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NEW EMBO MEMBER'S REVIEW

Role of integrins in regulating epidermal adhesion, growth and differentiation

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Mammalian epidermis is renewed throughout life by proliferation of a multipotential stem cell population and terminal differentiation of stem cell progeny. In recent years, extracellular matrix receptors of the integrin family have been identified as important regulators of epidermal homeostasis, influencing the balance between stem cell renewal and differentiation. Integrin expression is altered when the epidermis is damaged or diseased, and there is good evidence that specific integrins can contribute positively or negatively to pathogenesis. In this review I summarize what is known about the expression and function of epidermal integrins, and highlight the challenges for future research.

Keywords: cancer/differentiation/epidermis/integrins/stem cells

Introduction

Integrins are heterodimeric transmembrane receptors consisting of one α and one β subunit. The two subunits collaborate to bind ligands, which are extracellular matrix proteins or counter-receptors of the Ig superfamily. Ligand specificity is determined by heterodimer composition and cellular context. The strength of ligand binding is modulated by divalent cations, by receptor clustering and by the association of integrins with accessory molecules (Bazzoni and Hemler, 1998; Liu *et al*., 2000; van der Flier and Sonnenberg, 2001). Integrins can transduce signals between cells and the extracellular milieu, and the structural basis of how changes in the extracellular domain can influence the conformation of the cytoplasmic domain is the subject of intense investigation.

The focus of this review is the expression and function of integrins in keratinocytes, the cells that make up the epidermis and other stratified squamous epithelia. Back in 1977, it was demonstrated that when keratinocytes are held in suspension they withdraw from the cell cycle and initiate terminal differentiation (Green, 1977). More than 20 years later, we know that the suspension effect reflects the fact that anchorage of keratinocytes to extracellular matrix negatively regulates their differentiation (Adams and Watt, 1989; Watt *et al*., 1993; Levy *et al*., 2000). The advent of tissue-specific knockout mice and the characterization of certain human heritable skin disorders have expanded our understanding of the importance and multiple roles of integrins in the epidermis.

Which integrins are expressed?

The integrins expressed by keratinocytes can be placed into different categories, according to whether they are abundant or weakly expressed, and whether they are expressed constitutively, or induced by wounding or pathological changes (Table I). The most abundant, constitutive integrins in the epidermis are α2β1 (collagen receptor), α3β1 (predominantly a laminin 5 receptor) and α6β4 (laminin receptor) (Watt and Hertle, 1994). αvβ5 (vitronectin receptor) is also a constitutive epidermal integrin, but is expressed at lower levels than the others (Adams and Watt, 1991; Watt and Hertle, 1994).

α5β1 (fibronectin receptor) and αvβ6 (receptor for fibronectin and tenascin) are induced in culture and on wounding (Watt and Hertle, 1994; Breuss *et al*., 1995; Zambruno *et al*., 1995; Häkkinen *et al*., 2000). αvβ6 is often expressed in squamous cell carcinomas, tumours of keratinocytes (Jones *et al*., 1997). α9β1 (tenascin receptor; Palmer *et al*., 1993) is expressed in undamaged epidermis (Stepp *et al*., 1995) and is upregulated during wound healing, albeit with different kinetics from αvβ6 (Häkkinen *et al*., 2000).

Two other integrins that have been found in the epidermis are α8β1 (receptor for tenascin, fibronectin and vitronectin; Schnapp *et al*., 1995a) and αvβ8 (receptor for vitronectin and potentially also for laminin, collagen and fibronectin; Nishimura *et al*., 1994; Venstrom and Reichardt, 1995). Although originally reported in the epidermis of developing chick embryos (Bossy *et al*., 1991), α8β1 is confined to the arrector pili muscle in postnatal mammalian skin (Schnapp *et al*., 1995b). Unlike the other keratinocyte integrins, αvβ8 is absent from the basal layer of adult epidermis and is found exclusively in the suprabasal layers (Stepp, 1999).

Where are the integrins expressed?

In normal, undamaged epidermis, integrin expression is confined to the basal layer and outer root sheath of the hair follicles (an exception being αvβ8, as described above). α6β4 is primarily concentrated at the basement membrane zone and is a component of hemidesmosomes. The other integrins are distributed over the basal, lateral and apical surfaces of basal cells (Watt and Hertle, 1994). Whole-mount labelling of human epidermis reveals that on the basal surface of basal cells there are clusters of β1 integrins interspersed with hemidesmosomes, but the majority of β1 integrins appear to form an 'O' ring at the cell periphery (Jensen *et al*., 1999).

In vitro, α6β4 is found in rudimentary hemidesmosomes that are associated with intermediate filaments and also on the leading lamellae of migrating keratinocytes in association with filamentous actin (Adams and Watt,

Table I. Keratinocyte integrins

Integrin	Major ligand	Expression
α2β1	Collagen	Constitutive
α3β1	Laminin	Constitutive
α6β4	Laminin	Constitutive
αvβ5	Vitronectin	Weak
α5β1	Fibronectin	Induced in culture, on wounding, in pathological conditions
αvβ6	Fibronectin; tenascin	As α5β1
α9β1	Tenascin	Upregulated during wound healing
αvβ8	Vitronectin	Suprabasal

1991; Mercurio *et al*., 2001). The β1 integrins are found in focal adhesions, but also, as *in vivo*, concentrate in an 'O' ring at the peripheral membrane in contact with the culture substrate (Braga *et al*., 1998).

In addition to the upregulation of certain integrins that occurs in culture, during wound healing or in disease (see Table I), two changes in epidermal integrin expression are frequently observed. The first is expression of integrins in the suprabasal, differentiating cell layers, which is generally associated with benign hyperproliferation, for example during wound healing, in psoriatic lesions and in normal oral mucosa, and is also seen in some squamous cell carcinomas (Watt and Hertle, 1994; Figure 1A). The second is focal or generalized loss of integrins, and this is a feature of squamous cell carcinomas (Jones *et al*., 1993; Bagutti *et al*., 1998). Focal integrin loss in tumours tends to correlate with a loss of basement membrane proteins, and has been proposed to play a role in invasion (Downer *et al*., 1993; Jones *et al*., 1993).

Extracellular matrix adhesion and migration

The earliest experiments to define the role of integrins in keratinocyte adhesion and migration relied on the use of integrin-specific antibodies to perturb adhesion of cultured human keratinocytes on different extracellular matrix substrates (reviewed by Watt and Hertle, 1994). Studies with adhesion-blocking antibodies also established a role for integrins in keratinocyte motility, different integrins mediating movement on different substrates (Watt and Hertle, 1994; Nguyen *et al*., 2000).

Complementary roles for α3β1 and α6β4 in mediating keratinocyte adhesion and motility on laminin have been proposed, with α3β1 being required at the leading edge of the cell and α6β4 stabilizing attachment distally (DiPersio *et al*., 1997; Goldfinger *et al*., 1999; Nguyen *et al*., 2000). However, it is now clear that α6β4 has distinct functions according to whether it is associated with hemidesmosomes or with membrane protrusions, and α6β4 and α3β1 at the leading edge may collaborate to promote motility and wound healing (Nguyen *et al*., 2000; Mercurio *et al*., 2001). One of the mechanisms by which α6β4 promotes migration is through activation of phosphatidylinositol 3-kinase (PI3-K) (Mercurio *et al*., 2001); however, signalling via Rac and Rho (O'Connor *et al*., 2000) and the MAPK pathway (Mainiero *et al*., 1997) are also important.

The generation of mice that lack specific integrins has confirmed their importance in keratinocyte adhesion to the underlying basement membrane (Table II). Mice with a targeted deletion of the α6 or β4 integrin subunit die shortly after birth; they have severe blistering of the skin and other stratified squamous epithelia, and lack hemidesmosomes (Dowling *et al*., 1996; Georges-Labouesse *et al*., 1996; van der Neut *et al*., 1996). Humans who have pyloric atresia with junctional epidermolysis bullosa, an autosomal recessive disorder with epidermal blistering, have mutations in the α6 or β4 integrin genes, the degree of severity of the phenotype reflecting the nature of the mutation (reviewed by Ashton *et al*., 2001).

In contrast to the α6 and β4 knockouts, the β1 knockout is early embryonic lethal. To study the consequences of epidermal-specific β1 deletion, mice with floxed β1 alleles have been crossed with mice expressing Cre under the control of a promoter (keratin 5 or 14) that is active in the basal layer of the epidermis (Brakebusch *et al*., 2000; Raghavan *et al*., 2000). These animals have epidermal blistering, although not as severe as in the α6 or β4 knockouts. Some mice with epidermal deletion of β1 survive long enough to allow wound-healing studies to be performed and these confirm that β1 is essential for keratinocyte migration *in vivo* (Grose *et al*., 2002).

Other integrin knockouts have also shed light on which integrins mediate epidermal adhesion and wound healing *in vivo*. When the α3 subunit is deleted, there is occasional epidermal blistering and more extensive disorganization of the basement membrane (DiPersio *et al*., 1997). Interestingly, the double knockout of α3β1 and α6β4 has an epidermal phenotype that is no more severe than either one alone (DiPersio *et al*., 2000a). The α2 (Chen *et al*., 2002; Holtkötter *et al*., 2002), α9 (Huang *et al*., 2000b) and β5 (Huang *et al*., 2000a) knockouts have no reported skin phenotype, although *in vitro* β5-null keratinocytes show severely impaired migration (Huang *et al*., 2000a).

Terminal differentiation and apoptosis

When cultured human or mouse keratinocytes are placed in suspension as single cells, they withdraw from the cell cycle and undergo terminal differentiation (Green, 1977; Adams and Watt, 1989; Drozdoff and Pledger, 1993; Romero *et al*., 1999). The initiation of terminal differentiation can be partially inhibited by fibronectin or adhesion-blocking antibodies to β1 integrins (Adams and Watt, 1989; Watt *et al*., 1993; Levy *et al*., 2000). Fab fragments of anti-β1 antibodies are as effective as whole IgG in blocking differentiation, and the antibodies do not act by promoting actin polymerization in suspended cells (Adams and Watt, 1989; Watt *et al*., 1993). It thus appears that ligation of β1 integrins is a negative regulator of terminal differentiation and that the mechanism by which β1 integrins exert their effect differs from β1-mediated cell spreading in that it does not require receptor clustering or polymerization of the actin cytoskeleton.

The role of β1 integrins in regulating keratinocyte differentiation has been further investigated by introducing wild-type and mutant chicken β1 subunits into primary human keratinocytes (Levy *et al*., 2000). These studies demonstrate that the absolute number, and not the

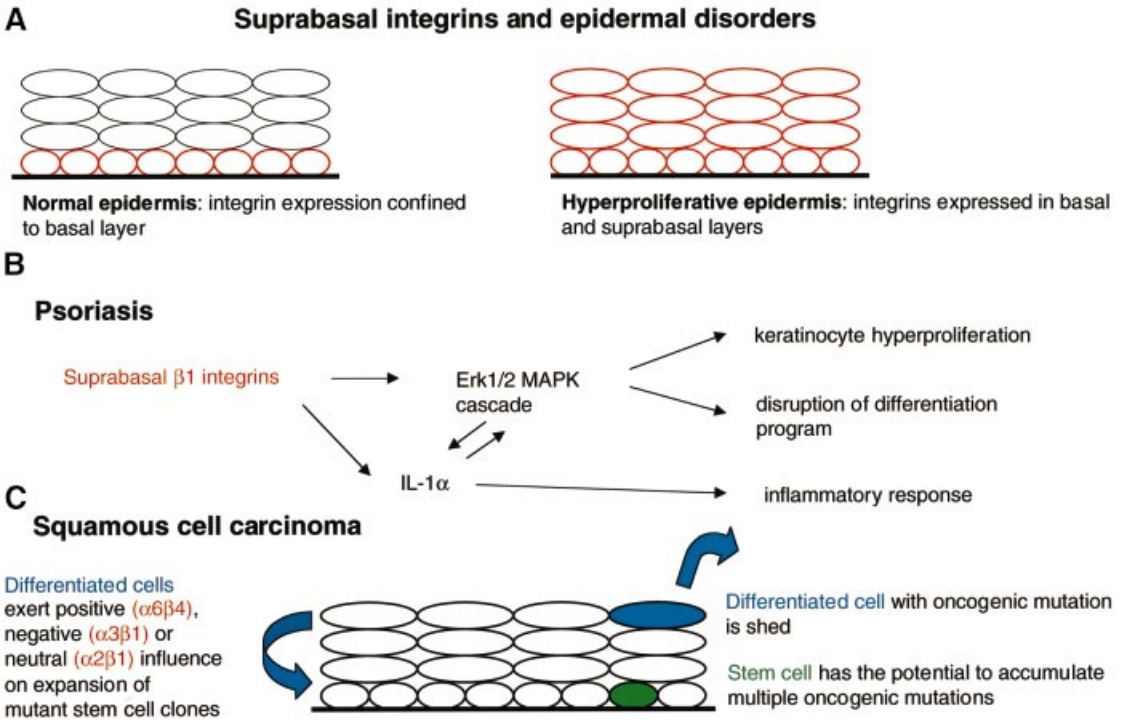

Fig. 1. Schematic representation of how suprabasal integrin expression in the epidermis (**A**) contributes to the pathogenesis of psoriasis (**B**) and squamous cell carcinoma (**C**).

Table II. Epidermal phenotype of integrin knockout mice

Integrin subunit	Phenotype	Reference
α3	Disorganized basement membrane; occasional epidermal/dermal blistering, primarily on legs and footpads	DiPersio *et al*. (1997)
β6	Juvenile hair loss due to macrophage infiltration into skin; wound healing normal	Huang *et al*. (1996)
α9	No defects observed	Huang *et al*. (2000b)
β5	Wound healing normal	Huang *et al*. (2000a)
β1	Floxed β1 × K5Cre	Brakebusch *et al*. (2000)
	Abnormal hair follicles; hair loss with removal of follicles by infiltrating macrophages; epidermal/dermal blisters; reduced proliferation and abnormal differentiation of IFE; disruption of basement membrane; reduced α6β4 expression; reduced hemidesmosomes; dermal fibrosis; impaired wound healing	Grose *et al*. (2002)
	Floxed β1 × K14Cre	Raghavan *et al*. (2000)
	Epidermal/dermal blistering; basement membrane disruption; reduced hemidesmosomes; thin epidermis; reduced number of hair follicles; reduced α6β4 expression	
α6	Severe epidermal blistering	Georges-Labouesse *et al*. (1996)
β4	Severe epidermal blistering; absence of hemidesmosomes	Van der Neut *et al*. (1996)
		Dowling *et al*. (1996)
α3 + α6	Epidermal blistering; proliferation, stratification and hair follicle morphogenesis normal in adherent epidermis	DiPersio *et al*. (2000a)
α2	No epidermal defects observed, wound healing not impaired	Chen *et al*. (2002)
		Holtkötter *et al*. (2002)

proportion, of occupied receptors regulates differentiation. The signal transduced by β1 integrins is an instruction not to differentiate (transduced by occupied receptors) rather than a positive signal to differentiate (transduced by unoccupied receptors). β1 subunits with point mutations inactivating one or both of the cytoplasmic domain NPXY motifs can still regulate differentiation even though they cannot target focal adhesions (Levy *et al*., 2000). Deletions affecting the juxtamembrane region are, however, inactive, and investigation of the signalling pathways affected by removal of this region should provide information as to the mechanism by which β1 integrins regulate differentiation.

Based on the *in vitro* experiments with human keratinocytes, one would predict that ablation of epidermal β1 integrins *in vivo* would stimulate the initiation of differentiation. Some of the mice in which the β1 gene is removed by crossing with keratin 5-Cre animals survive

beyond 6 weeks after birth (Brakebusch *et al*., 2000). They have severe hair loss due to reduced proliferation of hair matrix keratinocytes, and at 7 weeks lack hair follicles and sebaceous glands. Proliferation is also reduced in the interfollicular epidermis. The proportion of suprabasal, terminally differentiating keratinocytes is increased from 20 to 40%, although the differentiation process itself is executed largely normally. Interpreting the effects of β1 ablation is complicated by the fact that the mice display dermal fibrosis and inflammation, both of which can influence keratinocyte proliferation and differentiation. The β1 K14-Cre mice, which die shortly after birth, also show hair abnormalities, blistering and impaired epidermal proliferation; the differentiation programme itself is normal, but the proportion of differentiating cells has not been determined (Raghavan *et al*., 2000).

The primary effect of deletion of α6 or β4 is massive epidermal blistering, and those regions that remain attached to the basement membrane show normal differentiation (Georges-Labouesse *et al*., 1996; van der Neut *et al*., 1996). This is also the case in epidermis lacking both α6β4 and α3β1 (DiPersio *et al*., 2000a). Clusters of basal keratinocytes are found in the suprabasal layers of β4-null epidermis (Dowling *et al*., 1996). This might reflect clonal expansion of keratinocytes that have escaped detachment-induced cell death (Dowling *et al*., 1996); however, since similar clusters of displaced basal cells are found in the roof of newly formed epidermal suction blisters (Hertle *et al*., 1992), it may simply reflect catapulting of basal cells into the suprabasal layers as a result of disrupted basement membrane adhesion.

The conclusion from analysis of mice lacking β1 or α6β4 integrins is that β1 integrins are indeed important for normal epidermal proliferation, whereas the role of α6β4 is primarily one of anchorage. The finding that mice lacking the α3β1 and α6β4 integrins show no evidence of reduced epidermal proliferation or abnormal differentiation (DiPersio *et al*., 2000a) suggests that all the β1 integrins must be removed in order to decrease proliferation and increase the proportion of differentiating keratinocytes. The reasons why the β1-null keratinocytes do not all undergo spontaneous terminal differentiation, as occurs when cultured keratinocytes are held in suspension, could be that intact intercellular contacts are maintained *in vivo* and that a basement membrane, albeit an abnormal one, is present. *In vitro*, β1-null keratinocytes show greatly impaired adhesion to extracellular matrix proteins and a 5-fold higher proportion of terminally differentiating cells than β1-positive keratinocytes (Grose *et al*., 2002).

While there are clear parallels between epidermal terminal differentiation and apoptosis, the two processes are distinct and primary human keratinocytes do not undergo suspension-induced apoptosis, known as anoikis (Gandarillas *et al*., 1999). It has been suggested that in mouse epidermis, loss of β4 causes apoptosis (Dowling *et al*., 1996), but this has not been confirmed in α6-null epidermis or in epidermis lacking both α3 and α6 integrins (DiPersio *et al*., 2000a). Targeted deletion of all β1 integrins does not stimulate epidermal apoptosis either (Brakebusch *et al*., 2000). Although unligated integrins are reported to stimulate apoptosis in adherent cells by recruiting caspase-8 to the plasma membrane (Stupack *et al*., 2001), this is not seen in transgenic mouse epidermis in which a variety of integrins are expressed suprabasally in their unligated form (Carroll *et al*., 1995; Romero *et al*., 1999; Owens and Watt, 2001). Thus, integrins do not appear to play a significant role in regulating apoptosis within the epidermis, which is perhaps not surprising since cells that have left the basal layer in human skin remain viable and metabolically active for several days or weeks before being lost from the cell surface.

Stem cells

Stem cells are a subpopulation of keratinocytes that are responsible for renewing the epidermis throughout adult life, giving rise to the differentiating cells of the interfollicular epidermis, hair follicles and sebaceous glands (Watt, 2001). Stem cell daughters that have left the stem cell compartment can divide a small number of times prior to terminal differentiation and are known as transit-amplifying cells or committed progenitors.

Both in cultures of human keratinocytes and in human interfollicular epidermis, it is possible to enrich for stem cells by selecting the cells that have highest levels of β1 integrins (Jones and Watt, 1993; Jones *et al*., 1995; Jensen *et al*., 1999). In human hair follicles, there is also high β1 integrin expression in the region, known as the bulge, where stem cells are concentrated (Jones *et al*., 1995; Lyle *et al*., 1998; Akiyama *et al*., 2000). High β1 expression within the stem cell compartment is of functional significance for two reasons. First, if β1 expression and function are downregulated via a dominant-negative integrin mutation, the cells behave like transit-amplifying cells, differentiating within a few rounds of division (Zhu *et al*., 1999). β1 integrins and MAP kinase cooperate to maintain the epidermal stem cell compartment *in vitro* (Zhu *et al*., 1999). Secondly, high β1 integrin expression helps to maintain the patterned distribution of stem cells; stem cells are less motile than transit-amplifying cells and thus tend to remain clustered within the epidermal basal layer (Jensen *et al*., 1999). Human keratinocytes in culture that express high levels of β1 integrins also express high levels of α6β4 (Jones and Watt, 1993) and β1 integrin ablation in mouse keratinocytes reduces α6β4 expression (see for example Grose *et al*., 2002). However, opinions differ as to whether α6β4 can be used to enrich for human epidermal stem cells as effectively as β1 integrins (Jones and Watt, 1993; Li *et al*., 1998; Jensen *et al*., 1999).

In cultures of mouse keratinocytes, as in human (Jones and Watt, 1993), stem cells are more adhesive to extracellular matrix than transit-amplifying cells (Bickenbach and Chism, 1998). In mouse epidermis, β1 integrins are reported to be upregulated in the bulge region of the hair follicles (Huelsken *et al*., 2001). A decrease in β1 integrin expression is associated with c-Myc-mediated depletion of stem cells in mouse epidermis (Waikel *et al*., 2001) and with the inhibition of proliferation and stimulation of differentiation that results from inhibition of Ras (Dajee *et al*., 2002). High expression of α6β4 is also thought to be a marker of stem cells in mouse epidermis (Tani *et al*., 2000), although the functional significance of this is questionable, given that α6β4 ablation does not affect epidermal proliferation (van der Neut *et al*., 1996; DiPersio *et al*., 2000a). Conversely,

the reduced proliferation of epidermis lacking β1 integrins, the loss of hair follicles and impaired wound healing may all be indicative of depletion of the stem cell compartment, although detailed analysis remains to be carried out (Brakebusch *et al*., 2000; Raghavan *et al*., 2000; Grose *et al*, 2002).

Epidermal hyperproliferation and inflammation

Suprabasal integrin expression, which is a feature of hyperproliferative epidermis (Figure 1A), can contribute to the onset of psoriasis. This has been demonstrated by creating transgenic mice in which various integrin subunits are expressed under the control of the involucrin promoter (Carroll *et al*., 1995; Romero *et al*., 1999). The mice have sporadic epidermal hyperproliferation with accompanying histological features of psoriasis, including a lymphocytic infiltrate.

Recent experiments suggest a role for β1 integrin-mediated activation of the classical MAPK cascade in the pathogenesis of psoriasis (Haase *et al*., 2001; Figure 1B). In hyperproliferative wounded or psoriatic epidermis of human and mouse, but not in normal epidermis, MAPK is activated in basal and suprabasal keratinocytes. Constitutive activation of MAPK in cultured human keratinocytes results in increased proliferation and some of the abnormalities of terminal differentiation that are features of psoriasis. Ligation of suprabasal integrins activates MAPK, thus establishing that the receptors are capable of signal transduction. However, since most potential extracellular matrix ligands are not expressed suprabasally (Hertle *et al*., 1992), a second mechanism by which suprabasal integrins can activate MAPK, through stimulating keratinocytes to release interleukin (IL)-1α, may be more important (Haase *et al*., 2001; Figure 1B). This could explain the inflammation seen in affected transgenic mouse epidermis: IL-1α production by keratinocytes induces a dermal mononuclear infiltrate, leading to release of further cytokines and growth factors.

Even though suprabasal integrins are capable of activating MAPK directly or via stimulating release of IL-1α, examination of phenotypically normal transgenic epidermis establishes that suprabasal integrins do not activate MAPK constitutively (Haase *et al*., 2001). One explanation is that keratinocyte responsiveness to IL-1α does not correlate directly with the amount of IL-1α released (discussed by Haase *et al*., 2001). In addition, suprabasal integrins may sensitize the epidermis to other, as yet uncharacterized, environmental stimuli (Owens and Watt, 2001).

While suprabasal β1 expression may stimulate inflammation through IL-1α release (Haase *et al*., 2001), αvβ6 can contribute to skin inflammation via its interaction with transforming growth factor (TGF) β. β6-null mice have juvenile baldness and the degenerating hair follicles are surrounded by foci of monocytes and macrophages (Huang *et al*., 1996). αvβ6 binds and activates latent TGFβ1, thereby spatially restricting TGFβ1 activation (Munger *et al*., 1999). This, in turn, could have a profound effect on epidermal proliferation and skin inflammation.

Integrins and squamous cell carcinomas

Within the epidermis, it is the stem cells that have the greatest potential to found tumours. Stem cells are long-term residents and can thus accumulate multiple oncogenic mutations (Figure 1C), whereas transit-amplifying cells and their progeny are continually lost from the epidermis through terminal differentiation (see for example Brown *et al*., 1998; Jensen *et al*., 1999). Nevertheless, the differentiated keratinocytes of the epidermis do have a role to play in carcinogenesis as they can influence whether or not a potentially oncogenic clone of stem cell progeny expands to form a tumour or is held in check (Figure 1C).

Examination of human and mouse squamous cell carcinomas reveals considerable variation in integrin expression, both between tumours and in different regions of the same tumour. Normal expression, overexpression and focal or extensive loss of expression of the major keratinocyte integrins have all been observed, together with *de novo* expression of αvβ6 (see for example Jones *et al*., 1997; Bagutti *et al*., 1998). These changes could potentially influence growth and differentiation of the primary tumour and the ability of that tumour to invade and metastasize.

The integrin that has been most heavily implicated in epithelial carcinogenesis is α6β4 (Mercurio and Rabinovitz, 2001). Overexpression of α6β4, i.e. expression in suprabasal keratinocytes or keratinocytes that are not in the layer closest to the tumour stroma, is observed in human and mouse squamous cell carcinomas, correlating with poor prognosis in human oral cancer (van Waes *et al*., 1991) and with a high risk of malignant conversion in mouse carcinogenesis (Tennenbaum *et al*., 1993). Within a given tumour, both overexpression of α6β4 in the suprabasal layers and focal loss of α6β4 at the tumour margin can be observed, the latter pattern correlating with loss of the underlying basement membrane (Downer *et al*., 1993).

Expression of α6β4 is maintained in many invasive carcinomas in the absence of hemidesmosomes; in such cells it is associated with the actin cytoskeleton in areas of protrusive membrane activity (Mercurio and Rabinovitz, 2001). α6β4 is mobilized from the hemidesmosomes in response to chemotactic factors such as epidermal growth factor (EGF), and this is associated with increased phosphorylation of the cytoplasmic domain of β4 (Mainiero *et al*., 1996). α6β4 cooperates with growth factor receptors in activating PI3-K, and PI3-K activation is essential for invasion (Mercurio and Rabinovitz, 2001); other kinases are also be involved (Giancotti, 2000). In evaluating the role of α6β4 in tumours, attention must be paid not only to overall expression levels, but also to whether or not the integrin is in hemidesmosomes and to its ability to signal in both the ligated and non-ligated state (Mercurio and Rabinovitz, 2001).

The α6β4 studies have tended to emphasize the role of this integrin in promoting invasion by stimulating cell motility. However, another aspect of squamous cell carcinoma invasion is breakdown of the surrounding basement membrane, and there is some evidence that specific integrins influence matrix metalloproteinase expression by keratinocytes (DiPersio *et al*., 2000b;

Thomas *et al*., 2001). In addition to their effects on tumour invasion, integrins can influence the genesis of tumours and their differentiation status. Introduction of αv into a poorly differentiated αv-negative oral squamous cell carcinoma line results in cell surface expression of αvβ5 and inhibition of anchorage-independent growth (Jones *et al*., 1996b). In contrast, repair of a β4-negative tumour line did not stimulate differentiation (Jones *et al*., 1996a), although this is perhaps not surprising in view of the phenotype of α6β4-negative mice.

Since tumorigenesis is a multi-step process, the timing of altered integrin expression may be critical, and early changes may have a greater effect on the course of the disease than the pattern of integrin expression that characterizes a mature tumour. Evidence to support this comes from studies of the transgenic mice in which integrins are expressed suprabasally via the involucrin promoter (Figure 1C). The mice do not develop spontaneous tumours; however, integrin-specific effects become evident when tumours are induced by applying DMBA to initiate Ras mutations, followed by repeated treatments with phorbol ester to promote expansion of mutant clones. Benign tumours (papillomas) appear first; some of these regress, but others convert to malignant squamous cell carcinomas. Mice expressing transgenic α2β1 respond in the same way as wild-type animals (Owens and Watt, 2001). Mice expressing α3β1 develop papillomas at the same frequency as wild-type animals; however, the papillomas are more highly differentiated and show a decreased rate of conversion to malignant tumours. The mechanism of action of α3β1 is unknown, but there is correlative evidence that it involves suppression of the TM4SF protein CD81 (Owens and Watt, 2001). In contrast, overexpression of α6β4 increases papilloma and squamous cell carcinoma formation (D.M.Owens and F.M.Watt, in preparation). Thus, whereas stem cells are responsible for the genesis of most tumours, altered integrin expression in the differentiated cell layers can profoundly affect the course of the disease (Figure 1C).

Conclusions

From this review, it is clear that integrins do far more than simply anchor the epidermis to the underlying basement membrane. They are required for wound repair and contribute to skin inflammation. They regulate the balance between proliferation and differentiation, and perturbed integrin expression contributes to the pathogenesis of benign and neoplastic conditions. Ten years ago, scientists working in this area were largely preoccupied with identifying new integrins and cataloguing their expression; now the focus is on understanding how integrins exert their diverse functions in keratinocytes.

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