deposits, but not IgM, along the BMZ (Fig. 1c,d). Indirect immunofluorescence, using NaCl-split normal human skin as substrate revealed that the patient's serum (diluted 1:10 [IgG] and 1:40 [IgA]) contained circulating IgA and IgG anti-BMZ autoantibodies bound to the epidermal side of the split (data not shown). Based on these results, diagnosis of LAGBD was done. Oral prednisolone (15 mg/day) and topical steroid was initiated. Because the lesions had not changed in a week, diamino diphenyl sulfone (DDS) (50 mg/day) was added. A week later, DDS was increased to 75 mg/day. The treatment was effective, and the lesions disappeared in 2 months.

To examine antigens for these IgG and IgA anti-BMZ autoantibodies, we performed various immunoblot analyses.[6] The patient's serum showed no IgG or IgA reactivity against proteins in concentrated HaCaT cell culture media, or recombinant proteins of BP180-NC16a and BP180-C-terminal domains (data not shown), but showed reactivity against normal human epidermal extract (Fig. 2a). Immunoblotting using normal human epidermal extracts showed a clear IgA reactivity with the 230-kDa protein and no clear IgG reactivity. This finding suggested that linear IgA deposit at the BMZ was due to IgA antibodies to BP230. The weak IgG and IgA reactivity with the 190-kDa protein are considered to be a non-specific reaction, because the 190-kDa periplakin is localized in the epidermal cell surface. The IgA reactivity with the 230-kDa protein was confirmed as IgA anti-BP230 autoantibodies, because immunoelectronmicroscopy showed exclusive localization of IgA at the attachment plaque of hemidesmosome (Fig. 2b). For post-embedding immunoelectronmicroscopy,[7,8] normal human skin was incubated with diluted patient's serum (1:60), followed by labeling with 10-nm gold-conjugated goat antihuman IgA.

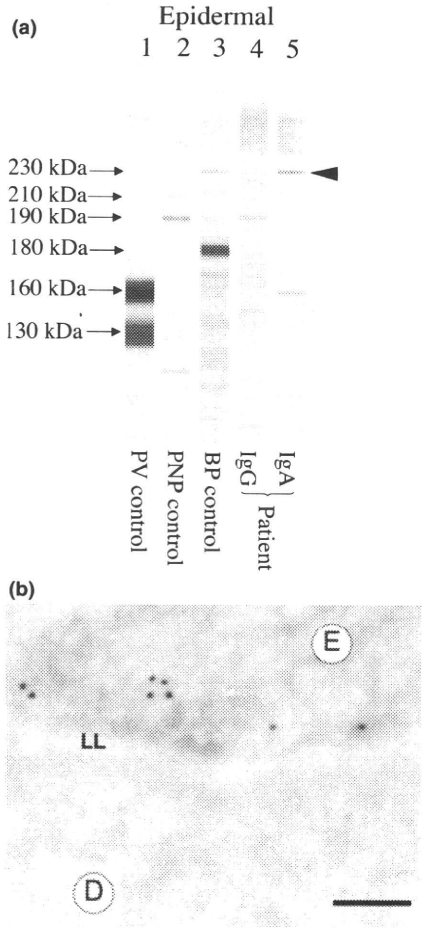

Figure 2. (a) Immunoblotting using epidermal extracts. Control pemphigus vulgaris sera reacted with the 160-kDa desmoglein 1 and 130-kDa desmoglein 3 (lane 1). Control paraneoplastic sera reacted with the 210-kDa envoplakin and 190-kDa periplakin (lane 2). Control bullous pemphigoid sera reacted with BP230 and BP180 (lane 3). Immunoglobulin (Ig)A antibodies of this case clearly reacted with BP230, but not BP180 (lane 5). IgG and IgA antibodies of this case showed a weak reactivity with the 190-Da protein (lanes 4 and 5). (b) Immunoelectronmicroscopy using patient serum and labeled anti-IgA secondary antibody. Gold particles (10 nm) were exclusively concentrated at the attachment plaque of hemidesmosome. BP, bullous pemphigoid; D, dermis; E, epidermis; LL, lamina lucida; PNP, paraneoplastic pemphigus; PV, pemphigus vulgaris. (Bar, 200 nm.)

Immunoglobulin A autoantibodies to LAD-1 may play a role in pathogenesis of LABD. LAD-1 does not

contain the MCW-1 region, the most immunogenic region of BP180 for bullous pemphigoid,[1] indicating different epitopes in BP180 between bullous pemphigoid and LABD. Besides, IgA autoantibodies produce only weak activation of complements by the alternative pathway, indicating that the inflammatory cascade in IgA-mediated diseases is different from the complement-induced chemotaxis in IgG-mediated diseases such as bullous pemphigoid.[1] In LABD, chemotaxis by cytokines from keratinocytes leads to neutrophilic inflammation and release of proteolytic enzymes from neutrophils, causing subepidermal blisters.[1] Based on this speculation, neutrophilic inflammation would be most important for blistering in LABD. In our case, the effectiveness of DDS and lack of IgG reactivity to the known pathogenic autoantigens in western blotting mean that IgA deposition is a key for neutrophilic inflammation even if IgG deposition is also observed. As a reasonable pathogenesis in our case, IgA anti-BP230 antibodies induced neutrophil-dependant inflammatory reaction, and subsequently caused deposits of IgG and C3 immunocomplex at the BMZ.

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ORIGINAL ARTICLE

Heterogeneity of Brunsting–Perry type pemphigoid: A case showing blister formation at the lamina lucida, immune deposition beneath the lamina densa and autoantibodies against the 290-kD polypeptide along the lamina densa

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ABSTRACT

An otherwise healthy 31-year-old man presented with multiple, vesicular, subepidermal blistering on the head, face, chest and oral cavity, leaving shallow scar formation, typical of Brunsting–Perry type pemphigoid. Direct immunofluorescence showed linear deposition of immunoglobulin (Ig)G and C3 along the basement membrane zone (BMZ), and indirect showed anti-BMZ autoantibodies (IgG, >40×) reacting with the dermal side under the salt-split study. Immunofluorescence staining for type IV collagen and laminins, as well as routine electron microscopy, demonstrated that the cleavage level of the blister was intra-lamina lucida. The immunoperoxidase method applied to lesional skin demonstrated IgG deposits along the lamina densa. The post-embedding immunogold method demonstrated that the autoantibodies against BMZ reacted with the lamina densa and the dermis just beneath it. Immunoblot studies demonstrated that the autoantibodies reacted with the 290-kD polypeptide (suggesting type VII collagen) when dermal extract was used as the substrate. The patient was treated with combination therapy consisting of 30 mg prednisolone, 900 mg nicotinamide and 750 mg tetracycline, and the number of newly forming blisters decreased. We concluded that Brunsting–Perry type pemphigoid, a rare autoimmune blistering disease, includes cases showing characteristics of epidermolysis bullosa acquisita as well as bullous pemphigoid. This case showed discrepancy between the blistering level (intra-lamina lucida) and location of antigen (lamina densa and sub-lamina densa areas).

Key words: Brunsting–Perry type pemphigoid, epidermolysis bullosa acquisita, type VII collagen.

INTRODUCTION

Brunsting–Perry type pemphigoid,[1] a rare variant of subepidermal blistering autoimmune bullous dermatosis, is characterized by pruritic, recurrent, circumscribed, vesicular blisters located mainly on the head, face, neck and upper trunk leaving atrophic scarring. Recent investigations have demonstrated that the causative antigen is the BP180 molecule,[2–4] which is the pathological antigen in cases of bullous pemphigoid (BP) and gestational pemphigoid, and seems to be related to some cases of mucous membrane pemphigoid and some linear IgA disease (LAD). The finding that the causative antigen is BP180 concurs with the previous finding obtained from immunoelectronmicroscopy that immune deposits in

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this type of pemphigoid are distributed in the lamina lucida, especially beneath the hemidesmosomes.[5,6] However, a few cases have been also reported to show clinical features very similar to those of Brunsting–Perry type pemphigoid, whereas the cleavage and immune deposition were at the sub-lamina densa area, suggesting that those should be diagnosed as epidermolysis bullosa acquisita (EBA).[7,8] Furthermore, in a case clinically resembling Brunsting–Perry type pemphigoid, sophisticated immunoblotting demonstrated that the causative antigen was type VII collagen, the antigen of EBA.[9] Here, we report an additional peculiar case showing Brunsting–Perry type pemphigoid with blister formation at the lamina lucida level, immune deposition along the lamina densa and probably type VII collagen as the related antigen.

CASE REPORT

A 31-year-old Japanese man without any contributing personal or family history showed recurrent small blister formation on the chest that persisted for several weeks. At the first consultation on May 2009, the patient presented crops of vesicular blisters with mild erythematous color on the cheeks, neck and mid-chest (Fig. 1a,b). The skin lesions showed mild itching and residual shallow scar formation. During the

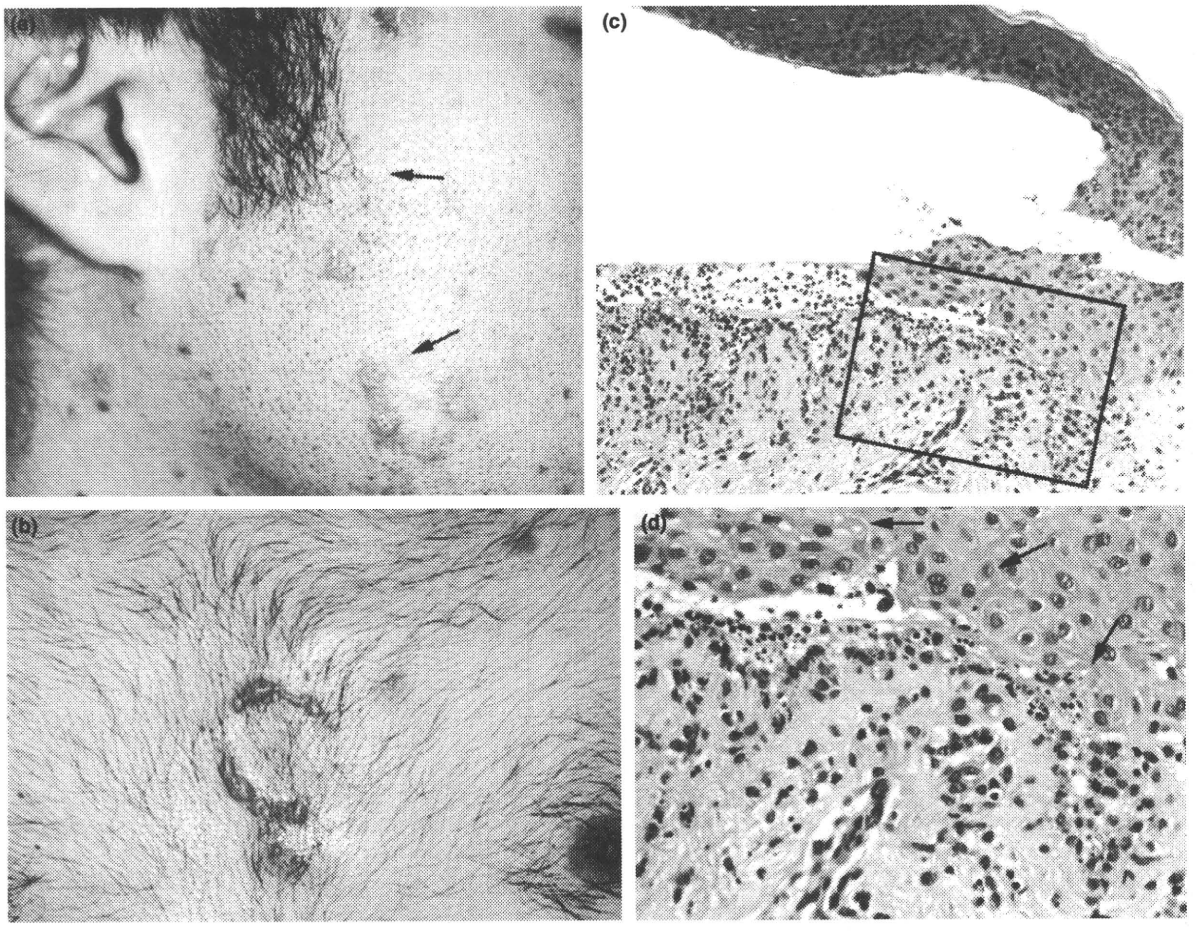

Figure 1. (a) Vesicular to herpetiform blisters on the right cheek. Shallow scars were also noted (arrows). (b) Annularly arranged vesicular blisters over the sternum. (c) On histological examination, a biopsied eruption showed subepidermal blister with inflammatory infiltrates in the blister cavity and dermis. (d) Enlargement of the rectangular area of (c) shows neutrophils and some eosinophils as well as mononuclear cells attached to the blister floor, and eosinophilic degeneration of the lowermost part of the blister roof (arrows) (hematoxylin–eosin, original magnifications: [c] ×200; [d] ×500).

clinical course, small blisters also occurred on the oral cavity. There were no other symptoms and the results of laboratory examinations did not suggest collagen diseases. Skin biopsy examination, performed on a small blister taken from the chest, showed subepidermal blister formation that contained numerous neutrophils, eosinophils and lymphoid cells. The infiltrating inflammatory cells attacked the floor of the blister so densely that the floor could not be clearly observed. In the lower most portion of the blister roof, degenerating basal cells were scattered (Fig. 1c,d). Direct immunofluorescence studies for detecting *in vivo*-bound IgG, IgA, IgM, C1q and C3 disclosed linear deposition only of IgG and C3 along the basement membrane zone (BMZ) of the peri-bullous area and on the floor of the blister (Fig. 2a). Indirect immunofluorescence on 1-mol/L salt-split skin demonstrated IgG class autoantibodies reacting with the dermal side of the split (>40×, Fig. 2b). Immunofluorescence mapping studies using anti-type IV collagen and anti-laminin rabbit antibodies to demonstrate the level of epidermal separation disclosed that these components of the lamina densa were located along the floor of the blister (Fig. 3a,b). Further investigation using electron microscopy from the cryosection detected highly folding lamina densa on the floor of the blister (Fig. 3c,d), strongly suggesting that the blister formation occurred intra-lamina lucida, namely between the basal cells and the lamina densa. Immunoelectron microscopy performed on the biopsied cryosection using antihuman IgG1 mouse monoclonal antibodies (1:50 dilution; Sigma-Aldrich, St Louis, MO, USA) and EnVision System-HRP kit (Dako North America, Carpentaria, CA, USA) demonstrated that the IgG deposits were distributed along the lamina densa, especially beneath it (Fig. 4a). A post-embedding immunogold technique was performed as follows: sections of normal human skin embedded in Lowicryl K11M were incubated with patient's serum (1:100), followed by incubation with rabbit polyclonal antihuman IgG antibodies (1:1500; Dako, Glostrup, Denmark), and labeled with 10-nm gold-conjugated goat antirabbit IgG (1:10; Amersham International, Buckinghamshire, UK). It demonstrated that the IgG autoantibodies of the patient's serum reacted with the lamina densa and the dermis just beneath it (Fig. 4b). Immunoblot analyses were performed using extracts of normal human epidermis and dermis, recombinant proteins of NC16a domain and C-terminus of BP180, purified laminin 332, and supernatant of cultured HaCaT cells. IgG antibodies reacted with a band of 290-kD molecular weight in extracts of normal human dermis, which was relatively weak but detected in these

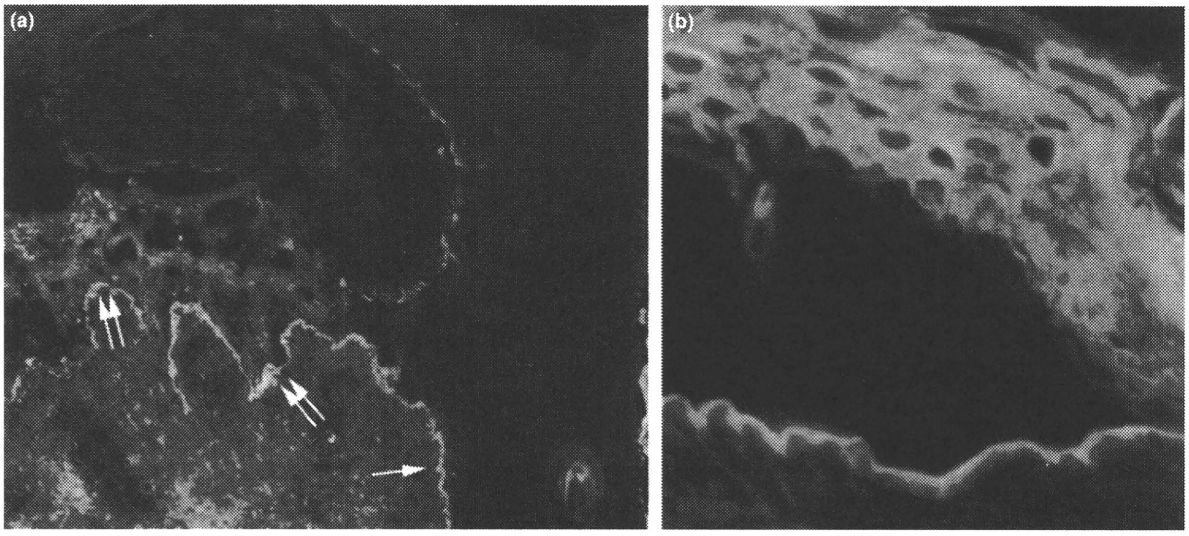

Figure 2. (a) Direct immunofluorescence showed linear deposition of immunoglobulin (Ig)G (and C3) along the basement membrane zone (BMZ) of the epidermis (single arrow), and along the floor of the blister (double arrows) (original magnification ×200). (b) Indirect immunofluorescence on 1-mol/L salt-split skin demonstrated IgG class autoantibodies reacting with the dermal side of the split.

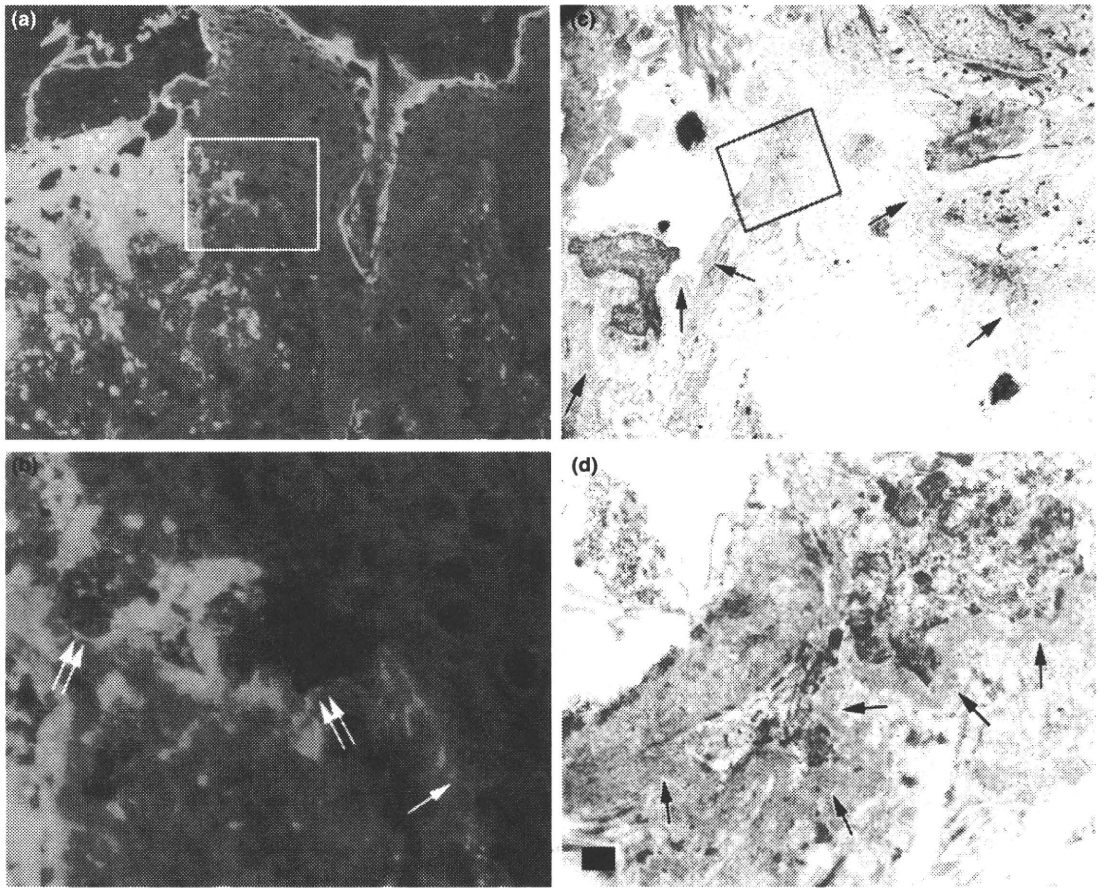

Figure 3. (a) Immunofluorescence staining of type IV collagen showed reactivity along the floor of the blister. (b) Enlargement of the rectangular area of (a). Type IV collagen was trailed along the blister floor (double arrows) from the basement membrane zone of the epidermis (single arrow). (c) By electron microscopy, tissue from almost the same area as (b) demonstrated highly folding lamina densa (arrows) on the floor of the blister. (d) Enlargement of the rectangular area of (c) (bar = 100 nm).

separate investigation (Fig. 5). Other analyses were negative. Enzyme-linked immunoassay for BP180, BP230 and desmogleins-1 and -3 were all negative.

Based on the clinical, histopathological and immunofluorescence findings, diagnosis of Brunsting–Perry type pemphigoid was made, and treatment with combination therapy consisting of 30 mg prednisolone, 900 mg nicotinamide and 750 mg tetracycline was started. The number of newly forming blisters decreased, and thereafter the dosage of prednisolone was slowly tapered.

DISCUSSION

Although the residual scar formation was not severe, the clinical features of herpetiform blisters and the histopathology of subepidermal separation allowed us to make the diagnosis of typical Brunsting–Perry type pemphigoid.[1] The most interesting characteristic of this case was inconsistency between the cleavage level of the blister and the location of immune deposits and antigen. Mapping studies of BMZ components as well as electron microscopy demonstrated that cleavage occurred not in the sub-lamina densa area but in the lamina lucida, confirming the histopathological finding that the basal surface of the blister roof was degenerated. However, both immunoperoxidase electron microscopy to detect *in vivo*-bound IgG and immunogold electron microscopy to demonstrate the location of the antigen disclosed immune deposits and causative antigens along the lamina densa, especially beneath it, like EBA, or

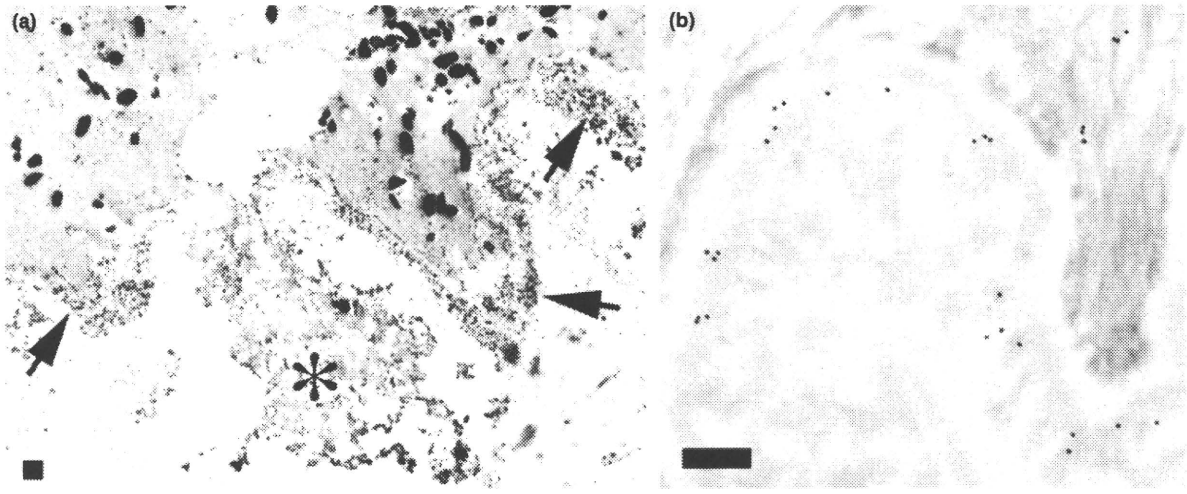

Figure 4. (a) Immunoelectron microscopy using antihuman immunoglobulin (Ig)G1 mouse monoclonal antibody as the first antibody and EnVision System-HRP kit demonstrated granular reaction of 3,3′-diaminobenzidine (DAB) suggesting deposits of IgG along the lamina densa (arrows), part of which was disrupted by infiltrating cells (asterisk) (bar = 100 nm). (b) Post-embedding immunogold technique using an ultrathin section of Lowicryl K11M-embedded normal human skin as substrate, and 10-nm gold particle conjugated antihuman IgG as the second antibody demonstrated that autoantibodies of the patient's serum reacted to the lamina densa and the dermis just beneath it (bar = 100 nm).

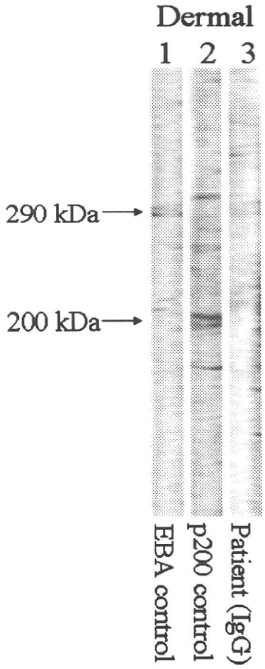

Figure 5. Result of immunoblot analysis using extracts of normal human dermis. A relatively weak band was observed at the 290-kD molecular weight (lane 3), the same position as that seen in epidermolysis bullosa acquisita (EBA) control serum (lane 1). A 200-kD protein was seen in anti-laminin γ-1 (p200) pemphigoid control serum (lane 2).

some cases of LAD. Such discrepancy was not observed in previous cases of Brunsting–Perry type pemphigoid, but has been demonstrated in cases of LAD[10,11] and EBA.[12] Although the detailed mechanisms underlying this discrepancy remain obscure, the high accumulation of inflammatory infiltrates containing neutrophils and eosinophils on the floor of the blister may be relevant.

Fine *et al.*[12] demonstrated that more than a half of the cases of EBA showed a cleavage level in the lamina lucida, rather than in the sub-lamina densa area, and they explained this finding by the intra-lamina lucida-separating effect of proteolytic enzymes of leukocytes. Honoki *et al.*[13] also reported a similar case of EBA showing intra-lamina lucida separation. However, if the proteolytic enzymes from neutrophils destroy the dermal–epidermal structures, fragmented lamina densa should be scattered on the floor as well as beneath the roof of the blister as seen in dermatitis herpetiformis.[14] On electron microscopy of this case, the lamina densa was detected only on the floor of the blister, although it was disrupted by the inflammatory infiltrates. The inflammatory cells may have attacked immune deposits along the lamina densa, resulting in molecular detachment between the lamina densa and hemidesmosomes.

Kurzhals *et al.*[7] and Joly *et al.*[8] reported cases clinically resembling Brunsting–Perry type pemphigoid, in which direct immunoelectron microscopic examination of peri-bullous skin showed a dermal cleavage level below the lamina densa, and granular deposits of IgG and C3 attached to and below the lamina densa in a pattern identical to EBA. The former concluded that EBA is much more clinically heterogeneous than previously suggested, and the latter concluded that Brunsting–Perry type pemphigoid might represent a clinical variant of EBA. These cases should be diagnosed as EBA, because of blister formation due to sub-lamina densa cleavage. Reporting such a case of Brunsting–Perry type pemphigoid, Tanaka *et al.*[9] demonstrated that the causative antigen was type VII collagen using sophisticated immunoblotting examinations. They also concluded that Brunsting–Perry type pemphigoid might be a clinical variant of EBA.

Strictly following the finding on immunoblotting that the molecular weight of the antigen was 290 kD and that on immunogold staining that the location of the antigen was along the lamina densa and sub-lamina densa area, this case should be diagnosed as EBA. However, it is also possible to think that Brunsting–Perry type pemphigoid is heterogeneous. Considering the findings of previous reports of Bursting–Perry type pemphigoid in which the cleavage level and location of immune deposits were discussed, it should be concluded that on one side of this disease, there are cases in which pathogenic mechanisms involve the lamina lucida,[2–6] like BP, and some cases of mucous membrane pemphigoid, and on the other side are cases in which these mechanisms occur beneath or along the lamina densa,[7–9] like EBA. Our case represents an intermediate form of Brunsting–Perry type pemphigoid between BP and EBA.

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Invited review article

Hemidesmosomes and focal contact proteins: Functions and cross-talk in keratinocytes, bullous diseases and wound healing

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ABSTRACT

The outer most layer of the skin, the epidermis, is attached to the dermis via a sheet of extracellular matrix proteins termed the basement membrane zone (BMZ). In the intact skin, adhesion of the keratinocytes in the basal layer of the epidermis to the BMZ is facilitated primarily by hemidesmosomes which associate with the keratin cytoskeleton. Cultured keratinocytes do not assemble bona fide hemidesmosomes although hemidesmosome protein clusters (stable anchoring contacts) are found along the substrate-attached surface of the cells and towards the leading edge of keratinocytes repopulating scratch wounds. Actin cytoskeleton-associated matrix adhesion devices termed focal contacts are not thought to play an important role in the adhesion of keratinocytes to the BMZ in intact skin but are prominent in cultured keratinocytes where they are believed to regulate cell migration. We review the molecular components, functions, dynamics and cross-talk of hemidesmosomes and focal contacts in keratinocytes. In addition, we briefly describe what is known about their role in autoimmune and genetic blistering diseases of the skin. We also discuss recent publications which indicate, contrary to expectation, that certain focal contact proteins retard keratinocyte migration while hemidesmosomal proteins regulate directed keratinocyte motility during wound healing.

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1. Introduction

Keratinocytes in the basal layer of the epidermis adhere to the connective tissue via interaction with extracellular matrix proteins organized into a sheet-like structure termed the basement membrane zone (BMZ). Keratinocyte-extracellular

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matrix interactions along the BMZ regulate a variety of cell behaviors, including proliferation, adhesion, differentiation, migration and apoptosis [1].

In intact skin, the adherence of keratinocytes to the BMZ is mediated by cell-matrix junctions termed hemidesmosomes, which also tether the keratin cytoskeleton to the cell surface [2]. Adhesion mediated by hemidesmosomes is considered stable and robust [3–5]. Ultrastructural examination of intact skin reveals hemidesmosomes dispersed all along the BMZ. Each has a tripartite electron dense cytoplasmic plaque to which tonofibrils adhere [5]. In contrast, keratinocytes in culture fail to assemble bona fide hemidesmosomes. Rather, hemidesmosome-enriched protein complexes, which one group has termed stable anchoring complexes (SACs), are found along the substrate-attached surface of keratinocytes maintained in culture [4]. SACs lack the ultrastructural features of hemidesmosomes found in tissues and possess immature plaques, at best. Moreover, SACs are not static, but exhibit dynamic properties during migration and chemical/antibody treatment as we detail below [2].

When grown in culture on glass or plastic, keratinocytes assemble matrix adhesion devices termed focal contacts [3]. Focal contacts differ from the hemidesmosomes of intact skin by being associated with the actin cytoskeleton. In vitro, focal contacts appear dynamic and move rapidly in the plane of the membrane [6]. Moreover, focal contacts are considered to function as hubs that direct numerous inside-out and outside-in signals [7]. Whether keratinocytes assemble focal contacts in skin tissue and whether focal contact proteins are involved in the adherence of keratinocytes to the BMZ is debatable. Indeed, certain focal contacts proteins, such as α3β1 and α2β1 integrin, are not associated with BMZ in intact skin but are found concentrated at sites of cell–cell contact [3].

In this review, we briefly describe the molecular components, function, dynamics and cross-talk of hemidesmosomes and focal contacts. We also detail the pathological significance of the proteins of both hemidesmosomes and focal contacts in relation to genetic and autoimmune blistering disease of the skin. We focus a large section of this review on a discussion of some recent novel, albeit controversial, data that hemidesmosomal components regulate the directed migration of keratinocytes while focal contact proteins in skin cells may actively retard keratinocyte motility.

2. The molecular composition of hemidesmosomes and focal contacts in keratinocytes

The core of each hemidesmosome consists of four single-span-transmembrane proteins, namely the 180 kDa-bullous pemphigoid antigen (BP180, type XVII collagen, BPAG2), the two subunits of the α6β4 integrin and a tetraspanin protein termed CD151 [8]. Both BP180 and α6β4 integrin interact with laminin-332 in the BMZ [9]. The α6β4 integrin associates with the keratin cytoskeleton, rather than actin, making this heterodimer unique among the integrin family [5]. This interaction is facilitated by the long 1000 amino acid cytoplasmic tail of the β4 integrin subunit which not only binds BP180 but also the 230 kDa bullous pemphigoid antigen (BP230 or BPAG1e) and plectin. The latter two proteins are plakin family members that mediate the indirect linkage of keratin to α6β4 integrin [5] (Fig. 1).

Focal contacts are complex molecular adhesion sites and each contains many more proteins than have so far been identified in the hemidesmosome. The focal contacts of cultured keratinocytes are no exception and contain numerous actin-binding proteins, including paxillin, vinculin, talin and actinin isoforms, via which they associate with actin [10]. A number of distinct integrin heterodimers including α2β1, α3β1 and α5β1 have been identified within each focal contact and these exhibit interactions

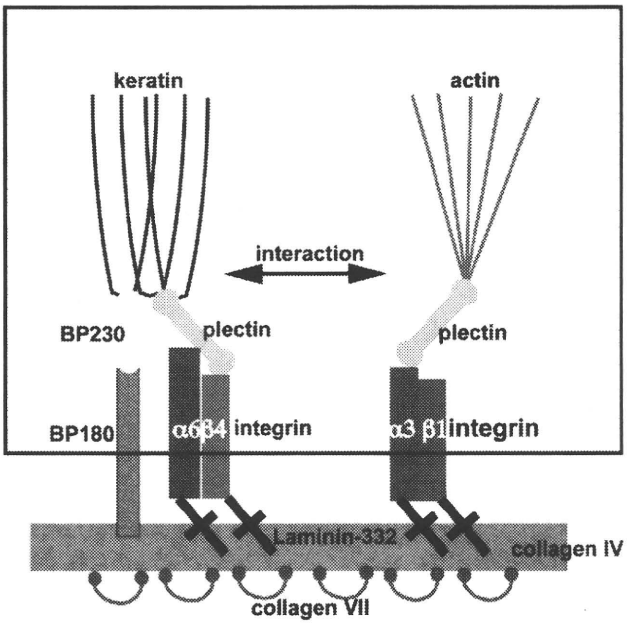

Fig. 1. Molecular composition of hemidesmosomes and focal contacts in keratinocytes.

with various matrix ligands including fibronectin, collagen and laminins [11]. Molecules involved in signaling, such as focal adhesion kinase and integrin-linked kinase, are also found in focal contacts [12].

3. The functions of hemidesmosomes and focal contacts in keratinocytes

Evidence for the adhesive function of hemidesmosomes comes from studies of several skin diseases where loss of hemidesmosomes results in dysadhesion of the epidermis and the development of blisters between the keratinocyte layers of the skin and the dermis [13]. In cultured skin cells, loss of hemidesmosome proteins does not dramatically impact adhesion of the cells to tissue culture surfaces. Rather, such loss inhibits several signaling pathways that are mediated by α6β4 integrin [14]. These data have led to the conclusion that the integrin component of the hemidesmosome is involved in signaling [14]. However, in all likelihood, α6β4 integrin primarily signals when outside the confines of the hemidesmosome.

There is considerable evidence that focal contacts in cultured cells, including keratinocytes, are involved in inside-out and outside-in signaling. For example, in keratinocytes, the mitogen-activated protein (MAP) kinase pathway is activated following ligation of α3β1 integrin by laminin-332 [15]. In addition, it has been reported that laminin-332-α3β1 integrin interaction induces activation of cdc42 and its effector the serine threonine kinase PAK1, thereby enhancing cell motility [16]. Laminin-332-α3β1 integrin mediated signaling may also activate the FAK/Src/Rac1 pathway and the formation of lamellipodia [17]. In addition to these outside-in signaling pathways, α3β1 integrin, through the activity of a protein termed T-lymphoma invasion and metastasis 1 (Tiam1), regulates assembly of laminin-332 matrix in an inside-out signaling mechanism [18,19].

4. The dynamics of cell-extracellular matrix attachment devices in keratinocytes

Focal contacts in both stationary and actively migrating cells are known to be highly dynamic. In brief, in cultured cells focal

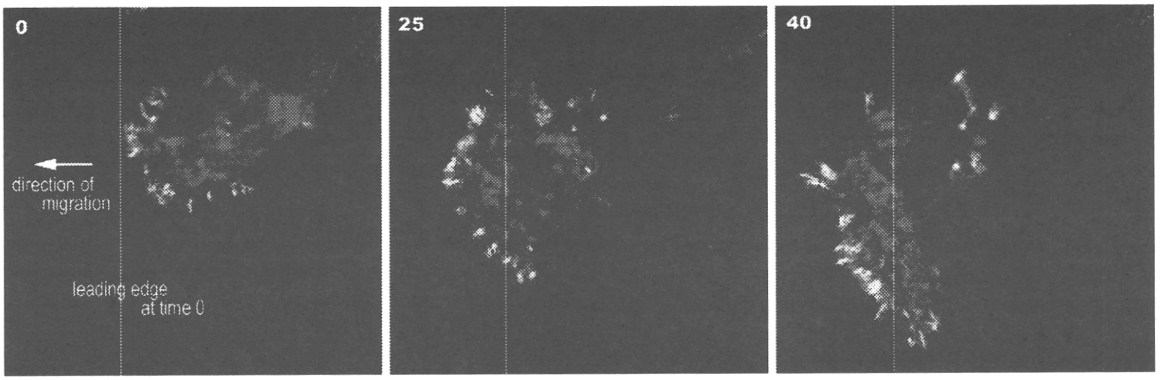

Fig. 2. Still images for the dynamics of hemidesmosomal proteins and focal contact proteins in live migrating keratinocytes.

contacts begin life as focal complexes at the cell surface, enlarge, and move primarily towards the cell center, although some also exhibit perimembranous movement [6]. In migrating cells, focal contacts at the trailing edge of the cell disassemble in a microtubule-dependent manner [20].

Hemidesmosomes in tissues are considered stable adhesion devices. However, in cultured keratinocytes, hemidesmosome protein-rich complexes or SACs show considerable dynamics in the plane of the membrane [2,21]. These dynamics have been reported to be dependent on the actin cytoskeleton and laminin-332-mediated clustering of α6β4 integrin heterodimers [2,21]. Moreover, fluorescent recovery after photobleaching (FRAP) studies suggest that β4 integrin protein can exchange rapidly between cell surface clusters and a cytoplasmic or membrane pool of protein [2].

In keratinocytes in vitro, the dynamics of focal contacts and hemidesmosome-rich protein complexes are tightly co-regulated during the closure of scratch wounds created in confluent monolayer cultures (Fig. 2) [22]. Both focal contact and hemidesmosome proteins are dispersed along the leading edge of cells populating a wound in vitro. Intriguingly, hemidesmosome protein complexes appear between or just behind actinin proteins in the advancing lamellipodia of motile skin cells [22]. In addition, inhibition of hemidesmosome proteins or their knockdown results in enhanced focal contact dynamics while blocking the α3 integrin component of the focal contacts of keratinocytes stabilizes hemidesmosome protein complexes in the plane of the membrane. Furthermore, both the dynamics of focal contacts and hemidesmosome proteins complexes and their interplay is energy and myosin dependent [22].

5. Skin diseases involving hemidesmosomes and focal contacts of cell-extracellular matrix adhesion (the diseases described here are summarized in Table 1 and shown in Fig. 3)

5.1. Inherited disorders affecting hemidesmosome or focal contact protein components

The involvement of hemidesmosome and focal adhesion proteins in human disease has been the subject of a number of excellent recent reviews. Thus, we will provide only a general overview of the literature.

Loss of function mutations affecting matrix adhesion components have been identified as pathogenic in a family of skin fragility and blistering disorders termed, collectively, epidermolysis bullosa (EB) [13,23]. Classification of the EB family of disorders has recently been rationalized and we will use this new classification [23]. Specifically, the EB disorders are broken down into four main classifications based upon ultrastructural examination of the blistering site: intraepidermal (EB simplex, EBS, Fig. 3a), junctional (JEB), dermolytic (dystrophic EB, DEB) and mixed (Kindler syndrome, Fig. 3b) [23]. Patients are further separated into minor EB subtypes based on clinical presentation and mode of inheritance.

In general, the phenotypic classification of patients provides an indication of the mutated gene and the type of mutation. Thus, basal EBS is associated with mutations in the basal keratinocyte keratins (K5 and K14), with mutations in those proteins (plectin or BP230) which mediate keratin association with hemidesmosomes or with specific mutations in β4 integrin at sites where it interacts with BP180 [13,24,25].

JEB patients present with mutations in any of the genes encoding the three subunits of laminin-332, BP180, or α6β4 integrin [13]. In the case of JEB, the most severe phenotype (termed JEB-Herlitz) result from loss of expression mutations in laminin α3, β3 or γ2, while the other forms (JEB-Other) occur either as a result of missense mutations in the laminin subunits (JEB non-Herlitz), missense/loss of expression mutations in BP180 (JEB non-Herlitz), or missense/loss of expression of α6β4 integrin (JEB with pyloric atresia) [13,23]. In addition, a rare form of JEB, pretibial EB with hereditary nephritis, is known to be caused by CD151 gene mutations [26].

Identifying specific phenotype/genotype correlations can be of great value to expanding our understanding of how matrix adhesion proteins interact and how they function. A good example

Table 1
Diseases whose pathogenesis involves hemidesmosomal or focal contact components.

	Inherit bullous diseases	Autoimmune bullous diseases
Hemidesmosome-related	Epidermolysis bullosa (EB) hereditaria	Bullous pemphigoid
	EB simplex (EBS)	Acquired EB
	Weber-Cockayne	Anti-laminin γ1 pemphigoid
	Koebner	Linear IgA bullous dermatosis
	Dowling-Meara	mucous membrane pemphigoid (MMP)
	Pretibial	
	EBS with muscular dystrophy	
	Junctional EB (JEB)	
	Herlitz type	
	JEB with pyloric atresia	
	Non-Herlitz type	
	Dystrophic EB (DEB)	
	Dominant DEB	
	Recessive DEB	
Focal contact-related	Kindler syndrome	None

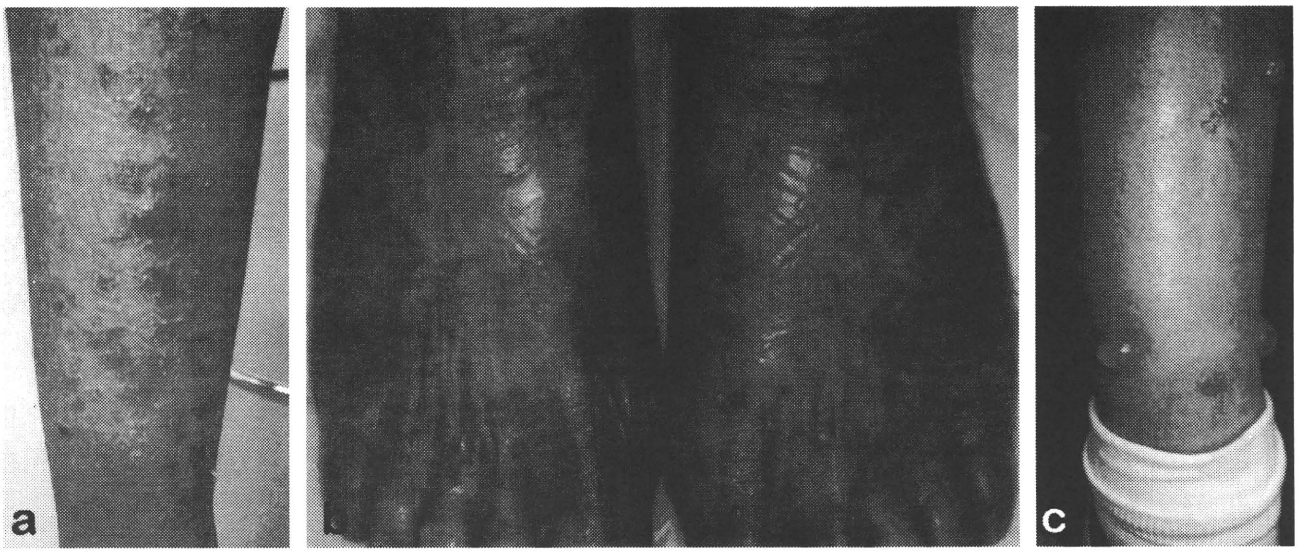

Fig. 3. Clinical photos for epidermolysis bullosa simplex (a), Kindler syndrome (b), and bullous pemphigoid (c).

of this is a rare disorder termed laryngo-onycho-cutaneous syndrome (LOC) which presents with non-healing cutaneous erosions and chronic overproduction of granulation tissue without apparent skin fragility [27]. Mutational analyses have revealed that LOC syndrome results from mutations in the first exon of the laminin α3a transcript implicating this region in the regulation of the granulation tissue response [27].

Mutations in every known component of the hemidesmosome have now been identified in human genetic disorders. In contrast, the only focal contact protein in which mutations resulting in a genodermatosis have been identified is kindlin-1, named after Kindler syndrome in which mutations in the gene were identified [28]. Similar to those afflicted with EBS, Kindler syndrome patients present with neonatal blistering [28].

Kindlin-1 protein possesses a central region containing an unusual arrangement of a pleckstrin homology (PH) domain flanked by two regions of homology to the four-point-one, ezrin, radixin, moesin (FERM) domain [29]. Both PH and FERM domains have been identified as being involved in membrane binding [30]. Kindlin-1 colocalizes with vinculin at focal contacts and interacts with the actin cytoskeleton via migfilin and filamin A [31]. Kindlin-1 therefore appears to play a critical role in the maintenance of the actin-extracellular matrix attachment [29]. The lack of identification of pathogenic mutations in other focal contact genes is likely a reflection of the developmental requirement for core focal contact components.

5.2. Autoimmune disorders affect keratinocyte-extracellular matrix adhesion

To date autoimmune disorders targeting focal contact proteins have not been identified. In contrast, a number of autoimmune disorders where circulating autoantibodies target hemidesmosome components have been described. The prototypical example is bullous pemphigoid (BP), which is characterized by subepidermal blisters with inflammatory infiltrate (Fig. 3c) [32]. Typically, there is a linear deposit of IgG at the BMZ and presence of circulating antibodies to BP230 and BP180. The role of anti-BPIgG antibody in bullae formation in BP has been considered to be cell-mediated cytolysis induced by the activation of complements, the migration of neutrophils and the effects of neutrophil elastases [32,33]. However, this model has been questioned since Iwata et al. have presented evidence that pathogenic autoantibodies against BP180 decrease the strength of adhesion of keratinocyte to matrix in the absence of complement and neutrophils [34]. Indeed, other mechanisms of pathogenesis of BP autoantibodies have been proposed. Several authors have suggested that BP autoantibodies may induce cellular signaling events that result in keratinocyte dysadhesion [35,36]. In addition, Kitajima et al. has provided some evidence that internalization of BP180 in basal keratinocytes occurs in affected skin samples of BP patients [37].

Autoantibodies in a variant of BP, termed mucous membrane pemphigoid (MMP), also target hemidesmosome proteins. Approximately 95% of MMP patients produce antibodies directed against BP180, while 5% of patients produce autoantibodies against laminin-332. MMP patients present with blistering, erosive and scarring lesions of mucous membranes [38].

6. Focal contact and hemidesmosome proteins in wound healing of the skin

Wound healing is a complex but regulated series of events that results in restoration of skin integrity. Re-epithelization is a part of the overall wound healing mechanism and is initiated by the dissociation of keratinocytes from the BMZ at the undamaged wound margins [39]. Dissociation involves hemidesmosome disassembly and matrix remodeling. By evading the constraints of the BMZ, keratinocytes move directionally over the provisional matrix in the wound bed and reestablish an intact epithelial sheet [40]. Upon wound coverage, the BMZ is reassembled and hemidesmosomes are reformed [41].

As part of the process that leads to hemidesmosome disassembly, the γ2 subunit of laminin-332 is enzymatically cleaved by members of the metalloprotease (MMP) family, including membrane-type 1 (MT1)-MMP, MMP-2, MMP-3, MMP-12, MMP-13, MMP-19, and MMP-20 [9]. In addition, the astacin family member, bone morphogenic protein (BMP)-1, and its related enzyme, mammalian tolloid (mTLD), also have been reported to cleave both the γ2 subunit of laminin-332, but the precise functional consequences of this cleavage are unknown [42]. In contrast, MT1-MMP and MMP-2 cleavage of the laminin γ2 subunit results in the production of 100-, 85-, 27-, and 25-kDa protein fragments [9]. Of these, the 27-kDa fragment is found in circulating blood in cancer patients and has been reported to

stimulate the epidermal growth factor receptor and, hence, cell migration [43]. In addition, epidermal growth factor mediated phosphorylation of β4 integrin cytoplasmic tale induces hemidesmosome disassembly [44]. Serine phosphorylation disrupts β4 integrin-plectin association and likely destabilizes the hemidesmosome [44]. Phosphorylation of other hemidesmosomal components may also play a role in inducing hemidesmosome disassembly. Finally, endocytotic uptake of hemidesmosome has also been observed and likely contributes to the dissociation of stable adhesion of keratinocytes to the BMZ at the wound margin [45].

Focal adhesion proteins are involved in directed migration, the process via which keratinocytes move over the wound bed, by mediating movement of cells over the extracellular matrix components, including fibronectin, fibrinogen and collagen in the wound bed [46]. Thus, it is not surprising that receptors of the latter molecules, such as α5β1, α2β1 and α3β1 integrin and several syndecans, are involved in regulating keratinocyte motility [47].

Laminin-332 is also present towards the leading front of the migrating sheet of keratinocytes that populate a wound bed [48]. Laminin-332, containing a full length α3 subunit is initially deposited along the edge of the migrating keratinocytes and is believed to support directed migration [49]. Subsequently, laminin-332 undergoes enzymatic processing such that the α3 laminin subunit is cleaved within its G domain. These include the serine protease, plasmin, BMP-1 and mTLD [42,49]. Following cleavage, laminin-332 supports the assembly of hemidesmosomes and thereby stabilizes the interaction of keratinocytes with the wound bed [49].

Until recently, integrin α3β1 association with laminin-332 at the site of focal contacts was considered to enhance keratinocyte migration whereas integrin α6β4 integrin association with laminin-332 was thought to retard keratinocyte motility by stabilizing attachment to the extracellular matrix [50]. However, both these viewpoints have been questioned recently. First, the Sonnenberg lab has presented that α3β1 integrin, rather than supporting migration, inhibits the motility of keratinocytes [51]. These workers generated mice exhibiting an epidermal targeted α3 integrin knockout. Wound closure in the knockout mice migrate faster than their wild type counterparts and exhibit enhanced directional migration. To confirm that this unexpected phenotype is because of the absence of α3 integrin, the α3 integrin subunit was re-expressed in the knockout skin cells. The rescued cells show slower migration in an in vitro scratch wound. How can this study be reconciled with the literature implicating α3 integrin in supporting migration on laminin-332 matrices? Primarily, data in support of the later conclusion comes from the use of an α3 integrin monoclonal antibody, P1B5. This antibody inhibits cell adhesion to laminin-332 and migration on laminin-332 extracellular matrix. We suspect that P1B5 may not only block α3β1 integrin adhesion to ligand but also, by clustering α3β1 integrin, may trigger signaling that inhibits the activity of a motility receptor. The notion that α3β1 integrin crosstalks and inhibits the activation state of other integrin receptors has been suggested by others [52,53].

There is also emerging data that α6β4 integrin and its associated proteins play essential roles in the mechanisms that positively regulate keratinocyte migratory behavior [14,54–56]. Specifically, in motile epithelial cells populating a wound in vitro, both α6β4 integrin and its matrix ligand laminin-332 are found towards the leading edge of their extending lamellipodium [14,48]. In addition, in migrating cells α6β4 integrin shows actin-dependent dynamics at the site of the nascent lamellipodium as determined by FRAP [14]. Moreover, we and others have demonstrated that α6β4 integrin through activation of Rac, regulates keratinocyte front-rear polarity and directed migration [54,57]. Taken together, these data suggest that α6β4 integrin is an important part of the steering machinery of keratinocytes.

α6β4 integrin is not the only hemidesmosomal component involved in regulating keratinocyte motile behavior. Specifically, BP230, which mediates the interaction of α6β4 integrin in hemidesmosomes with the keratin cytoskeleton in stationary cells, regulates Rac/α6β4 integrin association in motile keratinocytes and is found along the advancing lamellipodia of migrating cells [14]. Furthermore, BP230 deficiency results in a loss of front-rear polarity and inhibits directed migration of skin cells in vitro and inhibits wound closure in vivo [14,58].

How does Rac regulate skin cell motility? As in other cell systems, in skin keratinocytes Rac1 signals to the actin severing protein cofilin [54,59–61]. By severing actin filaments, cofilin increases the number of free actin barbed ends while simultaneously increasing the depolymerization rate of older actin filaments, thereby increasing the local pool of free actin monomers [54,60]. Polymerization of actin at the free ends of the freshly severed actin filaments extends the membrane of the lamellipodium [54,60]. This would suggest that loss of Rac activation induced by β4 integrin or BP230 deficiency would perturb lamellipodial formation and/or stability. Indeed, keratinocytes lacking either β4 integrin or BP230 exhibit abnormal lamellipodial numbers and lack front-rear polarity [14,56].

Cofilin phospholyration is regulated by phosphatases, slingshot (SSH) or chronophin (CIN) [62,63]. Keratinocytes express all members of the SSH family and CIN [56]. However, expression of phosphatase-dead versions of all three SSH proteins, but not dominant inactive CIN, results in phosphorylation/inactivation of cofilin, changes in actin cytoskeleton organization, loss of cell polarity and assembly of aberrant arrays of laminin-332 in human keratinocytes [56]. SSH activity is regulated by 14-3-3 protein binding, and intriguingly, 14-3-3/α6β4 integrin protein interaction is required for keratinocyte migration. This raises the intriguing possibility that 14-3-3 proteins function as molecular switches, regulating Rac1 mediated keratinocyte migration patterns. In support of this hypothesis, inhibition of Rac1 results in an increase in 14-3-3 protein association with SSH and a concomitant loss of 14-3-3 protein interaction with α6β4 integrin [55,56]. Moreover, using amino or carboxy terminal domains of 14-3-3ζ, it has been demonstrated that in keratinocytes, 14-3-3ζ/τ heterodimers bind SSH1 in the absence of Rac1 signaling [55]. This interaction leads to an inhibition of SSH1 activity, as measured by an increase in phosphorylated cofilin levels. Overexpression of the carboxy terminal domain of 14-3-3ζ acts as a dominant negative and inhibits the interaction between 14-3-3τ and SSH1, providing evidence that 14-3-3ζ/τ heterodimers function as key regulators of SSH1 activity in keratinocytes [55]. Taken together, these results indicate that α6β4 integrin signaling via Rac1, 14-3-3 proteins and SSH family members regulates cofilin activation, cell polarity and matrix assembly, leading to specific epidermal cell migration behavior.

Based on the above published data, we propose a working model that provides an overview of how α6β4 integrin and the BP antigens may regulate keratinocyte motility (Fig. 4). α6β4 integrin heterodimers move into the assembling lamellipodium (Fig. 4). They interact with ligand (laminin-332) in the matrix, recruit the BP antigens, and associate (indirectly) with the cytoskeleton (Fig. 4). We suggest that in the lamellipodium and along its base, α6β4 integrin and the BP antigens interact with actin (Fig. 4). The latter is consistent with reports of an association of actin with α6β4 integrin in actively migrating cancer cells [64]. It should be noted that α6β4 integrin at the site of the lamellipodium is unlikely to interact with the keratin cytoskeleton since intermediate filaments are extremely sparse within the lamellipodium [65].

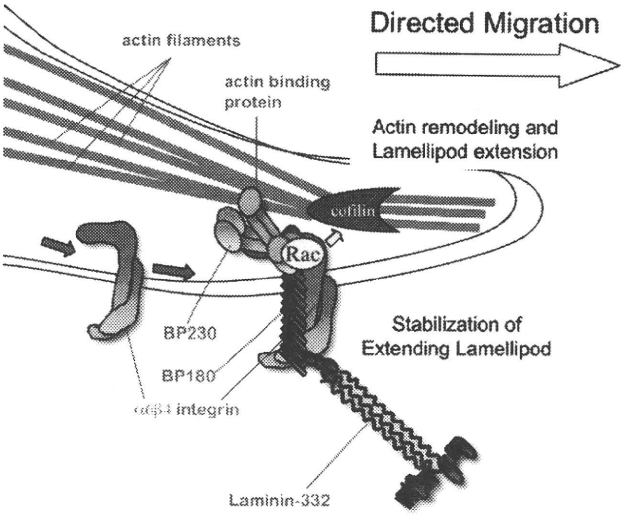

Fig. 4. Model for the role of hemidesmosome protein complexes in migration.

Moreover, keratin bundles in keratinocytes are found in the cytoplasm inside the circular arrays of actin that occur at the base of the lamellipodium [66] (unpublished observations).

The formation of α6β4 integrin/BP antigen/actin cytoskeleton/matrix complexes stabilizes the lamellipodium required for cell movement. In addition, by recruiting the BP antigens, α6β4 integrin activates Rac and subsequently cofilin. As mentioned above, the consequence of cofilin activation is the further extension of the lamellipodial surface and directed migration. As the cell moves forward the tethered integrin stays in place. This whole process is then repeated.

7. Conclusions

Two major inherited bullous diseases, epidermolysis bullosa and Kindler syndrome, have taught us much about the structure and functions of both focal contact and hemidesmosome proteins. Molecular genetic and live cell imaging analyses have elucidated new functions for the latter in both migration and adhesion. The dogma that hemidesmosome proteins are exclusively involved in stable adhesion while focal contacts are primarily involved in migration is being questioned. Novel signal pathways regulated by hemidesmosome protein complexes in migrating cells have recently been uncovered. It is to be hoped that this new knowledge may lead eventually to therapies that enhance wound closure. Numerous question, however, remain. Why are there unaffected areas of the skin in inherited blistering skin diseases, such as EB and Kindler syndrome? Is this due to compensation by other adhesion systems? Will identification of such compensatory mechanisms lead to new treatments for these devastating diseases? How precisely are the dynamics of hemidesmosome protein complexes and focal contacts coordinated? Is laminin-332 a key player in the latter since laminin-332 is a ligand for integrins of both the focal contact and hemidesmosome. What are the mechanisms that regulate the deposition of patterns of laminin-332 by moving skin cells and do these patterns determine motility behavior? By what molecular mechanism does α3 integrin retard skin cell motility? Finally, mechanisms regulating hemidesmosome disassembly have been identified [44]. However, we still do not understand the molecular mechanisms that control the switch that changes hemidesmosome protein function from being a "driver" of motility to being a mediator of stable attachment to the BMZ. Uncovering this mechanism will be an important avenue of research to pursue in the future.

Conflict of interest

The authors have no conflict of interest to declare.

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