The desmosome: cell science lessons from human diseases

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Summary

Human skin diseases have revealed fundamental mechanisms by which cytoskeletal proteins contribute to tissue architecture and function. In particular, the analysis of epidermal blistering disorders and the role of keratin gene mutations in these diseases has led to significant increases in our understanding of intermediate filament biology. The major cell-surface attachment site for intermediate filament networks is the desmosome, an adhesive intercellular junction prominent in the epidermis and the heart. During the past decade, substantial progress has been made in understanding the molecular basis of a variety of epidermal autoimmune diseases, skin fragility syndromes, and disorders that involve a combination of heart and skin defects caused by perturbations in desmosome structure and function. These human diseases reveal key roles for desmosomes in maintaining tissue integrity, but also suggest functions for desmosomal components in signal transduction pathways and epidermal organization.

Key words: Desmosome, Cadherin, Plakoglobin, Plakophilin, Pemphigus, Keratoderma, Cardiomyopathy

Introduction

Desmosomes are intercellular junctions that connect intermediate filaments to the cell surface and mediate strong cell-cell adhesion. They are particularly prominent in stratified squamous epithelia and the myocardium, tissues that are normally subjected to significant mechanical stress. Desmosomes are dynamic structures whose functions extend beyond adhesive interactions (Chidgey, 2002). Recent comprehensive reviews have highlighted the structural and functional properties of desmosomal proteins and the mechanisms of desmosome regulation revealed by cell biological and biochemical approaches (Garrod et al., 2002b; Getsios et al., 2004; Yin and Green, 2004). In this Commentary, we examine various human disorders in which desmosomal components are affected by mutations or autoimmune responses, and identify underlying principles of desmosome biology that these diseases reveal.

Several fundamental aspects of desmosome biology can be inferred from an examination of the clinical presentations of human diseases in which desmosome structure and function are altered. First, it is clear that the primary role of the desmosome is to resist mechanical stress. The two tissues subjected to routine and substantial mechanical forces are the heart and the skin. Invariably, mutations in, or autoantibodies directed at, desmosomal proteins lead to compromised cardiac or cutaneous function – and sometimes both. Second, different clinical presentations often arise when different desmosomal components are affected or even when different regions of the same molecule are mutated. By contrast, virtually identical clinical manifestations can result from mutations in different desmosomal components. Desmosomes may thus function as nodes where protein components participate in common functions, such as adhesion, but also engage in others, such as cytoskeletal organization, cell signaling, and tissue patterning. Last, the complex expression profiles of desmosomal genes are important for the differentiation program of tissues such as the epidermis. These observations suggest that individual members of each gene family have arisen to support specific aspects of the differentiation process. Below, we expand upon these themes by examining several autoimmune and inherited disorders affecting desmosome structure and function.

Desmosomal components and general organization

Desmosomes are highly symmetrical, electron-dense plasma membrane domains associated with intermediate filament networks. The core region of the desmosome includes the intercellular space and is a region of tight cell-cell adhesion; the cytoplasmic plaque couples these adhesive interactions to intermediate filament networks. Desmosomes are composed largely of proteins from three major gene families: desmosomal cadherins, armadillo family proteins and the plakin family of cytolinkers (Garrod et al., 2002a; Getsios et al., 2004; Godsel et al., 2004; Yin and Green, 2004). Cadherins are a large and diverse group of cell-cell adhesion molecules. The classical cadherins, such as E-cadherin, mediate Ca^{2+}-dependent contact between adjacent cells (Wheelock and Johnson, 2003). Desmogleins (Dsg) and desmocollins (Dsc) are the two types of desmosomal cadherin. These proteins have well-established adhesive functions, although the precise manner in which they mediate adhesion remains somewhat elusive (Garrod et al., 2002a). There are three known isoforms of desmocollin (1-3) and four of desmoglein (1-4) (Cheng and Koch, 2004; Garrod et al., 2002b), each cadherin subtype being encoded by a unique gene. Each desmocollin isoform also has two splice variants, the ‘a’ variant, which has a longer cytoplasmic tail, and the ‘b’ variant, which is shorter (Collins

et al., 1991). The biological significance of these splice variants is not yet known.

The cytoplasmic tails of the cadherins connect to the intermediate filament network through armadillo and plakin family proteins on the cytoplasmic face of the desmosome (Fig. 1). Plakoglobin is an armadillo family protein that binds directly to the cytoplasmic tails of both desmogleins and desmocollins (Peifer et al., 1992; Peifer et al., 1994) and is closely related to the adherens junction molecule β-catenin (Zhurinsky et al., 2000). There are four plakophilin (PKP) family members: PKP1, PKP2, PKP3 and p0071 (also referred to as PKP4) (Hatzfeld, 2005; Schmidt and Jager, 2005). These molecules are also armadillo family proteins, have diverse binding partners and are thought to facilitate the attachment of intermediate filaments to desmosomal plaques (Kowalczyk et al., 1999; McGrath et al., 1997; McGrath et al., 1999). Deeper in the cytoplasmic plaque of the desmosome is desmoplakin (North et al., 1999), a plakin family member and intermediate-filament-binding protein that appears to be an obligate component of desmosomes across a range of different tissues (Getsios et al., 2004). The general picture that has emerged over the past 10-15 years is that the desmosomal cadherins mediate Ca^{2+}-dependent cell-cell adhesion and bind directly to plakoglobin. Plakoglobin is thought to interact with desmoplakin and thereby link the cadherin tails to the intermediate filament network. The plakophilins bind tightly to desmoplakin and probably play a role in lateral interactions that cluster desmosomal components, thereby driving the formation of a densely packed structure (Fig. 1) (Kowalczyk et al., 1999).

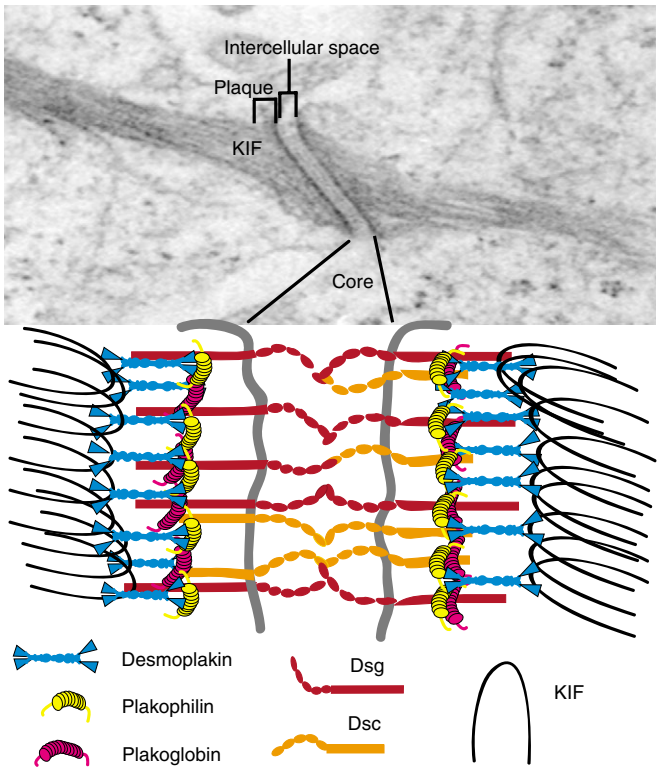

Fig. 1. Electron micrograph and schematic representation of the desmosome. The desmosome is an electron-dense complex (upper panel) found in tissues subjected to mechanical stress, such as stratified squamous epithelia cells and the myocardium. This intercellular junction is composed of a core region, which mediates tight cell-cell adhesion, and a plaque region, which mediates attachment to the intermediate filament cytoskeleton. The core region contains the extracellular domains of the desmosomal cadherins, the desmocollins and desmogleins. The cytoplasmic plaque region includes the C-terminal tails of the desmosomal cadherins, which associate directly and indirectly with various cytoplasmic proteins. The armadillo family proteins in the desmosome include plakoglobin and plakophilins. These proteins mediate interactions between the desmosomal cadherin tails and desmoplakin, a plakin family protein that binds directly to intermediate filaments. These components of the desmosome allow tethering of the intermediate filaments to the plasma membrane, thereby acting as a scaffold to provide structural integrity to cells and tissues. KIF, keratin intermediate filaments.

The epidermis represents an instructive example of the choreographed expression profiles of desmosomal genes (Fig. 2). Plakoglobin and desmoplakin are found throughout the epidermis, whereas the localization of desmogleins and plakophilins varies considerably. Dsg2 is widely expressed in simple epithelia and can be detected in the lower layers of the epidermis, along with high levels of Dsg3. Dsg1 is prominent in the upper layers, whereas Dsg4 is highly represented in the hair follicle. Dsc2 and Dsc3 are present in the lower layers; Dsc1 is highly expressed in the granular layer, along with Dsg1 (Garrod et al., 2002b). The plakophilins also exhibit specific expression patterns (reviewed by Schmidt and Jager, 2005). PKP1 is expressed throughout the epidermis but is localized preferentially to desmosomes only in the upper layers (Moll et al., 1997). PKP3 is widely expressed, whereas PKP2 is present in both complex and simple epithelia, the heart, and a variety of mesenchymal cell types (Bonne et al., 1999; Mertens et al., 1996). Studies of several human diseases are now providing clues to the significance of these complex expression patterns.

Desmosomal cadherins

Pemphigus: diseases of cell adhesion caused by autoimmune attack on desmogleins

In 1991, Amagai and Stanley reported that Dsg3 is the target of autoantibodies produced in the skin disease pemphigus vulgaris (PV) (Amagai et al., 1991), confirming previous studies indicating that the autoantigen involved was likely to be a desmosomal component (Jones et al., 1986). Pemphigus is a class of skin disorders characterized by the loss of cell-cell adhesion in the epidermis and mucous membranes (Payne et al., 2004; Stanley, 2003) (Fig. 3). The tissue affected depends on the isoform of desmoglein targeted by the autoantibodies. In pemphigus foliaceus, Dsg1 is targeted, which results in superficial blistering in the epidermis without effect on mucous membranes. By contrast, PV is characterized by oral erosions and sometimes blistering of the epidermis. Some PV patients exhibit only anti-Dsg3 autoantibodies, and clinical manifestations in these individuals are limited to mucous membranes. However, approximately 50% of PV patients go on to develop additional antibodies directed against Dsg1. These patients also exhibit severe epidermal blistering with the split occurring between basal and suprabasal cells (Mahoney et al., 1999a). Although the pattern of blister formation depends upon which desmoglein isoform is targeted, pemphigus provides striking evidence of the adhesive function of desmosomal cadherins. Recent studies of the infectious skin diseases *Staphylococcus* scalded-skin syndrome and its more

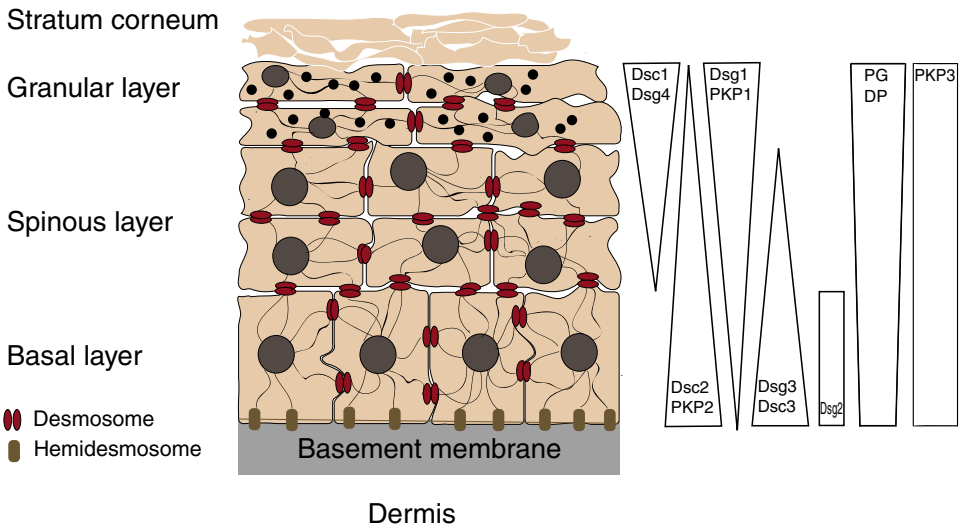

Fig. 2. Expression pattern of desmosomal components in the epidermis. Keratin filaments are shown connecting to desmosomes at sites of cell-cell contact and to hemidesmosomes at the basement membrane. The profiles and relative expression levels of various desmosomal proteins in the epidermal layers are depicted on the right.

limited counterpart, bullous impetigo, provide further evidence that desmogleins function in cell-cell adhesion. These two diseases are characterized by superficial blistering that is clinically and histologically identical to that seen in pemphigus foliaceus. Blistering in these patients is caused by exfoliative toxins (Amagai et al., 2000a; Amagai et al., 2002; Hanakawa et al., 2002), which are bacterial proteases that have exquisite specificity for Dsg1 and cleave its extracellular domain between repeats three and four (Hanakawa et al., 2004). This amazing convergence of autoimmune and infectious disease mechanisms provides striking evidence for a role of desmogleins in epidermal cell adhesion (Payne et al., 2004).

An early step in pemphigus might be the physical disruption of adhesion by blocking the adhesive interface of the desmogleins by autoantibodies (Shimizu et al., 2004). Biophysical, structural and mutagenesis experiments have resulted in a rather confusing picture of cadherin ectodomain interactions, but all point to a crucial role for sequences in the extreme N-terminal domain of the cadherins in the formation of the adhesive interface (He et al., 2003; Koch et al., 2004). Mapping studies indicate that this N-terminal region of the desmogleins is often targeted by pemphigus autoantibodies (Anzai et al., 2004; Kowalczyk et al., 1995; Li et al., 2003; Payne et al., 2005; Sekiguchi et al., 2001). Compelling evidence that the Dsg3 N-terminal domain is a key pathogenic epitope also comes from an experimental model system in which Dsg3-null mice were immunized with purified Dsg3 to

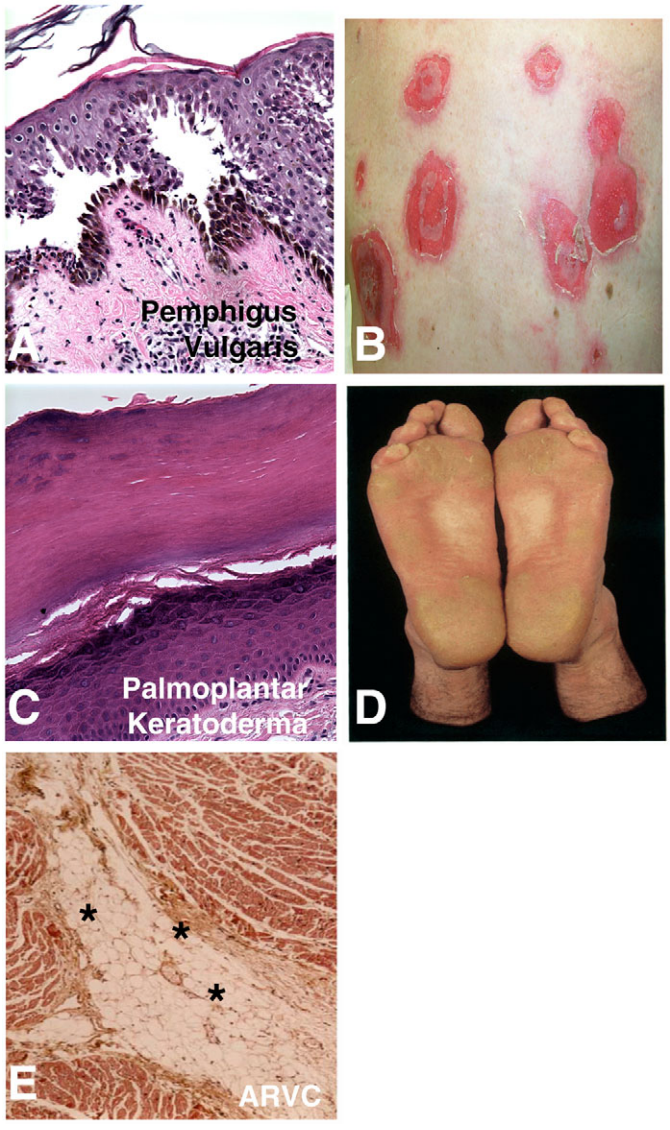

Fig. 3. Clinical appearance and pathohistology of various human desmosomal disorders. Pemphigus vulgaris (A and B) is characterized by the loss of intercellular adhesion between basal and suprabasal keratinocytes (A) and by skin blistering and erosions (B). The hallmark of palmoplantar keratoderma (C and D) is massive thickening of the stratum corneum (C), resulting in dramatically thickened skin on palms and soles (D). Arrhythmogenic right-ventricular cardiomyopathy (ARVC) is characterized by fibrofatty replacement (*) of the myocardium (E). Please refer to Table 1 for additional information on specific molecular targets of each disease. Panel D is reprinted from (Rickman et al., 1999) with permission from Oxford University Press. Panel E is reprinted from (Protonotarios and Tsatsopoulou, 2004) with permission from Elsevier.

generate a series of anti-Dsg3 monoclonal antibodies (Amagai et al., 2000b; Tsunoda et al., 2003). Several of these experimental antibodies cause PV-like blistering in wild-type recipient mice, and one antibody (AK23) is strongly pathogenic. Epitope-mapping experiments revealed that AK23 recognizes sequences within the proposed adhesive interface of Dsg3. Antibodies that are non-pathogenic or weakly pathogenic recognize epitopes in other portions of the Dsg3 extracellular domain (Tsunoda et al., 2003). These elegant studies indicate that antibodies targeting Dsg3 adhesive determinants are pathogenic.

A key question is whether autoantibody binding to desmoglein adhesive interaction sites is sufficient to cause epidermal blistering or whether subsequent events are required for the complete loss of desmosomal adhesion that is characteristic of the disease. Early research in pemphigus focused on plasminogen-activator-mediated proteolysis of cell-cell adhesion molecules following binding of autoantibody to the keratinocyte cell surface as a potential mechanism for the disruption of adhesion by pemphigus antibodies (Hashimoto et al., 1983; Morioka et al., 1987). More recent work suggests that the plasminogen activator system is not required for gross blistering in mouse models of the disease (Mahoney et al., 1999b). However, matrix metalloproteases (MMPs) were recently found to be involved in bullous pemphigoid, an autoimmune disease of the basement membrane zone (Liu et al., 2005), and additional studies are beginning to establish cross-talk between cadherins and MMPs (Hazan et al., 2004). Thus, proteases might exacerbate the effects of the autoantibodies, but this possibility may be difficult to establish in existing mouse model systems. Finally, very recent evidence suggests that pemphigus foliaceus autoantibodies, which are directed against Dsg1, do not actually disrupt desmoglein adhesive interactions. For example, atomic force microscopy experiments have indicated that pemphigus foliaceus autoantibodies do not disrupt Dsg1 ectodomain interactions (Waschke et al., 2005). Therefore, although substantial evidence indicates that pemphigus autoantibodies compromise adhesion, cellular responses might be required for these antibodies to disrupt cell-cell contact completely.

Another way pemphigus antibodies might disrupt desmosomal adhesion is by depleting surface cadherins. Desmosome assembly and disassembly is thought to be a coordinated process in which the kinetics of synthesis and turnover of various components are tightly regulated (Gloushankova et al., 2003; Godsel et al., 2005; Mattey et al., 1990; Pasdar et al., 1991; Pasdar and Nelson, 1989; Penn et al., 1989; Windoffer et al., 2002). Cadherin endocytosis is an important mechanism for modulating cell adhesion during development and in certain epithelial-cell-derived tumors (Bryant and Stow, 2004; D'Souza-Schorey, 2005; Kowalczyk and Reynolds, 2004). Binding of pemphigus autoantibodies probably alters the normal balance of desmosome assembly and disassembly kinetics. Indeed, in cultured epithelial cells, PV IgG was shown to deplete Dsg3 levels (Aoyama and Kitajima, 1999). Moreover, we have recently demonstrated that PV autoantibodies trigger Dsg3 endocytosis in normal human keratinocytes (Calkins et al., 2006). Thus, part of the pathogenic mechanism of PV IgG might be the depletion of pools of Dsg3 required for desmosome assembly or enhancement of the turnover of Dsg3 in pre-existing desmosomes.

If downstream events are required for the disruption of desmosomes by pemphigus antibodies, then specific signal transduction pathways should be activated upon binding of autoantibodies to desmogleins. In fact, Ca^{2+} influxes and phosphorylation of Dsg3 are observed upon binding of pemphigus autoantibodies to epithelial cells in vitro (Aoyama et al., 1999; Nguyen et al., 2004; Seishima et al., 1995). Furthermore, Rubenstein and colleagues recently demonstrated that heat shock protein 27 (Hsp27) is specifically phosphorylated after binding of PV autoantibodies to cultured human keratinocytes, which results in activation of the p38 mitogen-activated protein kinase (MAPK) pathway (Berkowitz et al., 2005). One function of the desmosome might thus be to relay messages from the outside of the cell to the inside through desmosomal cadherins. Indeed, recent studies have indicated that plakoglobin is required for pemphigus antibodies to disrupt desmosomal adhesion fully in cultured keratinocytes (Caldelari et al., 2001). Interestingly, even the effects of extracellular Ca^{2+} depletion, which disrupts cadherin-based adhesion, can be prevented by inhibiting protein kinase C (PKC) activity (Wallis et al., 2000). Intracellular signaling thus has a profound influence over the adhesive functions of cadherins. Reports are beginning to emerge that inhibition of various signaling pathways can prevent loss of adhesion in response to PV autoantibodies, using both in vitro and in vivo model systems (Berkowitz et al., 2005; Sanchez-Carpintero et al., 2004). Collectively, these and other studies are beginning to underpin a new approach to treating autoimmune blistering disorders. Manipulating desmosomes from the 'inside out' might thus be viewed as a valid therapeutic strategy. Similarly, the use of pemphigus IgG as a model system to disrupt desmosomes is likely to yield new insights into how changes in adhesion are executed by cells in other circumstances, such as wound healing, development and tumorigenesis.

Desmosomal cadherins regulate tissue morphogenesis

As discussed above, desmosomal cadherins are expressed in a tissue-specific and differentiation-stage-specific manner (Fig. 2). These differential expression patterns might play a role in tissue morphogenesis, and strong evidence supports the notion that expression patterns of desmoglein isoforms are crucial to normal epidermal homeostasis. For example, overexpression of Dsg3 in the upper layers of the epidermis of transgenic mice leads to hyperproliferation (Merritt et al., 2002), and the skin of mice expressing Dsg3 under the control of the involucrin promoter exhibits features similar to oral mucosa, which suggests that Dsg3 inhibits keratinocyte terminal differentiation (Elias et al., 2001). Studies using an organotypic model of human epidermal development indicate that Dsg1 might in fact be necessary for the proper differentiation of keratinocytes (S. Getsios and K. J. Green, personal communication). Dsg3 might thus function as a brake on keratinocyte differentiation, whereas Dsg1 promotes keratinocyte terminal differentiation in the upper layers of the epidermis.

The clinical presentations of several human diseases further support the idea that the desmosomal cadherins contribute to epidermal patterning and homeostasis. The autosomal-dominant skin disease striate palmoplantar keratoderma is caused by haploinsufficiency of the gene encoding Dsg1 (Hunt et al., 2001; Rickman et al., 1999). Rather than developing

blisters in the superficial layers of the epidermis, as occurs in response to pemphigus foliaceus antibodies targeting Dsg1, patients with Dsg1 haploinsufficiency exhibit a thickening of the stratum corneum on the palms and soles (Fig. 3). These locations are probably most sensitive to changes in the amounts of desmosomal components since they are subjected to repeated physical stress and pressure. Thickening of the stratum corneum over areas of repeated trauma is a normal response of healthy skin and results in callous formation. Palmoplantar keratoderma might thus represent this type of thickening response occurring at low levels of mechanical stress. This possibility is supported further by the observation that palmoplantar keratoderma can also result from mutations in genes encoding components of the desmosomal plaque (see below). Another possibility is that keratoderma is caused by an alteration in tissue morphology driven by aberrant ratios of desmoglein isoform expression. This idea would support the notion that desmogleins are responsible for more than simply adhesion. Clinical manifestations such as keratoderma might thus reflect compensatory responses to tissue fragility and/or alterations in the keratinocyte differentiation program driven by desmosomal components. Indeed, key principles of epidermal structure and function are likely to emerge from an increased understanding of how changes in keratinocyte adhesive strength relate to alterations in keratinocyte differentiation.

The hair follicle represents a fascinating example of tissue patterning, and desmosomal cadherins also play key roles here. For example, Dsg3-null mice exhibit not only a pemphigus-like phenotype but also hair loss (Koch et al., 1997). Although humans suffering from pemphigus do not typically lose their hair, human Dsg4 appears to play a key role in the hair follicle. Inherited hypotrichosis results from a mutation in Dsg4 that causes altered follicular keratinocyte differentiation and proliferation (Kljuic et al., 2003). Mice lacking Dsg4 exhibit similar changes, and Dsg4-null keratinocytes are unable to make a normal transition from proliferation to differentiation (Kljuic et al., 2003). These findings implicate Dsg4 as a key regulator of the keratinocyte differentiation program in the hair follicle. This hair follicle phenotype is in stark contrast to the clinical presentation observed in patients with mutations in the gene encoding Dsg1, which lead to striate palmoplantar keratoderma. The difference in phenotypes between patients who have mutations in different members of the desmoglein family underscores the different functional roles that various desmosomal cadherins play in the keratinocyte differentiation program.

Desmocollins also play important roles in epidermal integrity and differentiation. To date, no human desmocollin mutations have been reported. However, Dsc1-null mice exhibit loss of cell-cell adhesion in the granular layer (Chidgey et al., 2001) and hyperproliferative changes and increased expression of the wound keratins K6 and K16. Dsc1 may thus be crucial for strong adhesion and terminal differentiation. By contrast, Dsc3 expression might be more compatible with keratinocyte proliferation and dynamic cell-cell adhesion. These suggestions are consistent with the reciprocal localization of these cadherins in the upper differentiated layers (Dsc1) and lower proliferative layers of the epidermis (Dsc3) (Fig. 2). Aberrant expression of Dsc3 in the suprabasal epidermal layers results in altered keratinocyte differentiation (Hardman et al., 2005) and hair follicle changes similar to those in mice over-expressing β-catenin. Indeed, Hardman et al. have established a link between Dsc3 and β-catenin stability, providing further evidence for integration of desmosomal cadherins and signaling pathways fundamental to epidermal proliferation and differentiation programs.

Desmosomal plaque proteins

The adhesive functions of the desmosomal cadherins are supported by cytoplasmic linkages that couple these adhesion molecules to the cytoskeleton. The ability of many desmosomal plaque proteins to translocate to other subcellular compartments supports the hypothesis that the desmosome is an intersection for adhesion and signaling. Human mutations in genes encoding desmosomal plaque proteins, along with mouse genetic model systems, expose the importance of desmosomal plaque proteins in supporting desmosomal adhesion and signaling activities.

The plakophilins

The first genetic disorder of the desmosome to be identified was reported by McGrath and colleagues. Patients exhibiting a severe autosomal recessive ectodermal dysplasia and skin fragility syndrome were found to have mutations in the armadillo family protein PKP1 (McGrath et al., 1997; McGrath et al., 1999). An affected individual had two copies of PKP1 with premature stop codons, which resulted in a complete absence of the protein. The skin of patients lacking PKP1 cannot withstand minor trauma, which supports the notion that PKP1 plays a key role in desmosomal adhesion. Light and electron microscopy analysis revealed widened intercellular spaces and smaller than normal desmosomes, as well as aberrant localization of desmoplakin. In many keratinocytes, intermediate filaments are condensed in a perinuclear location rather than being attached to desmosomes at the cell periphery. These data suggest that PKP1 is required for proper attachment of intermediate filaments to the desmosomes, perhaps through desmoplakin. In vitro data support this notion and demonstrate that PKP1 binds tightly to desmoplakin and strongly augments desmoplakin recruitment to sites of cell contact (Hatzfeld et al., 1994; Hatzfeld et al., 2000; Kowalczyk et al., 1999; Wahl, 2005). Recent evidence further implicates PKP1 in stabilizing desmosomes and modulating keratinocyte migration (South et al., 2003; South, 2004). In addition to its role in adhesion and attachment to intermediate filaments, the ectodermal dysplasia in patients lacking PKP1 suggests that this protein plays a role in epidermal morphogenesis. These patients have sparse hair and dystrophic nails and cannot sweat normally. All of these defects indicate compromised development of the epidermis and its appendages, thus implicating PKP1 in epidermal patterning.

PKP1 exhibits striking nuclear localization, which suggests that some changes in the skin of patients lacking PKP1 might be a result of its functions outside the desmosome (Schmidt and Jager, 2005). Indeed, growing evidence has suggested nuclear functions for the plakophilin family proteins, all of which have been reported to exhibit nuclear localization (Hatzfeld, 2005). For example, PKP2 localizes to subnuclear particles and interacts with RNA polymerase III (Mertens et al., 2001). PKP2 also associates with β-catenin and might

thereby influence β-catenin signaling and/or adherens junction assembly (Chen et al., 2002). Nonetheless, it remains clear that a fundamental function of PKP2 is in desmosome assembly and in the maintenance of tissue integrity. Whereas PKP1 mutations are manifest in the skin, human PKP2 mutations cause arrhythmogenic right-ventricular cardiomyopathy (ARVC) (Gerull et al., 2004) (Fig. 3E). In most cases, the mutations affect the C-terminal region of the protein, although other mutations are observed throughout the gene. In addition, one case appears to reflect PKP2 haploinsufficiency. Experiments ablating the gene encoding PKP2 in mice indicate that the cardiomyopathy in patients with PKP2 mutations is most likely a result of mechanical fragility. Mice lacking PKP2 exhibit mid-gestational embryonic lethality owing to heart morphogenesis defects and apparent mechanical fragility of the myocardium (Grossmann et al., 2004). In common with mutations in the gene encoding human PKP1 that lead to impaired association of keratin with the plasma membrane in keratinocytes, lack of PKP2 in mouse cardiomyocytes causes intermediate filaments to retract from the membrane. Thus, both PKP1 and PKP2 play essential roles in mediating desmoplakin association with desmosomes in the epidermis and heart, respectively. Nonetheless, it is likely that these proteins have additional nuclear or cytoskeletal roles that contribute to these phenotypes. It will be interesting to determine the roles of other plakophilin family members in the heart, skin and other organs, and to determine whether they harbor tissue-specific functions.

Plakoglobin

Plakoglobin is the most studied armadillo family protein in the desmosome. Mouse-knockout studies have provided important clues that plakoglobin has key roles in both the epidermis and the heart. Ablation of the plakoglobin gene results in mouse embryonic lethality owing to mechanical fragility of the myocardium. In some genetic backgrounds, mouse pups are viable but display serious epidermal fragility, heart defects and early postnatal lethality (Bierkamp et al., 1996; Ruiz et al., 1996). Consistent with a crucial function for plakoglobin in both heart and skin is the finding that a plakoglobin mutation in humans causes the autosomal recessive Naxos disease (McKoy et al., 2000). Sequence analysis of Naxos patient DNA revealed a plakoglobin mutation resulting in the expression of a truncated protein that lacks the C-terminal domain (McKoy et al., 2000). Clinically, this disease is characterized by ARVC, woolly hair and palmoplantar keratoderma (Protonotarios and Tsatsopoulou, 2004). The heart defects appear to result from fragility of the myocyte syncytium that leads to its degradation and fibrofatty replacement. The palmoplantar keratoderma is also likely to be a consequence of weakened cell adhesion, although epidermal manifestations in Naxos disease are not as severe as those observed in plakoglobin-null mice. Plakoglobin-knockout mice exhibit skin blistering from acantholysis (loss of cell-cell adhesion), indicating that desmosomes are disrupted (Bierkamp et al., 1996). Acantholysis is not observed in patients with Naxos disease, which suggests that some plakoglobin functions in cell adhesion are maintained in the epidermis of these patients. Desmosomes in normal palmar and plantar skin are larger than those in other locations, perhaps reflecting their role in resistance to mechanical stress (Wan et al., 2003). Perhaps patients with Naxos disease have intact desmosomes, but these structures are unable to support the more extreme mechanical demands placed on the epidermis of the palms and soles. Because the heart is subjected to substantial and continual mechanical forces, it is possible that even minor decreases in the strength of desmosomal adhesion become clinically apparent when the myocardium is affected. Similarly, weakening of adhesion might be apparent in the skin only in areas subjected to routine physical stress, such as the palms and soles, where hyperkeratosis is evident.

An interesting aspect of Naxos disease is the presence of a hair phenotype (McGrath and Wessagowit, 2005). These patients present with keratoderma on the palms and soles, but also exhibit ‘woolly’ hair. The hair is often lighter in color, and is shorter and finer than in unaffected individuals. Why these changes occur is unknown, but it is interesting to note that plakoglobin and the closely related adherens junction protein β-catenin both have been implicated in hair formation. In addition to supporting association of E-cadherin with the actin cytoskeleton, β-catenin translocates to the nucleus and interacts with members of the T-cell factor (TCF) family of transcription factors (Huelsken and Behrens, 2002). Although plakoglobin does not appear to function identically to β-catenin in these signaling pathways, there is evidence that plakoglobin can modify β-catenin and/or TCF signaling (Miravet et al., 2002; Yin and Green, 2004). Furthermore, overexpression of plakoglobin in mouse epidermis decreases keratinocyte proliferation and shortens the anagen (growth) phase of the hair cycle, thereby causing decreased hair growth (Charpentier et al., 2000). By contrast, β-catenin overexpression causes hyperproliferation and hair follicle differentiation (Gat et al., 1998). It is currently unclear whether there is a balance between plakoglobin and β-catenin signaling that is disrupted in patients with Naxos disease or whether the woolly hair phenotype reflects changes in hair structure owing to compromised keratinocyte cell adhesion in the hair follicle or hair shaft.

Interestingly, the woolly hair phenotype also results from mutations in desmoplakin (see below). Furthermore, mutations in the genes encoding plakoglobin, Dsg1 and desmoplakin sometimes cause keratoderma on the palms and soles. Similarly, several keratin mutations lead to keratodermas (Lane and McLean, 2004). An interesting question is whether the keratoderma and woolly hair phenotypes are the result of defects in epidermal differentiation or a manifestation of mechanical fragility. It might be that mechanical fragility in hair follicles or palmar skin causes dysregulation of epidermal morphogenesis. Alternatively, it is possible that mutations in desmosomal components alter morphogenesis independently of effects on keratinocyte adhesion. The latter notion is supported by the fact that pemphigus patients exhibit loss of cell-cell adhesion but do not exhibit dramatic changes in epidermal morphogenesis.

Desmoplakin

The desmosomal cadherin-plakoglobin complex is coupled to the intermediate filament network by desmoplakin, a member of the plakin family of cytolinkers (Leung et al., 2001; Leung et al., 2002). The N-terminal domain of desmoplakin binds to plakoglobin and plakophilin, whereas the C-terminal domain interacts with intermediate filaments (Getsios et al.,

2004; Yin and Green, 2004). Desmoplakin is expressed ubiquitously in all tissues that have desmosomes. Mice lacking desmoplakin die just after implantation at day E6.5 and have significantly fewer desmosomes than do wild-type mice (Gallicano et al., 1998). The few desmosomes present are not attached to keratin intermediate filaments, which confirms in vitro studies suggesting that desmoplakin is crucial for linking keratin filaments to the plasma membrane (Bornslaeger et al., 1996). Desmoplakin-null mice also reveal a role for desmoplakin in tissue morphogenesis. The embryos fail to undergo the massive increase in cellular proliferation normally observed at embryonic days 5-6, and subsequent studies indicate that desmoplakin plays crucial roles in a variety of tissues, including skin, neuroepithelium, heart and blood vessels (Gallicano et al., 2001; Vasioukhin et al., 2001). Interestingly, desmoplakin-null animals exhibit far more severe phenotypes than animals lacking intermediate filaments such as keratin 8/18 (Baribault et al., 1993) or vimentin (Colucci-Guyon et al., 1994). For example, desmoplakin is crucial for endothelial organization during vascular development, whereas vimentin appears largely dispensable. These findings provide a striking demonstration that desmoplakin plays roles in cell adhesion and tissue organization that extend beyond its role linking intermediate filament networks to the plasma membrane.

Desmoplakin mutations cause an array of human diseases, which vary in severity (Cheong et al., 2005). Desmoplakin haploinsufficiency leads to striate palmoplantar keratoderma (Armstrong et al., 1999; Whittock et al., 1999), whereas a more severe keratoderma with skin fragility and woolly hair results from compound heterozygosity of nonsense and missense mutations (Whittock et al., 2002). Recently, a lethal condition termed acantholytic epidermolysis bullosa (Jonkman et al., 2005) was shown to result from compound heterozygous mutations that truncate the desmoplakin C-terminus, the region that binds to intermediate filaments. The patient with these desmoplakin mutations died 10 days postpartum from transcutaneous fluid loss as a result of extensive skin erosion. In addition, the patient exhibited complete alopecia, neonatal teeth and nail loss. Electron microscopy revealed that desmosomes formed and appeared relatively normal. However, keratin filaments were retracted towards the nucleus and desmosomes were often torn out of adjacent cells owing to the lack of cytoskeletal attachment to the plaque. The desmoplakin N-terminal domain and the central α-helical rod domain thus seem to be sufficient for desmosome morphology, but attachment of intermediate filaments is crucial for tissue integrity.

Desmoplakin mutations can also lead to disorders similar to those caused by mutations in plakoglobin or Dsg1. For example, Carvajal syndrome is an autosomal recessive disorder characterized by dilated cardiomyopathy, woolly hair and keratoderma (Norgett et al., 2000). In this case, compound heterozygous mutations result in a truncated desmoplakin lacking part of the C-terminus. Clinically, this disorder appears similar to Naxos disease, which results from a mutation in plakoglobin (McKoy et al., 2000; Protonotarios and Tsatsopoulou, 2004). A difficult issue to resolve is why different desmoplakin mutations affect different tissues. Some desmoplakin mutations affect the heart, other mutations are restricted to the skin, and some mutations affect both organ systems (Table 1 and Fig. 3). Heart manifestations can involve mutations in either the N-terminal or C-terminal region of desmoplakin. Furthermore, some C-terminal mutations cause heart and skin defects (Alcalai et al., 2003), whereas others lead only to skin disorders (Whittock et al., 2002). One explanation is that different regions of the desmoplakin C-terminal domain exhibit different binding affinities for various intermediate filaments (Choi et al., 2002; Fontao et al., 2003; Meng et al., 1997). Additional studies of how various disease-causing mutations alter interactions between desmoplakin and intermediate filaments are needed if we are to understand both the cell biology of the interactions and the underlying reasons for these complicated variations in clinical presentation.

Table 1. Mutations and target antigens in desmosomal diseases

Protein	Perturbation	Diseases
Dsg1		
	Haploinsufficiency	SPPK
	Autoantibodies	Pemphigus foliaceus
	Exfoliative toxin	*Staphylococcus* scalded-skin syndrome
Dsg3	Autoantibodies	Pemphigus vulgaris
Dsg4	Hair follicle abnormalities, hair loss	Autosomal recessive hypotrichosis
Plakoglobin	C-terminal truncation	Naxos disease (PPK, woolly hair, ARVC)
Plakophilin-1	Compound heterozygous mutations (premature stop codons and absence of protein)	Ectodermal dysplasia and skin fragility syndrome
Plakophilin-2	Various heterozygous mutations and haploinsufficiency	ARVC
Desmoplakin	Haploinsufficiency	SPPK
	Compound heterozygosity with missense (N-terminal) and nonsense (C-terminal) mutations	Keratoderma, keratin retraction, skin fragility and woolly hair/alopecia
	N-terminal missense mutation	Autosomal-dominant ARVC
	C-terminal missense	Autosomal-dominant ARVC
	C-terminal truncation	Dilated left ventricular cardiomyopathy, SPPK, woolly hair (Carvajal syndrome)
	Compound heterozygous mutations with C-terminal truncation	Lethal acantholytic epidermolysis bullosa

Abbreviations: ARVC, arrhythmogenic right-ventricular cardiomyopathy; PPK, palmoplantar keratoderma; SPPK, striate palmoplantar keratoderma.

Conclusions

Desmosomes have long been suspected to play crucial roles in tissue integrity. Over the past 15 years, careful analysis of human diseases and mouse genetic model systems has demonstrated key roles for desmosomes in heart and skin integrity. Alterations in desmosomal protein function often lead to tissue fragility, with significant clinical consequences. Although desmosomes function as robust adhesive structures, they are also subject to dynamic regulation and undergo continual turnover. In addition, desmosomes are emerging as mediators of various signaling pathways. These advances in our understanding of desmosome structure, function and signaling have important consequences for both fundamental cell science and therapeutic efforts directed towards heart and skin diseases caused by defects in desmosomal components.

Recent progress has also raised new questions. At times, the clinical presentation of disease seems to make sense and reinforces prevailing notions of desmosome structure and function. For example, inactivation of desmogleins by autoantibodies leads to blistering diseases that almost certainly arise from the loss of cell-cell adhesion. However, in other cases, clinical phenotypes can overlap or diverge in a puzzling manner. Mutations in genes encoding different desmosomal proteins can cause similar clinical phenotypes, such as the development of palmoplantar keratoderma resulting from mutations in the genes for either Dsg1 or desmoplakin. Does this disease presentation represent cellular responses to mechanical fragility, or do both Dsg1 and desmoplakin play overlapping roles in signaling pathways that regulate epidermal differentiation? By contrast, different mutations in the same gene can lead to divergent phenotypes, which suggests that different domains of individual desmosomal proteins have tissue-specific functions. Other important and unresolved questions center around the diversity in tissue-specific and differentiation-stage-specific expression patterns of desmosomal genes. Transgenic mouse models clearly demonstrate that differential expression of desmosomal cadherins contributes to tissue patterning and differentiation programs. How exactly do these molecules participate in signaling pathways that drive epidermal differentiation? Identifying downstream targets and molecular determinants of desmosomal cadherin signaling will have important implications for our understanding of tissue morphogenesis during development, as well as the underlying mechanisms of both autoimmune and genetic skin disorders.

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