MOLECULAR AND CELLULAR BIOLOGY, Apr. 2006, p. 2877–2886 Vol. 26, No. 8
0270-7306/06/$08.00+0 doi:10.1128/MCB.26.8.2877–2886.2006

MINIREVIEW

Multiple Functions of the Integrin α6β4 in Epidermal Homeostasis and Tumorigenesis

Kevin Wilhelmsen,† Sandy H. M. Litjens,† and Arnoud Sonnenberg*

Division of Cell Biology, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands

Since the discovery of the α6β4 integrin in the late 1980s, our understanding of its role in providing stable adhesion of epithelial cells to basement membranes (BM) has significantly increased. α6β4 plays a key role in the formation and stabilization of junctional adhesion complexes called hemidesmosomes (HDs) that are connected to the intermediate filament (IF) system, as well as in the regulation of a variety of signaling processes. However, it is not clear as yet whether α6β4 participates in cell signaling by serving as a substrate for tyrosine kinases and as an adaptor for their associated signaling proteins or whether its role in cellular processes is passive, involving regulation of the assembly and disassembly of HDs. In this review, we will discuss the roles attributed to α6β4 and the controversies in the field.

HISTORY OF THE α6β4 INTEGRIN

The integrin α6β4 was discovered in the late 1980s by two different groups and was called either αEβ4 or Ic-Ic binding protein (Ic-IcBP) (36, 85). The Ic subunit had previously been shown to form a complex with glycoprotein IIa on platelets (86), which was subsequently identified as the common β1 subunit of the integrin family (63). Since the Ic subunit was immunologically and biochemically different from the five integrin α subunits known at that time, it was named α6 and the complex of α6 with β1 was called VLA-6 (30). Subsequently IcBP, which for an integrin subunit had the unusual size of approximately 200 kDa, was found to be identical to the β4 subunit of the αEβ4 complex (29). The discovery of the integrin α6β4 demonstrated that a particular α subunit can dimerize with more than one β subunit, a property that was then thought to be unique for β subunits. In further studies, the tumor antigens TSP-180 and A9 were found to be identical to α6β4 (39, 93). Increased expression of α6β4 and changes in its distribution were then correlated with increased aggressiveness of tumors and poor prognosis (15, 97). At the same time, α6β4 was also found to be a component of HDs (34, 84, 87). Although α6β4, like α6β1, can interact with different laminin isoforms, its preferred ligand in the epidermal BM is laminin-5 (4, 57, 71).

Sequencing of β4 revealed that its large size is due to an unusually long cytoplasmic domain of over 1,000 amino acids (31, 89). This domain contains two pairs of type III fibronectin (FNIII) domains, separated by a connecting segment (CS) (Fig. 1). A Na-Ca exchanger (CalX) motif precedes the first FNIII domain, but its function is still not clear (77). Importantly, α6β4 was found to be associated with keratin IFs instead of with actin like other integrins (26, 84). The association with IFs is mediated by the hemidesmosomal components plectin and BP230 (27, 58, 70). The importance of β4 for adhesion to the BM became evident in β4 knockout mice that developed severe blistering of the skin (14, 92). This was in line with findings, just prior to these studies, that a mutation in the β4 gene (*ITGB4*) is responsible for the pyloric atresia associated with junctional epidermolysis bullosa (EB) syndrome in humans. Like that of the knockout mice, the skin of these patients is fragile (94). More recent studies have elucidated the interactions between β4 and other hemidesmosomal components and revealed a potential role of β4 in signaling events associated with cell growth, survival, and migration under physiological and pathological conditions.

ROLE OF α6β4 IN HDs

Lessons from patients. Our current knowledge of the role of α6β4 in HD formation and organization derives from transfection, biochemical, and yeast two-hybrid assays. Furthermore, the analysis and dissection of the molecular consequences of the mutations in the α6, β4, plectin, or BP180 genes that have been identified in patients suffering from EB have greatly contributed to our understanding of HD assembly (Table 1). For example, mutations that result in R1225H and R1281W substitutions in β4 were found to disrupt the interaction with plectin (41). As a result, the adhesion of α6β4 to laminin-5 cannot be strengthened by an interaction with the cytoskeleton, leading to a nonlethal form of junctional EB (55, 64, 65). Similarly, absence or reduced expression of plectin also results in a congenital skin disease characterized by skin fragility and blistering (1, 20, 50, 82). Together, these findings demonstrate that the interaction between α6β4 and plectin is critical for proper HD assembly.

At least two sites on plectin and three on β4 mediate their interaction. The actin binding domain (ABD) of plectin binds to the first pair of FNIII domains and part of the CS of β4 (23). Binding of β4 and F-actin to the plectin ABD is mutually exclusive, probably because their binding sites overlap (23, 44). The β4-plectin-ABD interaction is enforced by the plectin plakin domain that binds to part of the CS together with the fourth FNIII domain and the C-terminal tail of β4 (42, 70). The importance of

* Corresponding author. Mailing address: Division of Cell Biology, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands. Phone: (31) 20-512-1942. Fax: (31) 20-512-1944. E-mail: a.sonnenberg@nki.nl.

† These two authors contributed equally to this study.

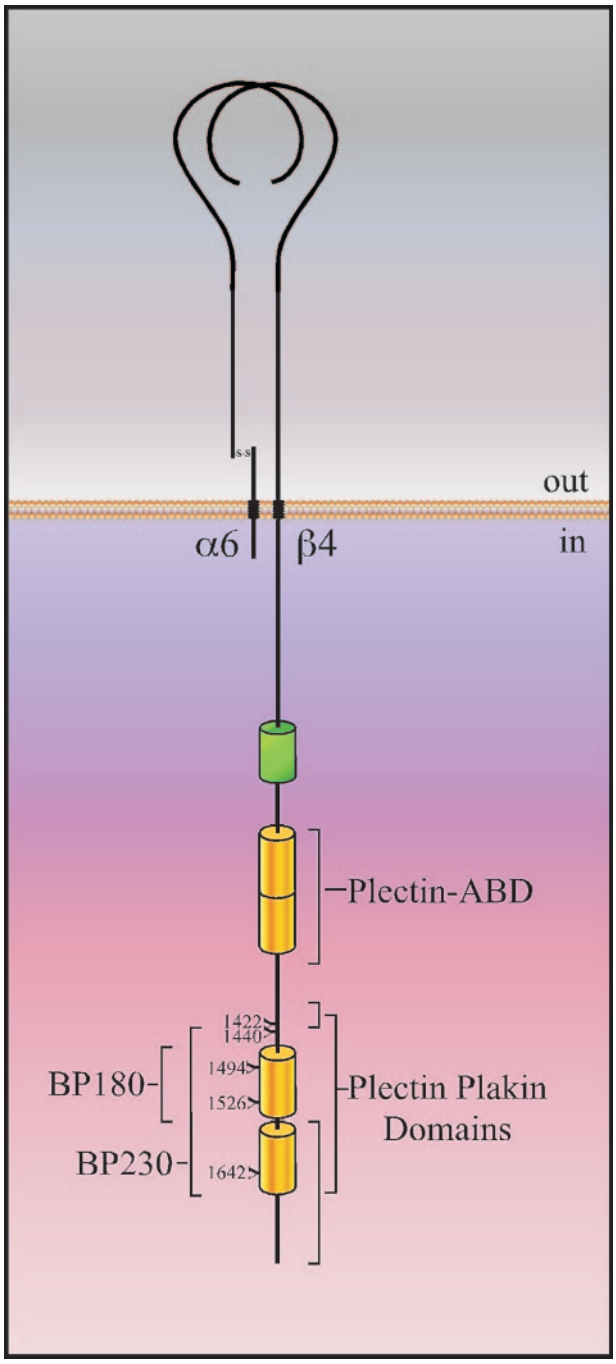

FIG. 1. Structure of the integrin α6β4. The structures of the α6 and β4 subunits are depicted. The positions of important tyrosine residues reported in the literature and the locations of the regions that are critical for the association with other hemidesmosomal components are shown. The yellow cylinders depict the four FNIII domains, numbered in ascending order from the plasma membrane, and the pink cylinder shows the location of the CalX motif.

this latter association is supported by the identification of different pathogenic mutations in patients with EB (Table 1).

Two other HD components, BP180 and BP230, bind to the third FNIII domain of β4 and to a region comprising the C-terminal end of the CS and the second pair of FNIII domains, respectively (40). One patient suffering from a mild form of EBS has a 50-amino-acid deletion in the third FNIII domain of β4, which presumably affects the association between β4 and BP180 (35). Likewise, another EBS patient carries a mutation causing the deletion of the region comprising amino acids 18 to 407 of BP180, which abrogates the binding to β4, plectin, and BP230 (17). Even though the mutant BP180 was localized in HDs, probably due to an interaction with the α6 subunit or a putative ligand in the epidermal BM, the assembly and stability of HDs were affected. The region in BP180 involved in binding to α6 (NC16A) contains a major epitope for autoantibodies in patients with bullous pemphigoid (76). These autoantibodies may impair the interaction between BP180 and α6β4, thereby destabilizing HDs (32). Furthermore, the α6 subunit of α6β4 interacts with the tetraspanin CD151 (88). Its function in HDs is not clear, but mutations in the CD151 gene are associated with skin fragility in humans (37). Interestingly, CD151 null mice do not show skin fragility, probably due to compensation by other molecules (98). To date, no mutations in BP230 and β4 that specifically disrupt their interaction have been found.

A hierarchical model for the assembly of HDs. The phenotypes that result from the lack of α6β4 in patients and mice clearly indicate that α6β4 is critical for the assembly of HDs. One of the first steps in HD assembly is the recruitment of plectin by α6β4, for which prior binding to the extracellular ligand laminin-5 is not needed (60, 72). Likewise, ligation of α6β4 by laminin-5 does not require binding of plectin (23, 41). As discussed in subsequent sections, the phosphorylation of β4 causes the disassembly of HDs; therefore, phosphatase activity may be required to initiate their assembly. The resulting change in conformation would allow the plectin ABD to interact with the first pair of FNIII domains on β4. When plectin is not recruited by α6β4, the localization of BP180 and BP230 is severely impaired. BP180 is only efficiently recruited when plectin is present. In turn, the incorporation of BP230 into a stable HD can only occur after the recruitment of BP180 (42). These results indicate that HD assembly entirely depends on α6β4 and that BP180 has no role in the initiation of this process (Fig. 2). However, in human skin, hemidesmosomal complexes containing BP180, BP230, and plectin were observed

TABLE 1. Mutations in hemidesmosomal components that affect protein-protein interactions

Protein (mutation)[a]	Protein-protein interaction(s) affected	Disease[b]	Reference
β4 (R1281W)	Plectin ABD	Nonlethal PA-JEB	64
β4 (R1225H)	Plectin ABD	Nonlethal PA-JEB	55
β4 (Q1714X)	Plectin plakin domain	Lethal EBS	55
β4 (Δ1450–1499)	BP180?	Nonlethal EBS	35
BP180 (Δ18–407)	β4, plectin, BP230	Nonlethal EBS	18
CD151 (Δ128–253)	α6	HN-PEB	37
Plectin (Δ893–895)	β4?	MD-EBS	65

[a] The canonical β4A subunit (89) and the plectin 1C variant (50) were taken as the basis for numbering the amino acids of β4 and plectin. In all cases, except where there is homozygosity for a single mutation (R1281W; Q1714X), there is a confounding effect by a second mutation in the gene, which determines whether the disease is lethal or not.

[b] PA-JEB, pyloric atresia associated with junctional epidermolysis bullosa; HN-PEB, hereditary nephritis associated with pretibial epidermolysis bullosa; MD-EBS, muscular dystrophy associated with epidermolysis bullosa simplex. In JEB the epidermis is separated from the dermis at the level of the basement membrane, whereas in EBS splitting occurs through the basal keratinocytes.

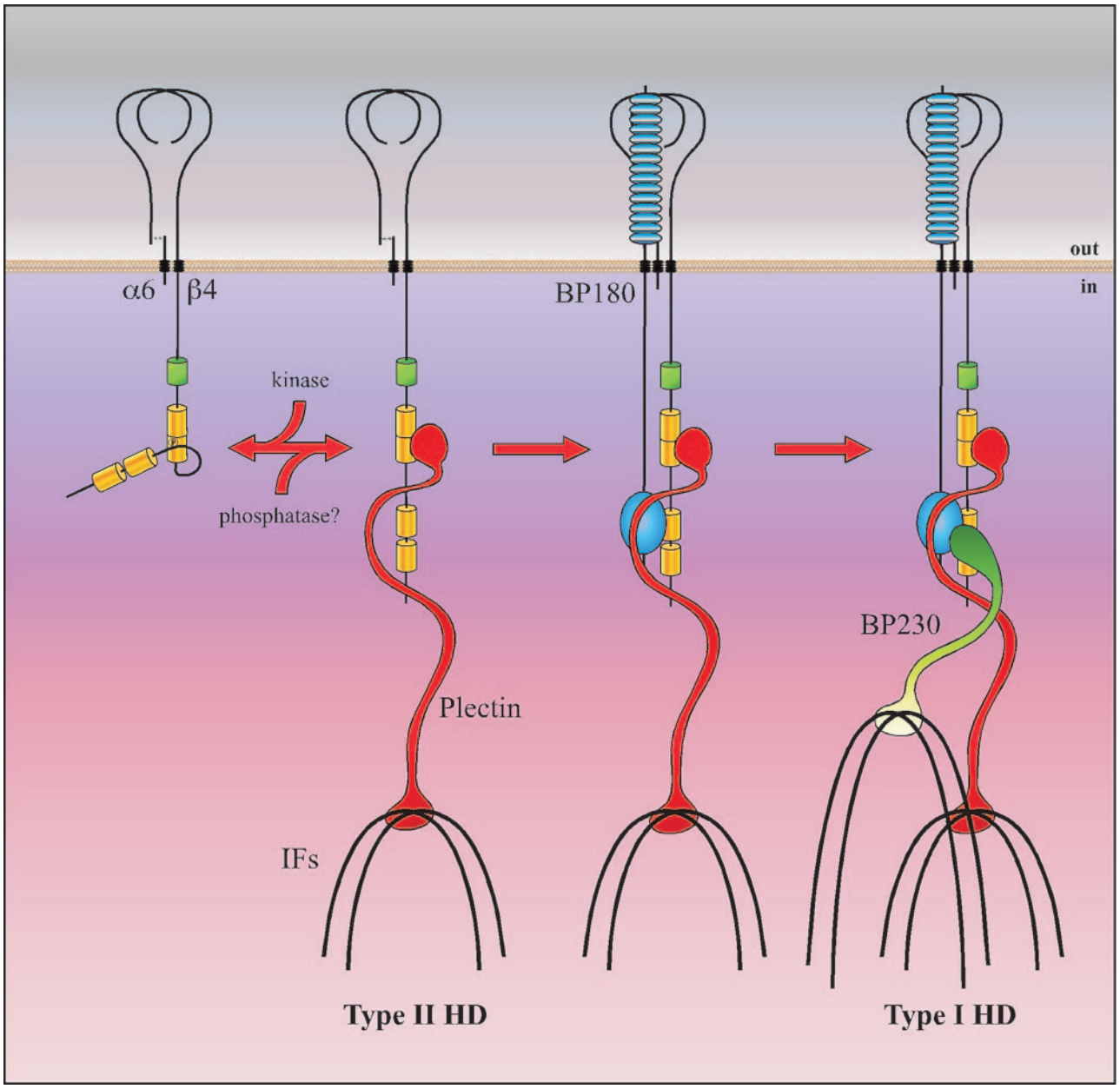

FIG. 2. Model of the hierarchical assembly of HDs. After the CS of β4 becomes dephosphorylated by an unidentified phosphatase, the plectin ABD is free to make associations with the first pair of FNIII domains on β4. The interaction of β4 and plectin is enforced by additional interactions between the plectin plakin domain and the β4 CS and the C-tail, which results in the formation of a type II HD. Once plectin is bound, BP180 is recruited into the complex, which makes associations with both the third FNIII domain of β4 and plectin. Lastly, a type I HD is formed once BP230 becomes incorporated into the complex through associations to both β4 and BP180. BP230 and plectin both can bind to IFs through an interaction site at their respective C termini.

in the absence of α6β4, suggesting an alternative means of HD assembly (59). Binding of BP180 to an as yet unidentified ligand in the BM may initiate the formation of these less robust hemidesmosomal complexes (40). At another level, the correct targeting of HD components to the basal plasma membrane must be regulated. A recent study in zebrafish identified penner/lethal giant larvae-2 (Lgl-2) as a critical regulator of HD assembly (83). The Lgl family currently consists of four members, several of which can bind to syntaxins that mediate fusion of exocytic vesicles with the basolateral plasma membrane (38, 54). Another molecule that may be involved in the correct targeting of HD components is ERBIN, which binds to both β4 and BP230 (16). The exact functions of these molecules in the transport of HD proteins to the plasma membrane still have to be clarified.

ROLE OF α6β4 IN BIOLOGICAL PROCESSES

Keratinocyte migration. In apparent contrast to its function in stable adhesion, α6β4 appears to promote migration signaling pathways that are either independent of or synergistic with

those that act downstream of growth factor receptors. Which function prevails depends on the cell type, or even the species, and on the experimental conditions. This makes it difficult to define a precise role of α6β4 in keratinocyte migration. Indeed, while one study showed that removal of β4 increased migration of immortalized mouse keratinocytes (69), another study reported that reexpression of β4 in β4-null human keratinocytes increased migration (72).

In an attempt to separate the adhesive and signaling functions of β4, immortalized murine keratinocytes that express β4 with a C-terminal deletion from residue 1355 ($\beta4^{\Delta1355}$) onwards were studied. The deleted region is not necessary for the high-affinity interaction with plectin (75) and hence, this β4 can still initiate the formation of HDs. Furthermore, this region of β4 is critical for downstream signaling events triggered by α6β4 ligation (46, 47). Therefore, $\beta4^{\Delta1355}$ should be unable to initiate promigratory signaling cascades. Indeed, migration of keratinocytes expressing $\beta4^{\Delta1355}$ was significantly decreased, as was wound healing in vivo (62). Another report showed that when either the binding of α6β4 to laminin-5 or its association with the IF system is compromised, the migration rate is higher than that of cells expressing wild-type α6β4 (24). These mutant β4 subunits contained the complete cytoplasmic domain, so they can initiate promigratory signaling cascades downstream of α6β4 but are unable to form robust HDs. These studies support the hypothesis that β4 can promote and hinder migration through signaling and adhesion, respectively.

Although HD disassembly is a prerequisite for cell migration, only a few reports have addressed this subject. Serine phosphorylation of the β4 CS has been implicated as a critical event in this process (67). For example, epidermal growth factor (EGF)-induced activation of protein kinase C and subsequent serine phosphorylation of β4 in cells plated on laminin-1 results in the mobilization of α6β4 from HDs to lamellipodia, where it associates with actin-rich protrusions (66). Activation of the Ron (MSP) receptor on keratinocytes plated on laminin-5 also resulted in a protein kinase C-dependent translocation of α6β4 to lamellipodia, where 14-3-3 proteins mediate association of α6β4 with the Ron receptor to ensure its sequestration from HDs (74). Both results suggest that serine phosphorylation of β4 promotes HD disassembly and translocation of α6β4 to lamellipodia, where it may help to stabilize these dynamic structures.

Rho family GTPases have an important role in cell migration, since they control the interplay between growth factor receptors, integrins, and the cytoskeleton. In keratinocytes that either are β4 negative or express an adhesion-defective β4 ($\beta4^{AD}$) or β4 without a cytoplasmic domain ($\beta4^{\Delta CD}$), EGF-induced Rac1 activity is weaker than in cells with wild-type β4 (72, 101). However, in keratinocytes expressing $\beta4^{\Delta1355}$, Rac1 activity is not affected by EGF, and in fact it is higher than in wild-type control cells in the absence of growth factor (62). These results suggest that EGF-induced Rac1 activity is positively regulated by integrin ligation and the regions in β4 upstream of residue 1355 but negatively regulated by regions downstream of this residue.

Another important integrin that binds laminin-5 is α3β1, which is associated with the actin cytoskeleton. The Rac1-dependent localization of α3β1 in lamellipodia is essential for their stabilization in migrating epithelial cells (6, 25). Although α3β1 can regulate Rac1 activity by itself via TIAM-1 (28), the results of the above studies suggest that growth factor-mediated HD disassembly and the α6β4-dependent increase in Rac1 activity promote the formation of lamellipodia and the localization of α3β1 to these structures. In lamellipodia, α3β1 binds to the newly deposited laminin-5, which results in a RhoA-dependent upregulation of laminin-5 and the formation of focal adhesions, which drives migration (56). Notably, the continuous deposition of laminin-5 beneath the migrating cell will result in a gradient of laminin-5 that may be critical for directional migration to occur (19). Therefore, Rac1 activity seems to be controlled by the concerted action of both α3β1 and α6β4.

Carcinoma invasion. Studies on the invasion of carcinoma cells, most of which were performed prior to those on keratinocyte migration, also revealed a role for α6β4-dependent activation of phosphatidylinositol (PI)-3 kinase, which is an upstream regulator of Rac1 (81). This suggests that invading carcinoma cells use migration mechanisms similar to those of keratinocytes (Fig. 3). Studies using breast carcinoma cells showed that tyrosine 1494 in the third FNIII domain of β4 is necessary for insulin receptor substrate (IRS) phosphorylation, binding of IRS to the p85 subunit of PI-3 kinase, and the subsequent activation of IRS after β4 cross-linking by monoclonal antibodies (MAbs). Moreover, cells expressing the $\beta4^{Y1494F}$ mutant displayed reduced invasion in assays in vitro (80). Despite the importance of understanding invasion, the mechanism by which IRS is activated downstream of tyrosine 1494 is not currently known.

Since growth factors activate enzymes that initiate the disassembly of HDs, the presence of overactive growth factor receptors may explain why α6β4 is unable to initiate HD formation in many carcinoma cells. Presumably, α6β4 can easily associate with the overactive tyrosine kinases under these circumstances, and the subsequent phosphorylation of β4 may help to amplify signaling pathways that promote invasion. In fact, α6β4 cooperates with several different tyrosine kinases in carcinoma cells to promote invasion, including c-met (hepatocyte growth factor [HGF] receptor). This receptor was reported to be constitutively associated with α6β4 and to induce tyrosine phosphorylation of β4 upon HGF stimulation (91). Moreover, expression of α6β4 in breast carcinoma cells was found to induce the formation of lung metastases in nude mice. However, since no cell lines expressing adhesion-defective β4 subunits were utilized, the metastases may be purely a result of α6β4-mediated adhesion to laminin-5 that is exposed in some areas of the lung (95). In contrast, a more exhaustive study has suggested that HGF promotes invasion of carcinomas independently of α6β4, and in fact, no association between α6β4 and c-met could be detected in cells from different carcinomas (8). Independent of its effect on invasion, a very recent study shows that β4 also cooperates with c-met in the transformation of rodent fibroblasts and is necessary to maintain the tumorigenic phenotype of carcinoma cells, for which tyrosines 1257, 1440, and 1494 proved to be essential (3). Interestingly, this study also shows that β4 has some transforming capacity by itself. Besides the supposed cooperation with c-met in the promotion of carcinoma invasion, α6β4 can stimulate the invasiveness of NIH 3T3 fibroblasts transformed by ErbB2 in a PI-3 kinase-dependent manner. Interestingly, PI-3 kinase activity was de-

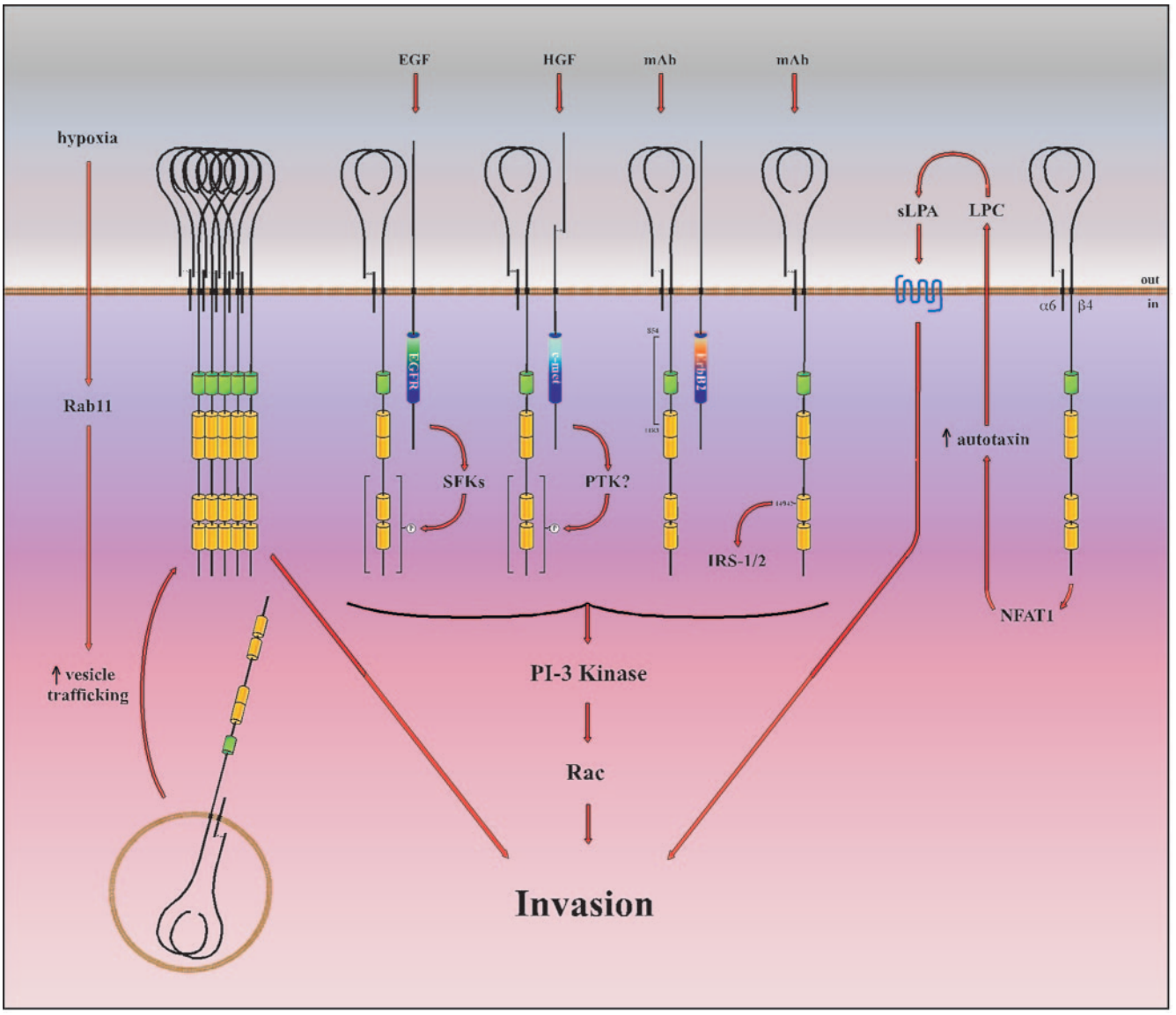

FIG. 3. In vitro carcinoma cell invasion. The various signaling pathways reported for α6β4-dependent invasion of carcinoma cells are shown. EGF receptor- and c-met-dependent invasion pathways rely upon the activation of PI-3 kinase, as does MAb cross-linking of the β4 subunit. Under hypoxic conditions, the upregulation of Rab11 results in the increased surface expression of α6β4, which has been reported to enhance invasion. Activation of α6β4 can also increase the extracellular expression of autotoxin, which results in the production of the proinvasive protein stearoly-lysophosphatidic acid (sLPA). PTK, protein-tyrosine kinase; LPC, lysophosphatidylcholine.

pendent not on tyrosine 1494 but on a region of β4 comprising the juxtamembrane residues 854 to 1183 (22). Upregulation of another Erb family member, ErbB1 (EGF receptor), has been implicated in the promotion of α6β4-dependent invasion of carcinomas through the activation of the Src family kinase (SFK) Fyn (49). Expression of a dominant-negative Fyn in transformed cells prevented β4 tyrosine phosphorylation in response to EGF and inhibited the invasion of cells expressing α6β4 in vitro, but whether the impaired invasion is directly a result of the decrease in β4 phosphorylation remains to be elucidated (49).

There is evidence that proteins other than tyrosine kinases can cooperate with α6β4 to promote invasion. For example, invasion of breast carcinoma cells is stimulated by promoting Rab11-mediated vesicular trafficking under hypoxic conditions (100). The increased trafficking results in increased surface expression of α6β4, but it is difficult to ascertain its relation with increased invasion since the levels of other proteins may also have increased. One intriguing study suggests that autotaxin is upregulated through the α6β4-dependent activation of the transcriptional regulator NFAT1 in breast carcinoma cells (5). Autotaxin generates lysophosphatidic acid, and stearoyl-lysophosphatidic acid has been shown to increase invasion by carcinomas (52). However, the mechanisms responsible for the α6β4-dependent upregulation of autotaxin have not been characterized.

In summary, the evidence for a correlation between α6β4 expression and invasion of carcinomas is strong. However, it is

not clear whether this invasion is regulated by signaling that is dependent on cooperation between α6β4 and growth factor receptors or by α6β4-mediated adhesion. In addition, in carcinoma cells tyrosine phosphorylation of β4 by activated kinases may allow β4 to function as an adaptor protein that amplifies proinvasive signaling cascades, while in normal keratinocytes, this function of β4 may be of less importance.

Keratinocyte survival. Studies using mouse models suggest that α6β4 does not directly influence the survival of normal keratinocytes in vivo (13, 62, 69). In β4 knockout and conditional β4 knockout mice, the detachment of the epidermis caused by the loss of HDs results in the apoptosis of basal keratinocytes; however, this is due to the total detachment of these cells from the underlying BM, and the decrease in cell survival cannot be attributed solely to the absence of α6β4 because signaling by other integrins necessary for survival is also compromised (14, 69, 92). However, the survival of keratinocytes expressing mutant forms of β4 is different in vitro. For example, keratinocytes expressing $\beta4^{\Delta1355}$, plated on laminin-5 under conditions of growth factor deprivation, undergo massive apoptosis. This does not occur in keratinocytes with wild-type β4 (62). Apparently, the region of β4 downstream of residue 1355 has a positive influence on cell survival. Also, inhibition of PI-3 kinase in growth factor-deprived primary keratinocytes expressing wild-type β4, plated on laminin-5, induces massive apoptosis (62). Consistently, Akt activation is decreased after laminin-5 ligation by keratinocytes expressing the $\beta4^{\Delta1355}$ mutant (61), suggesting that survival is mediated by the PI-3 kinase/Akt pathway. Although the role of β4 in keratinocyte survival in vivo is not evident, the results from the above studies suggest that under stress, α6β4 is involved in activation of this survival pathway in vitro.

Carcinoma survival. Similar to what is seen in normal keratinocytes under duress, Akt is activated downstream of α6β4 in carcinoma cells in vitro (Fig. 4). However, this activity and the resulting effect on cell survival are dependent on the status of p53. When p53 is functional, α6β4 stimulates apoptosis, but when p53 is nonfunctional or absent, it promotes cell survival (2, 9). p53 promotes apoptosis in α6β4-expressing carcinoma cells through the downregulation of Akt (2). Curiously, several studies even suggest that α6β4 may indirectly promote survival through enhanced expression of growth factors and cytokines. It was reported that tyrosine 1494 on β4 is critical for the upregulation of vascular endothelial growth factor (VEGF) in carcinoma cells, which was subsequently shown to be important for the survival of these cells (7, 43). Furthermore, in vitro ligation of α6β4 promotes the survival of thymocytes and the proliferation of thymic epithelial cells by increasing interleukin 6 (IL-6) production and secretion, which depends on the activation of p38 mitogen-activated protein (MAP) kinase and the transcriptional regulators NF-IL-6 and NF-κB (45, 68). α6β4-dependent activation of NF-κB is also the main mechanism for the promotion of cell survival in tumor acini, and, interestingly, survival has been linked to the ability of α6β4 to induce HDs and drive tissue polarity (96, 101). The implication of these findings is that the mechanism responsible for α6β4-dependent NF-κB activation cannot be accurately assessed in monolayer cultures (96). Taken together, α6β4 can regulate carcinoma survival through the interplay between the PI-3 kinase/Akt pathway and p53, while in polarized tumor tissues, the forma-

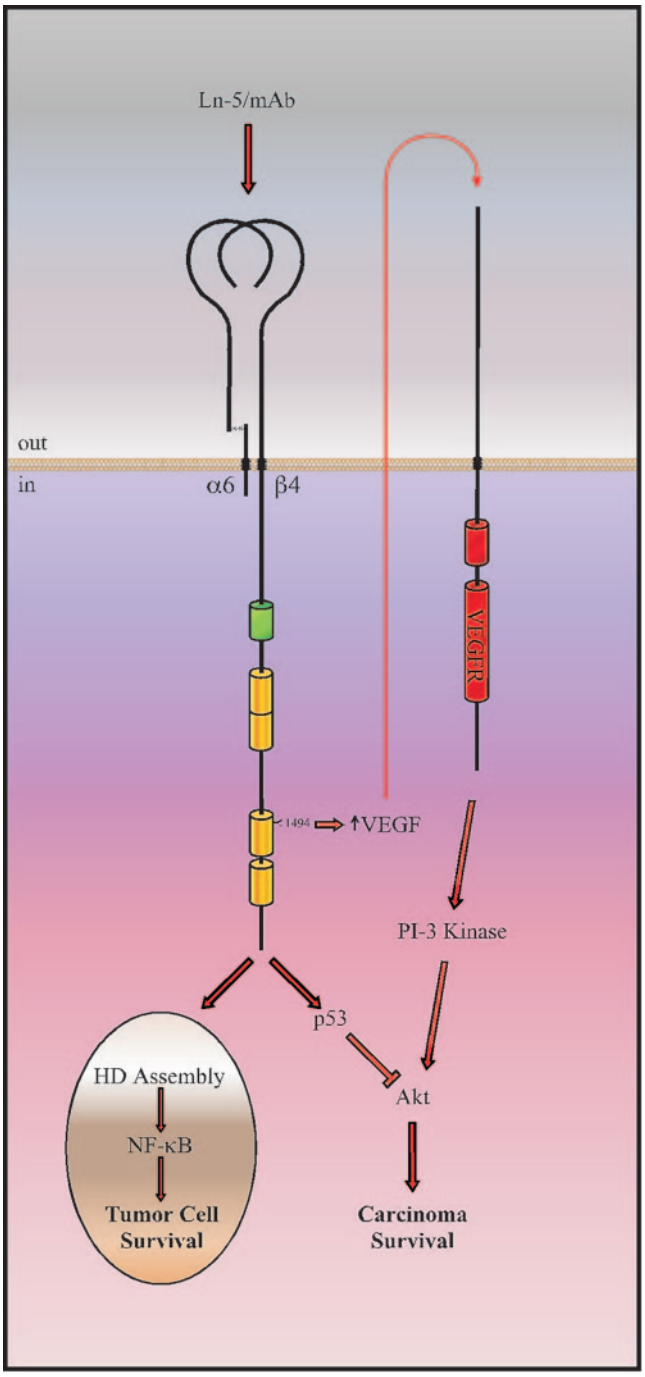

FIG. 4. In vitro carcinoma and tumor cell survival. Three major α6β4-dependent cancer cell survival mechanisms have been reported. Carcinoma survival has been reported to depend on the upregulation of VEGF downstream of tyrosine 1494 in β4. Exocytosis of VEGF results in the activation of its receptor, which leads to carcinoma survival through activation of the PI-3 kinase/Akt survival pathway. Interestingly, if p53 is functionally present, Akt is downregulated and carcinoma cells undergo apoptosis; this effect is also dependent upon α6β4 in these cells. In contrast to carcinomas, tumor cell survival depends on the formation of HDs and the activation of NF-κB. VEGFR, VEGF receptor.

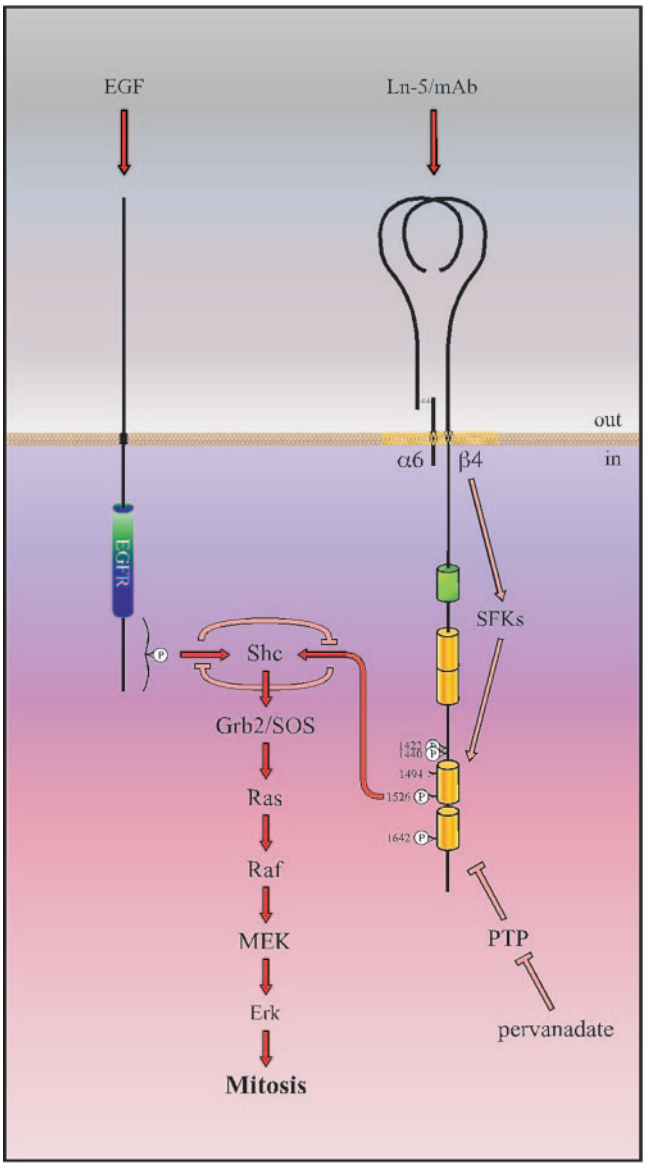

FIG. 5. In vitro keratinocyte mitosis. Laminin-5 ligation or MAb cross-linking of β4 (in lipid rafts) results in the activation and recruitment of Shc to tyrosine 1526 on β4. The data suggest that this event is sufficient to activate the classical MAP kinase cell cycle progression pathway. Importantly, the activated EGF receptor (EGFR) competes with tyrosine phosphorylated β4 for Shc binding and can independently induce mitosis. PTP, protein tyrosine phosphatase.

tion of HDs and the activation of NF-κB are the most important determinants for cell survival.

Mitosis. Studies to assess the role of α6β4 in cell cycle control were based on the activation (i.e., tyrosine phosphorylation) of β4 either by cross-linking with MAb, by plating on a laminin-5-rich matrix, or by pervanadate treatment of cells in tissue culture (Fig. 5). Cross-linking of β4 by MAbs on cells in suspension results in tyrosine phosphorylation of β4 and Shc and recruitment of Shc to β4. This activated Shc is associated with Grb2 and signals through Erk to promote progression through the cell cycle (11, 46–48). All studies undertaken to date suggest that phosphorylation of Y1526 in β4 is necessary and sufficient for Shc activation, recruitment of Shc to α6β4 through its phosphotyrosine binding domain, and stimulation of cell division by the classical MAP kinase pathway. However, the question of whether in vitro ligation and activation of α6β4 and the subsequent activation of the MAP kinase cascade are physiologically relevant remains. Therefore, the α6β4-dependent regulation of cell cycle progression in response to growth factors was studied in basal keratinocytes. Treatment with EGF results in the activation of the SFK member Fyn, which in turn can induce phosphorylation of β4 (49). Incidently, SFKs can also be activated after α6β4 clustering in lipid rafts (21). However, in contrast to the studies using β4 MAb cross-linking, EGF treatment did not cause the recruitment of Shc to β4 but instead inhibited the association of Shc with ligated α6β4, possibly due to competition for binding sites on the activated EGF receptor (48, 73). This suggests that Y1526 of β4 is not critical for mitosis in response to EGF, and this may be especially relevant for cells that overexpress the EGF receptor (i.e., A431), in which competition for binding Shc will be greater.

Interestingly, defects in Erk activation upon ligation on laminin-5 were observed in primary keratinocytes isolated from the $\beta4^{\Delta1355}$ transgenic mice (61). However, EGF-induced Erk activation was normal, which is consistent with the lack of effect of the deleted β4 subunit (69). The $\beta4^{\Delta1355}$-expressing keratinocytes did display defects in the translocation of active Erk to the nucleus after activation of the EGF receptor. Notably, MEK inhibitors had only a minimal effect on mitosis in the keratinocytes expressing wild-type β4, implying that the translocation of Erk to the nucleus is not critical for cell cycle progression in primary keratinocytes in vitro (62). The same study demonstrates that α6β4 also regulates the translocation of NF-κB to the nucleus and that this is important for cell cycle progression in primary keratinocytes plated on laminin-5 (62). However, there are considerable data that suggest NF-κB activation inhibits keratinocyte proliferation in both murine and human skin in vivo and in vitro (78, 79, 90, 102). In fact, the inhibition of NF-κB in the presence of activated Ras can trigger tumorigenesis in the human epidermis, which is dependent on α6β4 (10).

Studies with genetically modified mice suggest that the observations in vitro do not accurately reflect the in vivo situation. The mice displaying skin fragility (i.e., $\beta4^{-/-}$ and $\beta4^{\Delta CD}$ mice) and the mice without gross abnormalities in skin attachment (i.e., conditional knockout β4 and $\beta4^{\Delta1355}$ mice) showed no obvious defects in either the size of the pups or in the number of cells present in the epidermal layer compared to wild-type mice, as would be expected if cell cycle progression were impaired in cells incapable of initiating promitotic signaling events downstream of α6β4 (14, 53, 61, 69, 92). However, differences in keratinocyte proliferation were detected at the microscopic level in skin sections. The number of actively proliferating basal keratinocytes in $\beta4^{\Delta CD}$ and $\beta4^{\Delta1355}$ transgenic mice was low, while in the knockout and conditional knockout mice it was normal. The reason for this discrepancy is not clear; however, it was proposed that the difference might be due to the stage of embryogenesis or the postnatal period at which the proliferative potential of these cells was assessed (62). Taken together, the data show that growth factor-induced cell cycle progression in keratinocytes may not be wholly dependent on α6β4.

CONCLUDING REMARKS

When assessing the role of HDs in keratinocytes, it should be kept in mind that not all the components of the BM may be present in vitro and that some proteins typically found in type I HDs in vivo may be absent in vitro. For example, in the $\beta4^{\Delta1355}$-expressing keratinocytes, the HDs lack BP180 and BP230 because the binding sites for these type I HD components are absent, and therefore, these HDs are structurally more similar to the simpler type II HDs (33, 40, 62). The $\beta4^{\Delta1355}$ mutant also misses several binding sites that reinforce the β4-plectin interaction (42), so that HDs containing this mutant may even be less robust than type II HDs. Furthermore, α6β4 binds to the BM component netrin-1 (99). The function of this interaction has not yet been assessed and may represent another level of HD regulation in vivo that is not appreciated in cultured keratinocytes. It seems likely that the mechanisms responsible for the assembly and disassembly of type I HDs are more complex than those reported for the simpler type II HDs, because type I HDs contain additional proteins and more interactions that have to be regulated. Therefore, the effects of α6β4 on the regulation of cellular processes in keratinocytes in vitro may not accurately represent the function of α6β4 in vivo.

The different experimental conditions and cell types used in vitro also make it difficult to integrate all the data obtained regarding signaling events downstream of α6β4 in a comprehensive model. The task becomes even more daunting when trying to integrate all the in vitro findings obtained with genetically modified mice. One reason for this difficulty is that plating isolated keratinocytes on laminin-5 or MAb or cross-linking α6β4 by MAbs on cells in suspension does not accurately mimic the in vivo situation simply because the cells are not permanently attached to the BM and neighboring cells, whereas basal keratinocytes in vivo are. Also, other factors present in the extracellular matrix in vivo may regulate signaling pathways in basal keratinocytes that are dominant over or independent of the signaling adaptor function of α6β4. These notions may explain why observations in in vivo mouse models suggest that α6β4 has a modest effect on cell survival and mitosis in neonatal and adult mice. Murine knockin studies using point mutants of β4 (especially Y1494F and Y1526F) are critical to elucidate to what extent signaling downstream of α6β4 contributes to these cellular processes. The type I HDs in these mice are more likely to be similar to those in the wild-type steady-state situation, and therefore, the adhesive and signaling functions of α6β4 can be independently assessed.

Several growth factor receptors induce phosphorylation of β4. It seems likely that in aggressive carcinomas, which almost invariably show enhanced protein-tyrosine kinase signaling, the adaptor function of β4 is more important. In carcinoma cells, HDs have usually lost their structural integrity, which is due in part to a decrease in the expression levels of BP180 and 230, and α6β4 is often upregulated and no longer concentrated at the basal side in these cells (51). This may allow easier access of β4 to highly active kinases present all over the cell surface. Moreover, excessive amounts of laminin-5 that can ligate α6β4 in the absence of an organized BM are frequently secreted, and this may allow activation of α6β4-dependent signaling pathways that are not utilized in normal keratinocytes (12). These notions do not imply that α6β4 has no signaling function in normal keratinocytes. They only suggest that the effects from signaling cascades downstream of α6β4 may be much more subtle than is currently appreciated and that the adhesive function of α6β4 in HDs dominates any potential signaling function for α6β4 in normal keratinocytes in vivo.

ACKNOWLEDGMENTS

We thank Ed Roos, Anton Berns, Paul Engelfriet, Luca Borradori, Reinhard Fässler, and Johan de Rooij for critical reading of the manuscript.

REFERENCES

1. **Andra, K., H. Lassmann, R. Bittner, S. Shorny, R. Fassler, F. Propst, and G. Wiche.** 1997. Targeted inactivation of plectin reveals essential function in maintaining the integrity of skin, muscle, and heart cytoarchitecture. Genes Dev. **11:**3143–3156.
2. **Bachelder, R. E., M. J. Ribick, A. Marchetti, R. Falcioni, S. Soddu, K. R. Davis, and A. M. Mercurio.** 1999. p53 inhibits α6β4 integrin survival signaling by promoting the caspase 3-dependent cleavage of AKT/PKB. J. Cell Biol. **147:**1063–1072.
3. **Bertotti, A., P. M. Comoglio, and L. Trusolino.** 2005. β4 integrin is a transforming molecule that unleashes Met tyrosine kinase tumorigenesis. Cancer Res. **65:**10674–10679.
4. **Carter, W. G., M. C. Ryan, and P. J. Gahr.** 1991. Epiligrin, a new cell adhesion ligand for integrin α3β1 in epithelial basement membranes. Cell **65:**599–610.
5. **Chen, M., and K. L. O'Connor.** 2005. Integrin α6β4 promotes expression of autotaxin/ENPP2 autocrine motility factor in breast carcinoma cells. Oncogene **24:**5125–5130.
6. **Choma, D. P., K. Pumiglia, and C. M. DiPersio.** 2004. Integrin α3β1 directs the stabilization of a polarized lamellipodium in epithelial cells through activation of Rac1. J. Cell Sci. **117:**3947–3959.
7. **Chung, J., R. E. Bachelder, E. A. Lipscomb, L. M. Shaw, and A. M. Mercurio.** 2002. Integrin α6β4 regulation of eIF-4E activity and VEGF translation: a survival mechanism for carcinoma cells. J. Cell Biol. **158:**165–174.
8. **Chung, J., S. O. Yoon, E. A. Lipscomb, and A. M. Mercurio.** 2004. The Met receptor and α6β4 integrin can function independently to promote carcinoma invasion. J. Biol. Chem. **279:**32287–32293.
9. **Clarke, A. S., M. M. Lotz, C. Chao, and A. M. Mercurio.** 1995. Activation of the p21 pathway of growth arrest and apoptosis by the β4 integrin cytoplasmic domain. J. Biol. Chem. **270:**22673–22676.
10. **Dajee, M., M. Lazarov, J. Y. Zhang, T. Cai, C. L. Green, A. J. Russell, M. P. Marinkovich, S. Tao, Q. Lin, Y. Kubo, and P. A. Khavari.** 2003. NF-κB blockade and oncogenic Ras trigger invasive human epidermal neoplasia. Nature **421:**639–643.
11. **Dans, M., L. Gagnoux-Palacios, P. Blaikie, S. Klein, A. Mariotti, and F. G. Giancotti.** 2001. Tyrosine phosphorylation of the β4 integrin cytoplasmic domain mediates Shc signaling to extracellular signal-regulated kinase and antagonizes formation of hemidesmosomes. J. Biol. Chem. **276:**1494–1502.
12. **Davis, T. L., A. E. Cress, B. L. Dalkin, and R. B. Nagle.** 2001. Unique expression pattern of the α6β4 integrin and laminin-5 in human prostate carcinoma. Prostate **46:**240–248.
13. **DiPersio, C. M., R. van der Neut, E. Georges-Labouesse, J. A. Kreidberg, A. Sonnenberg, and R. O. Hynes.** 2000. α3β1 and α6β4 integrin receptors for laminin-5 are not essential for epidermal morphogenesis and homeostasis during skin development. J. Cell Sci. **113:**3051–3062.
14. **Dowling, J., Q. C. Yu, and E. Fuchs.** 1996. Beta4 integrin is required for hemidesmosome formation, cell adhesion and cell survival. J. Cell Biol. **134:**559–572.
15. **Falcioni, R., S. J. Kennel, P. Giacomini, G. Zupi, and A. Sacchi.** 1986. Expression of tumor antigen correlated with metastatic potential of Lewis lung carcinoma and B16 melanoma clones in mice. Cancer Res. **46:**5772–5778.
16. **Favre, B., L. Fontao, J. Koster, R. Shafaatian, F. Jaunin, J. H. Saurat, A. Sonnenberg, and L. Borradori.** 2001. The hemidesmosomal protein bullous pemphigoid antigen 1 and the integrin β4 subunit bind to ERBIN. Molecular cloning of multiple alternative splice variants of ERBIN and analysis of their tissue expression. J. Biol. Chem. **276:**32427–32436.
17. **Fontao, L., D. Geerts, I. Kuikman, J. Koster, D. Kramer, and A. Sonnenberg.** 2001. The interaction of plectin with actin: evidence for cross-linking of actin filaments by dimerization of the actin-binding domain of plectin. J. Cell Sci. **114:**2065–2076.
18. **Fontao, L., K. Tasanen, M. Huber, D. Hohl, J. Koster, L. Bruckner-Tuderman, A. Sonnenberg, and L. Borradori.** 2004. Molecular consequences of deletion of the cytoplasmic domain of bullous pemphigoid 180 in a patient with predominant features of epidermolysis bullosa simplex. J. Investig. Dermatol. **122:**65–72.

19. **Frank, D. E., and W. G. Carter.** 2004. Laminin 5 deposition regulates keratinocyte polarization and persistent migration. J. Cell Sci. **117:**1351–1363.
20. **Gache, Y., S. Chavanas, J. P. Lacour, G. Wiche, K. Owaribe, G. Meneguzzi, and J. P. Ortonne.** 1996. Defective expression of plectin/HD1 in epidermolysis bullosa simplex with muscular dystrophy. J. Clin. Investig. **97:**2289–2298.
21. **Gagnoux-Palacios, L., M. Dans, W. van't Hof, A. Mariotti, A. Pepe, G. Meneguzzi, M. D. Resh, and F. G. Giancotti.** 2003. Compartmentalization of integrin α6β4 signaling in lipid rafts. J. Cell Biol. **162:**1189–1196.
22. **Gambaletta, D., A. Marchetti, L. Benedetti, A. M. Mercurio, A. Sacchi, and R. Falcioni.** 2000. Cooperative signaling between α6β4 integrin and ErbB-2 receptor is required to promote phosphatidylinositol 3-kinase-dependent invasion. J. Biol. Chem. **275:**10604–10610.
23. **Geerts, D., L. Fontao, M. G. Nievers, R. Q. Schaapveld, P. E. Purkis, G. N. Wheeler, E. B. Lane, I. M. Leigh, and A. Sonnenberg.** 1999. Binding of integrin α6β4 to plectin prevents plectin association with F-actin but does not interfere with intermediate filament binding. J. Cell Biol. **147:**417–434.
24. **Geuijen, C. A., and A. Sonnenberg.** 2002. Dynamics of the α6β4 integrin in keratinocytes. Mol. Biol. Cell **13:**3845–3858.
25. **Goldfinger, L. E., S. B. Hopkinson, G. W. deHart, S. Collawn, J. R. Couchman, and J. C. Jones.** 1999. The α3 laminin subunit, α6β4 and α3β1 integrin coordinately regulate wound healing in cultured epithelial cells and in the skin. J. Cell Sci. **112:**2615–2629.
26. **Gomez, M., P. Navarro, M. Quintanilla, and A. Cano.** 1992. Expression of α6β4 integrin increases during malignant conversion of mouse epidermal keratinocytes: association of β4 subunit to the cytokeratin fraction. Exp. Cell Res. **201:**250–261.
27. **Guo, L., L. Degenstein, J. Dowling, Q. C. Yu, R. Wollmann, B. Perman, and E. Fuchs.** 1995. Gene targeting of BPAG1: abnormalities in mechanical strength and cell migration in stratified epithelia and neurologic degeneration. Cell **81:**233–243.
28. **Hamelers, I. H., C. Olivo, A. E. Mertens, D. M. Pegtel, R. A. van der Kammen, A. Sonnenberg, and J. Collard.** 2005. The Rac activator Tiam1 is required for α3β1-mediated laminin-5 deposition, cell spreading, and cell migration. J. Cell Biol. **171:**871–881.
29. **Hemler, M. E., C. Crouse, and A. Sonnenberg.** 1989. Association of the VLA α6 subunit with a novel protein. A possible alternative to the common VLA β1 subunit on certain cell lines. J. Biol. Chem. **264:**6529–6535.
30. **Hemler, M. E., C. Crouse, Y. Takada, and A. Sonnenberg.** 1988. Multiple very late antigen (VLA) heterodimers on platelets. Evidence for distinct VLA-2, VLA-5 (fibronectin receptor), and VLA-6 structures. J. Biol. Chem. **263:**7660–7665.
31. **Hogervorst, F., I. Kuikman, A. E. von dem Borne, and A. Sonnenberg.** 1990. Cloning and sequence analysis of β4 cDNA: an integrin subunit that contains a unique 118 kd cytoplasmic domain. EMBO J. **9:**765–770.
32. **Hopkinson, S. B., S. E. Baker, and J. C. Jones.** 1995. Molecular genetic studies of a human epidermal autoantigen (the 180-kD bullous pemphigoid antigen/BP180): identification of functionally important sequences within the BP180 molecule and evidence for an interaction between BP180 and α6 integrin. J. Cell Biol. **130:**117–125.
33. **Hopkinson, S. B., K. Findlay, G. W. deHart, and J. C. Jones.** 1998. Interaction of BP180 (type XVII collagen) and α6 integrin is necessary for stabilization of hemidesmosome structure. J. Investig. Dermatol. **111:**1015–1022.
34. **Jones, J. C., M. A. Kurpakus, H. M. Cooper, and V. Quaranta.** 1991. A function for the integrin α6β4 in the hemidesmosome. Cell Regul. **2:**427–438.
35. **Jonkman, M. F., H. H. Pas, M. Nijenhuis, G. Kloosterhuis, and G. Steege.** 2002. Deletion of a cytoplasmic domain of integrin β4 causes epidermolysis bullosa simplex. J. Investig. Dermatol. **119:**1275–1281.
36. **Kajiji, S., R. N. Tamura, and V. Quaranta.** 1989. A novel integrin (αEβ4) from human epithelial cells suggests a fourth family of integrin adhesion receptors. EMBO J. **8:**673–680.
37. **Karamatic Crew, V., N. Burton, A. Kagan, C. A. Green, C. Levene, F. Flinter, R. L. Brady, G. Daniels, and D. J. Anstee.** 2004. CD151, the first member of the tetraspanin (TM4) superfamily detected on erythrocytes, is essential for the correct assembly of human basement membranes in kidney and skin. Blood **104:**2217–2223.
38. **Katoh, M.** 2004. Identification and characterization of human LLGL4 gene and mouse Llgl4 gene in silico. Int. J. Oncol. **24:**737–742.
39. **Kennel, S. J., L. J. Foote, R. Falcioni, A. Sonnenberg, C. D. Stringer, C. Crouse, and M. E. Hemler.** 1989. Analysis of the tumor-associated antigen TSP-180. Identity with α6β4 in the integrin superfamily. J. Biol. Chem. **264:**15515–15521.
40. **Koster, J., D. Geerts, B. Favre, L. Borradori, and A. Sonnenberg.** 2003. Analysis of the interactions between BP180, BP230, plectin and the integrin α6β4 important for hemidesmosome assembly. J. Cell Sci. **116:**387–399.
41. **Koster, J., I. Kuikman, M. Kreft, and A. Sonnenberg.** 2001. Two different mutations in the cytoplasmic domain of the integrin β4 subunit in nonlethal forms of epidermolysis bullosa prevent interaction of β4 with plectin. J. Investig. Dermatol. **117:**1405–1411.
42. **Koster, J., S. van Wilpe, I. Kuikman, S. H. Litjens, and A. Sonnenberg.** 2004. Role of binding of plectin to the integrin β4 subunit in the assembly of hemidesmosomes. Mol. Biol. Cell **15:**1211–1223.
43. **Lipscomb, E. A., K. J. Simpson, S. R. Lyle, J. E. Ring, A. S. Dugan, and A. M. Mercurio.** 2005. The α6β4 integrin maintains the survival of human breast carcinoma cells in vivo. Cancer Res. **65:**10970–10976.
44. **Litjens, S. H., J. Koster, I. Kuikman, S. van Wilpe, J. M. de Pereda, and A. Sonnenberg.** 2003. Specificity of binding of the plectin actin-binding domain to β4 integrin. Mol. Biol. Cell **14:**4039–4050.
45. **Mainiero, F., M. Colombara, V. Antonini, R. Strippoli, M. Merola, O. Poffe, G. Tridente, and D. Ramarli.** 2003. p38 MAPK is a critical regulator of the constitutive and the β4 integrin-regulated expression of IL-6 in human normal thymic epithelial cells. Eur. J. Immunol. **33:**3038–3048.
46. **Mainiero, F., C. Murgia, K. K. Wary, A. M. Curatola, A. Pepe, M. Blumemberg, J. K. Westwick, C. J. Der, and F. G. Giancotti.** 1997. The coupling of α6β4 integrin to Ras-MAP kinase pathways mediated by Shc controls keratinocyte proliferation. EMBO J. **16:**2365–2375.
47. **Mainiero, F., A. Pepe, K. K. Wary, L. Spinardi, M. Mohammadi, J. Schlessinger, and F. G. Giancotti.** 1995. Signal transduction by the α6β4 integrin: distinct β4 subunit sites mediate recruitment of Shc/Grb2 and association with the cytoskeleton of hemidesmosomes. EMBO J. **14:**4470–4481.
48. **Mainiero, F., A. Pepe, M. Yeon, Y. Ren, and F. G. Giancotti.** 1996. The intracellular functions of α6β4 integrin are regulated by EGF. J. Cell Biol. **134:**241–253.
49. **Mariotti, A., P. A. Kedeshian, M. Dans, A. M. Curatola, L. Gagnoux-Palacios, and F. G. Giancotti.** 2001. EGF-R signaling through Fyn kinase disrupts the function of integrin α6β4 at hemidesmosomes: role in epithelial cell migration and carcinoma invasion. J. Cell Biol. **155:**447–458.
50. **McLean, W. H., L. Pulkkinen, F. J. Smith, E. L. Rugg, E. B. Lane, F. Bullrich, R. E. Burgeson, S. Amano, D. L. Hudson, K. Owaribe, J. A. McGrath, J. R. McMillan, R. A. Eady, I. M. Leigh, A. M. Christiano, and J. Uitto.** 1996. Loss of plectin causes epidermolysis bullosa with muscular dystrophy: cDNA cloning and genomic organization. Genes Dev. **10:**1724–1735.
51. **Mercurio, A. M., and I. Rabinovitz.** 2001. Towards a mechanistic understanding of tumor invasion—lessons from the α6β4 integrin. Semin. Cancer Biol. **11:**129–141.
52. **Mills, G. B., and W. H. Moolenaar.** 2003. The emerging role of lysophosphatidic acid in cancer. Nat. Rev. Cancer **3:**582–591.
53. **Murgia, C., P. Blaikie, N. Kim, M. Dans, H. T. Petrie, and F. G. Giancotti.** 1998. Cell cycle and adhesion defects in mice carrying a targeted deletion of the integrin β4 cytoplasmic domain. EMBO J. **17:**3940–3951.
54. **Musch, A., D. Cohen, C. Yeaman, W. J. Nelson, E. Rodriguez-Boulan, and P. J. Brennwald.** 2002. Mammalian homolog of Drosophila tumor suppressor lethal (2) giant larvae interacts with basolateral exocytic machinery in Madin-Darby canine kidney cells. Mol. Biol. Cell **13:**158–168.
55. **Nakano, A., L. Pulkkinen, D. Murrell, J. Rico, A. W. Lucky, M. Garzon, C. A. Stevens, S. Robertson, E. Pfendner, and J. Uitto.** 2001. Epidermolysis bullosa with congenital pyloric atresia: novel mutations in the β4 integrin gene (ITGB4) and genotype/phenotype correlations. Pediatr. Res. **49:**618–626.
56. **Nguyen, B. P., X. D. Ren, M. A. Schwartz, and W. G. Carter.** 2001. Ligation of integrin α3β1 by laminin 5 at the wound edge activates Rho-dependent adhesion of leading keratinocytes on collagen. J. Biol. Chem. **276:**43860–43870.
57. **Niessen, C. M., F. Hogervorst, L. H. Jaspars, A. A. de Melker, G. O. Delwel, E. H. Hulsman, I. Kuikman, and A. Sonnenberg.** 1994. The α6β4 integrin is a receptor for both laminin and kalinin. Exp. Cell Res. **211:**360–367.
58. **Niessen, C. M., E. H. Hulsman, E. S. Rots, P. Sanchez-Aparicio, and A. Sonnenberg.** 1997. Integrin α6β4 forms a complex with the cytoskeletal protein HD1 and induces its redistribution in transfected COS-7 cells. Mol. Biol. Cell **8:**555–566.
59. **Niessen, C. M., M. H. van der Raaij-Helmer, E. H. Hulsman, R. van der Neut, M. F. Jonkman, and A. Sonnenberg.** 1996. Deficiency of the integrin β4 subunit in junctional epidermolysis bullosa with pyloric atresia: consequences for hemidesmosome formation and adhesion properties. J. Cell Sci. **109:**1695–1706.
60. **Nievers, M. G., R. Q. Schaapveld, L. C. Oomen, L. Fontao, D. Geerts, and A. Sonnenberg.** 1998. Ligand-independent role of the β4 integrin subunit in the formation of hemidesmosomes. J. Cell Sci. **111:**1659–1672.
61. **Nikolopoulos, S. N., P. Blaikie, T. Yoshioka, W. Guo, and F. G. Giancotti.** 2004. Integrin β4 signaling promotes tumor angiogenesis. Cancer Cell **6:**471–483.
62. **Nikolopoulos, S. N., P. Blaikie, T. Yoshioka, W. Guo, C. Puri, C. Tacchetti, and F. G. Giancotti.** 2005. Targeted deletion of the integrin β4 signaling domain suppresses laminin-5-dependent nuclear entry of mitogen-activated protein kinases and NF-κB, causing defects in epidermal growth and migration. Mol. Cell. Biol. **25:**6090–6102.
63. **Pischel, K. D., H. G. Bluestein, and V. L. Woods, Jr.** 1988. Platelet glycoproteins Ia, Ic, and IIa are physicochemically indistinguishable from the very late activation antigens adhesion-related proteins of lymphocytes and other cell types. J. Clin. Investig. **81:**505–513.

64. **Pulkkinen, L., F. Rouan, L. Bruckner-Tuderman, R. Wallerstein, M. Garzon, T. Brown, L. Smith, W. Carter, and J. Uitto.** 1998. Novel ITGB4 mutations in lethal and nonlethal variants of epidermolysis bullosa with pyloric atresia: missense versus nonsense. Am. J. Hum. Genet. **63:**1376–1387.
65. **Pulkkinen, L., F. J. Smith, H. Shimizu, S. Murata, H. Yaoita, H. Hachisuka, T. Nishikawa, W. H. McLean, and J. Uitto.** 1996. Homozygous deletion mutations in the plectin gene (PLEC1) in patients with epidermolysis bullosa simplex associated with late-onset muscular dystrophy. Hum. Mol. Genet. **5:**1539–1546.
66. **Rabinovitz, I., A. Toker, and A. M. Mercurio.** 1999. Protein kinase C-dependent mobilization of the α6β4 integrin from hemidesmosomes and its association with actin-rich cell protrusions drive the chemotactic migration of carcinoma cells. J. Cell Biol. **146:**1147–1160.
67. **Rabinovitz, I., L. Tsomo, and A. M. Mercurio.** 2004. Protein kinase C-α phosphorylation of specific serines in the connecting segment of the β4 integrin regulates the dynamics of type II hemidesmosomes. Mol. Cell. Biol. **24:**4351–4360.
68. **Ramarli, D., M. T. Scupoli, E. Fiorini, O. Poffe, M. Brentegani, A. Villa, G. Cecchini, G. Tridente, and P. C. Marchisio.** 1998. Thymocyte contact or monoclonal antibody-mediated clustering of α3β1 or α6β4 integrins activate interleukin-6 (IL-6) transcription factors (NF-κB and NF-IL6) and IL-6 production in human thymic epithelial cells. Blood **92:**3745–3755.
69. **Raymond, K., M. Kreft, H. Janssen, J. Calafat, and A. Sonnenberg.** 2005. Keratinocytes display normal proliferation, survival and differentiation in conditional β4-integrin knockout mice. J. Cell Sci. **118:**1045–1060.
70. **Rezniczek, G. A., J. M. de Pereda, S. Reipert, and G. Wiche.** 1998. Linking integrin α6β4-based cell adhesion to the intermediate filament cytoskeleton: direct interaction between the β4 subunit and plectin at multiple molecular sites. J. Cell Biol. **141:**209–225.
71. **Rousselle, P., G. P. Lunstrum, D. R. Keene, and R. E. Burgeson.** 1991. Kalinin: an epithelium-specific basement membrane adhesion molecule that is a component of anchoring filaments. J. Cell Biol. **114:**567–576.
72. **Russell, A. J., E. F. Fincher, L. Millman, R. Smith, V. Vela, E. A. Waterman, C. N. Dey, S. Guide, V. M. Weaver, and M. P. Marinkovich.** 2003. α6β4 integrin regulates keratinocyte chemotaxis through differential GTPase activation and antagonism of α3β1 integrin. J. Cell Sci. **116:**3543–3556.
73. **Sakaguchi, K., Y. Okabayashi, Y. Kido, S. Kimura, Y. Matsumura, K. Inushima, and M. Kasuga.** 1998. Shc phosphotyrosine-binding domain dominantly interacts with epidermal growth factor receptors and mediates Ras activation in intact cells. Mol. Endocrinol. **12:**536–543.
74. **Santoro, M. M., G. Gaudino, and P. C. Marchisio.** 2003. The MSP receptor regulates α6β4 and α3β1 integrins via 14-3-3 proteins in keratinocyte migration. Dev. Cell **5:**257–271.
75. **Schaapveld, R. Q., L. Borradori, D. Geerts, M. R. van Leusden, I. Kuikman, M. G. Nievers, C. M. Niessen, R. D. Steenbergen, P. J. Snijders, and A. Sonnenberg.** 1998. Hemidesmosome formation is initiated by the β4 integrin subunit, requires complex formation of β4 and HD1/plectin, and involves a direct interaction between β4 and the bullous pemphigoid antigen 180. J. Cell Biol. **142:**271–284.
76. **Schachter, M., J. C. Brieva, J. C. Jones, D. Zillikens, C. Skrobek, and L. S. Chan.** 2001. Pemphigoid nodularis associated with autoantibodies to the NC16A domain of BP180 and a hyperproliferative integrin profile. J. Am. Acad. Dermatol. **45:**747–754.
77. **Schwarz, E. M., and S. Benzer.** 1997. *Calx*, a Na-Ca exchanger gene of *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **94:**10249–10254.
78. **Seitz, C. S., H. Deng, K. Hinata, Q. Lin, and P. A. Khavari.** 2000. Nuclear factor κB subunits induce epithelial cell growth arrest. Cancer Res. **60:** 4085–4092.
79. **Seitz, C. S., Q. Lin, H. Deng, and P. A. Khavari.** 1998. Alterations in NF-κB function in transgenic epithelial tissue demonstrate a growth inhibitory role for NF-κB. Proc. Natl. Acad. Sci. USA **95:**2307–2312.
80. **Shaw, L. M.** 2001. Identification of insulin receptor substrate 1 (IRS-1) and IRS-2 as signaling intermediates in the α6β4 integrin-dependent activation of phosphoinositide 3-OH kinase and promotion of invasion. Mol. Cell. Biol. **21:**5082–5093.
81. **Shaw, L. M., I. Rabinovitz, H. H. Wang, A. Toker, and A. M. Mercurio.** 1997. Activation of phosphoinositide 3-OH kinase by the α6β4 integrin promotes carcinoma invasion. Cell **91:**949–960.
82. **Smith, F. J., R. A. Eady, I. M. Leigh, J. R. McMillan, E. L. Rugg, D. P. Kelsell, S. P. Bryant, N. K. Spurr, J. F. Geddes, G. Kirtschig, G. Milana, A. G. de Bono, K. Owaribe, G. Wiche, L. Pulkkinen, J. Uitto, W. H. McLean, and E. B. Lane.** 1996. Plectin deficiency results in muscular dystrophy with epidermolysis bullosa. Nat. Genet. **13:**450–457.
83. **Sonawane, M., Y. Carpio, R. Geisler, H. Schwarz, H. M. Maischein, and C. Nuesslein-Volhard.** 2005. Zebrafish penner/lethal giant larvae 2 functions in hemidesmosome formation, maintenance of cellular morphology and growth regulation in the developing basal epidermis. Development **132:** 3255–3265.
84. **Sonnenberg, A., J. Calafat, H. Janssen, H. Daams, L. M. van der Raaij-Helmer, R. Falcioni, S. J. Kennel, J. D. Aplin, J. Baker, M. Loizidou, et al.** 1991. Integrin α6β4 complex is located in hemidesmosomes, suggesting a major role in epidermal cell-basement membrane adhesion. J. Cell Biol. **113:**907–917.
85. **Sonnenberg, A., F. Hogervorst, A. Osterop, and F. E. Veltman.** 1988. Identification and characterization of a novel antigen complex on mouse mammary tumor cells using a monoclonal antibody against platelet glycoprotein Ic. J. Biol. Chem. **263:**14030–14038.
86. **Sonnenberg, A., H. Janssen, F. Hogervorst, J. Calafat, and J. Hilgers.** 1987. A complex of platelet glycoproteins Ic and IIa identified by a rat monoclonal antibody. J. Biol. Chem. **262:**10376–10383.
87. **Stepp, M. A., S. Spurr-Michaud, A. Tisdale, J. Elwell, and I. K. Gipson.** 1990. α6β4 integrin heterodimer is a component of hemidesmosomes. Proc. Natl. Acad. Sci. USA **87:**8970–8974.
88. **Sterk, L. M., C. A. Geuijen, L. C. Oomen, J. Calafat, H. Janssen, and A. Sonnenberg.** 2000. The tetraspan molecule CD151, a novel constituent of hemidesmosomes, associates with the integrin α6β4 and may regulate the spatial organization of hemidesmosomes. J. Cell Biol. **149:**969–982.
89. **Suzuki, S., and Y. Naitoh.** 1990. Amino acid sequence of a novel integrin β4 subunit and primary expression of the mRNA in epithelial cells. EMBO J. **9:**757–763.
90. **Takao, J., T. Yudate, A. Das, S. Shikano, M. Bonkobara, K. Ariizumi, and P. D. Cruz.** 2003. Expression of NF-κB in epidermis and the relationship between NF-κB activation and inhibition of keratinocyte growth. Br. J. Dermatol. **148:**680–688.
91. **Trusolino, L., A. Bertotti, and P. M. Comoglio.** 2001. A signaling adapter function for α6β4 integrin in the control of HGF-dependent invasive growth. Cell **107:**643–654.
92. **van der Neut, R., P. Krimpenfort, J. Calafat, C. M. Niessen, and A. Sonnenberg.** 1996. Epithelial detachment due to absence of hemidesmosomes in integrin β4 null mice. Nat. Genet. **13:**366–369.
93. **Van Waes, C., K. F. Kozarsky, A. B. Warren, L. Kidd, D. Paugh, M. Liebert, and T. E. Carey.** 1991. The A9 antigen associated with aggressive human squamous carcinoma is structurally and functionally similar to the newly defined integrin α6β4. Cancer Res. **51:**2395–2402.
94. **Vidal, F., D. Aberdam, C. Miquel, A. M. Christiano, L. Pulkkinen, J. Uitto, J. P. Ortonne, and G. Meneguzzi.** 1995. Integrin β4 mutations associated with junctional epidermolysis bullosa with pyloric atresia. Nat. Genet. **10:** 229–234.
95. **Wang, H., W. Fu, J. H. Im, Z. Zhou, S. A. Santoro, V. Iyer, C. M. DiPersio, Q. C. Yu, V. Quaranta, A. Al-Mehdi, and R. J. Muschel.** 2004. Tumor cell α3β1 integrin and vascular laminin-5 mediate pulmonary arrest and metastasis. J. Cell Biol. **164:**935–941.
96. **Weaver, V. M., S. Lelievre, J. N. Lakins, M. A. Chrenek, J. C. Jones, F. Giancotti, Z. Werb, and M. J. Bissell.** 2002. β4 integrin-dependent formation of polarized three-dimensional architecture confers resistance to apoptosis in normal and malignant mammary epithelium. Cancer Cell **2:**205–216.
97. **Wolf, G. T., T. E. Carey, S. P. Schmaltz, K. D. McClatchey, J. Poore, L. Glaser, D. J. Hayashida, and S. Hsu.** 1990. Altered antigen expression predicts outcome in squamous cell carcinoma of the head and neck. J. Natl. Cancer Inst. **82:**1566–1572.
98. **Wright, M. D., S. M. Geary, S. Fitter, G. W. Moseley, L.-M. Lau, K.-C. Sheng, V. Apostolopoulos, E. G. Stanley, D. E. Jackson, and L. K. Ashman.** 2004. Characterization of mice lacking the tetraspanin superfamily member CD151. Mol. Cell. Biol. **24:**5978–5988.
99. **Yebra, M., A. M. Montgomery, G. R. Diaferia, T. Kaido, S. Silletti, B. Perez, M. L. Just, S. Hildbrand, R. Hurford, E. Florkiewicz, M. Tessier-Lavigne, and V. Cirulli.** 2003. Recognition of the neural chemoattractant Netrin-1 by integrins α6β4 and α3β1 regulates epithelial cell adhesion and migration. Dev. Cell **5:**695–707.
100. **Yoon, Y., and M. A. McNiven.** 2001. Mitochondrial division: new partners in membrane pinching. Curr. Biol. **11:**R67–R70.
101. **Zahir, N., J. N. Lakins, A. Russell, W. Ming, C. Chatterjee, G. I. Rozenberg, M. P. Marinkovich, and V. M. Weaver.** 2003. Autocrine laminin-5 ligates α6β4 integrin and activates RAC and NF-κB to mediate anchorage-independent survival of mammary tumors. J. Cell Biol. **163:**1397–1407.
102. **Zhang, J. Y., C. L. Green, S. Tao, and P. A. Khavari.** 2004. NF-κB RelA opposes epidermal proliferation driven by TNFR1 and JNK. Genes Dev. **18:**17–22.