The α3 laminin subunit, α6β4 and α3β1 integrin coordinately regulate wound healing in cultured epithelial cells and in the skin

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SUMMARY

Previously, we demonstrated that proteolytic processing within the globular domain of the α3 subunit of laminin-5 (LN5) converts LN5 from a cell motility-inducing factor to a protein complex that can trigger the formation of hemidesmosomes, certain cell-matrix attachment sites found in epithelial cells. We have prepared a monoclonal antibody (12C4) whose epitope is located toward the carboxy terminus of the globular domain of the α3 laminin subunit. This epitope is lost from the α3 subunit as a consequence of proteolytic processing. Antibody 12C4 stains throughout the matrix of cells that fail to process the α3 laminin subunit, but does not recognize the matrix of confluent cultures of MCF-10A cells, which efficiently process their α3 laminin chain. In subconfluent populations of MCF-10A cells, 12C4 only stains matrix deposited at the outer edges of cell colonies. In these cells, integrin α3β1 occasionally colocalizes with the staining generated by the 12C4 antibody but α6β4 integrin does not. In wounded MCF-10A cell cultures, the 12C4 antibody stains the extracellular matrix beneath those cells at the very edge of the cellular sheet that moves to cover the wound site. A similar phenomenon is observed in human skin wounds, since we also detect expression of the unprocessed α3 laminin subunit at the leading tip of the sheet of epidermal cells that epithelializes skin wounds in vivo. In addition, using α3 laminin subunit and integrin function-inhibiting antibodies, we provide evidence that LN5 and its two integrin receptors (α6β4 and α3β1) appear necessary for wound healing to occur in MCF-10A cell culture wounds. We propose a model for healing of wounded epithelial tissues based on these results.

Key words: Matrix, Proteolysis, Cell adhesion, Hemidesmosome, Epidermal repair

INTRODUCTION

Basal cells in stratified epithelial tissues are intimately associated with the basement membrane, a thin acellular layer that separates the epithelium from the connective tissue. The basement membrane is a dense network of complex macromolecules including collagens, proteoglycans, laminins, vitronectin, fibronectin and hyaluronic acids (Kleinman et al., 1991; Kuhn, 1994; Marinkovich et al., 1993a; Timpl et al., 1979; Timpl, 1996; Uitto and Pulkkinen, 1996). Originally the basement membrane was considered to be little more than structural support for the epithelial tissue. However, basement membrane proteins are now considered to be involved in the regulation of a variety of epithelial cell processes such as migration, proliferation, differentiation and adhesion (Beattie et al., 1996; Blaschke et al., 1994; Fuchs, 1990; Hay, 1993; Zieske et al., 1994).

One of the major matrix components of epithelial basement membranes is an isoform of laminin called laminin-5 (LN5; epiligrin/kalinin/nicein/ldasin) (Carter et al., 1991; Marinkovich et al., 1993b; Masunaga et al., 1996; Rousselle et al., 1991; Vailly et al., 1994). The LN5 molecule is a heterotrimeric glycoprotein that adopts a cruciform shape following assembly from its α3, β3 and γ2 subunits (Rouselle et al., 1991; Baker et al., 1996). The C terminus of the α3 subunit provides LN5 with a large globular domain, called the G domain (Ryan et al., 1994; Galliano et al., 1995; Baker et al., 1996). This region of the molecule is believed to be the binding site for both the α3β1 and α6β4 integrins (Baker et al., 1996; Mizushima et al., 1997). Furthermore, the G domain appears to be essential for the ability of LN5, via its association with α6β4 integrin, to induce formation of a multi-protein membrane-spanning adhesive device called the hemidesmosome in epithelial cells (Baker et al., 1996; Jones et al., 1991, 1998; Langhofer et al., 1994). Hemidesmosomes are considered to enforce stable attachment of basal cells to the basement membrane in stratified epithelia such as skin, tongue, cornea and bladder, as well as in some simple epithelial tissues (Green and Jones, 1996; Jones et al., 1998; Langhofer et al., 1994; Owaribe et al., 1991).

Although many epithelial tissues rely on hemidesmosomes for stabilizing their interaction with connective tissue, there are
occasions in which anchorage of basal cells to the basement membrane via these structures is undesirable, or at least requires regulation. A good example of this is wound healing in the skin. It has been shown that keratinocytes distal from the wound margin experience a spurt of proliferation, while the keratinocytes at the wound edge quickly migrate over the wound space and provide a barrier layer, which allows the underlying tissue to heal. To initiate migration, the latter cells first, presumably, must disassemble their hemidesmosomes, remodel their matrix interactions, and then crawl from the margins of the wound over a provisional extracellular matrix, which is deposited on the wound bed and which includes LN5 (Cavani et al., 1993; Garlick and Taichman, 1994; Haapasalmi et al., 1996; Rainulainen et al., 1998).

We have shown previously that LN5 containing an unprocessed α3 subunit promotes the migration of epithelial cells (Goldfinger et al., 1998). Furthermore, by proteolytically cleaving the α3 subunit in vitro, we have been able to modulate LN5 matrix function such that it not only impedes cell migration but also becomes competent to induce assembly of hemidesmosomes (Goldfinger et al., 1998). In this study we have investigated the relative distribution of unprocessed laminin α3 subunit and the two integrin receptors for LN5 (α3β1 and α6β4) in epithelial cells. We provide data indicating that unprocessed α3 laminin subunit is present beneath the leading edge of migrating epithelial cells and is a component of the provisional wound matrix in cultured cells and in skin tissue. We also present experimental evidence, using a cell culture model, that the α3 laminin subunit as well as α3β1 and α6β4 integrin play an important role in epithelial migration and wound closure.

MATERIALS AND METHODS

Cell culture

The human breast epithelial cell line MCF-10A, obtained from the American Tissue Culture Collection (Rockville, MD), was maintained at 37°C in Ham’s DMEM:F12 ( Gibco BRL, Gaithersburg, MD), with 5% heat-inactivated equine serum, 100 ng/ml cholera toxin, 500 ng/ml hydrocortisone, 100 i.u./ml penicillin, 100 μg/ml streptomycin and 5% heat-inactivated equine serum, 100 ng/ml cholera toxin, 500 ng/ml hydrocortisone and 100 i.u./ml penicillin at 37°C in Ham’s DMEM:F12 (Gibco BRL, Gaithersburg, MD). Hybridoma cells producing G5 monoclonal antibody, 12C4, was prepared against the G5 subdomain of the laminin α3 subunit using purified G5 recombinant protein as antigen (see below). The G5 subdomain is located at the C terminus of the α3 subunit. Female BALB/C mice were injected subcutaneously with G5 protein. The spleen from a mouse whose serum showed reactivity against recombinant G5 protein in western immunoblots (see below) was removed and the splenocytes were fused with SP2 mouse myeloma cells using polyethylene glycol according to standard procedures (Harlow and Lane, 1988). The fused cells were grown in RPMI 1640 (Sigma Chemical Co., St Louis, MO) with 10% heat-inactivated fetal bovine serum (Cascade Biologics, Portland, OR), HAT selection supplement (Sigma), 1 mM sodium pyruvate, 10 U/ml antibiotic/antimycotic and 20 mM L-glutamine (Gibco BRL, Gaithersburg, MD). Hybridoma cells producing G5 domain antibody were identified by western blotting using recombinant G5 protein. Cells were cloned three times and the cloned cells were then used to generate a high titer ascites fluid (Desmos, Inc., San Diego, CA). 3D5, a monoclonal antibody specific to the human laminin γ2 subunit, was generated using MCF-10A cell matrix as antigen. Briefly, female BALB/C mice were immunized with MCF-10A matrix protein preparations. Hybridoma cells were produced as above and antibody production was characterized by western blotting. Cells were cloned three times and the cloned cells used to produce a high titer ascites fluid (Desmos, Inc., San Diego, CA).

GB3, a monoclonal antibody against the human laminin γ2 subunit, was obtained from Harlan Sprague Dawley, Inc. (Indianapolis, IN) (Matsui et al., 1995; Verrando et al., 1987). The anti-human keratin 17 antibody was a gift from Dr Pierre A. Coulombe (Johns Hopkins University, Baltimore, MD). Monoclonal antibodies P1B5 and P4C10 against the α3 and β1 integrin subunits respectively were purchased from Gibco BRL. Monoclonal antibody LM609 against integrin αvβ3 and a rabbit polyclonal antisera AB1920 against human α3 integrin were obtained from Chemicon (Temecula, CA). The specificity of the latter was assessed by immunoprecipitation (result not shown). A rat monoclonal antibody specific to the extracellular domain of human α6 integrin, GoH3, was obtained from Immunotech (Westbrook, ME).

In vitro scrape wound assays

MCF-10A cells were grown to confluence either on tissue culture-treated plastic, or on glass coverslips. Medium was aspirated, and the cell-coated surface was scraped with a pipette tip in either a single stripe or a grid pattern. The scrape-wounded surface was washed with PBS and incubated in trypsin at 37°C for 30 seconds to remove debris from the wound area and wound edges. The surface was washed again twice with serum-containing medium, and then the wounds in the cultures were allowed to heal for various times. For antibody-blocking studies of wound healing, confluent cell cultures were scrape-wounded as above, then culture medium containing the appropriate antibodies was added to the culture. In some experiments, antibody-blocked scrape-wounded cultures were fixed after a set time and processed for immunofluorescence as described below. Antibodies in blocking studies were added at a concentration of 25-100 μg/ml to culture medium, depending on the instructions of the suppliers.

Skin wound specimens

Patients participating in this study were undergoing laser resurfacing for the treatment of wrinkles and discolorations. Briefly, patients were pretreated with Retin-A micro or Renova (Ortho Pharmaceutical Corp., Raritan, NJ) followed by treatment of the preauricular sites 2 weeks later with a Nidek Unipulse CO2 laser (Fremont, CA). The eschar was removed manually with a saline-soaked gauze between passes, and the laser-treated face was occluded with Flexzan (Dow Hickam Pharmaceuticals Inc., Sugarland, TX). Preauricular punch biopsies (3 mm) were taken 2 days post-resurfacing. The specimens were frozen in Tissue-Tek OCT Compound (Miles, Elkhart, IN) and were stored at −70°C. Consecutive frozen sections of 6 μm thickness were prepared using a Reichert-Jung Leica cryostat at −20°C and placed on slides. Sections were either extracted in methanol at 4°C for 10 minutes, or fixed in 3.7% formalin at 25°C for 8 minutes followed by a 5 minute extraction in 0.1% SDS. Sections were then processed for indirect immunofluorescence as described below, mounted with Vectashield mounting medium (Vector Laboratories, Burlingame, CA), and sealed.

Antibodies

The monoclonal antibodies against the LN5 α3 subunit, 10B5 and RG13, have been described previously (Goldfinger et al., 1998; Gonzales et al., 1999). RG13 recognizes the G2 subdomain of the globular domain of the α3 laminin subunit (Gonzales et al., 1999; our unpublished observations). J18 polyclonal antisera was raised in a rabbit using rat LN5 purified from extracellular matrix preparations of 804G cells, as previously described (Langhofer et al., 1993). A monoclonal antibody, 12C4, was prepared against the G5 subdomain of the laminin α3 subunit using purified G5 recombinant protein as antigen (see below). The G5 subdomain is located at the C terminus of the α3 subunit. Female BALB/C mice were injected subcutaneously with G5 protein. The spleen from a mouse whose serum showed reactivity against recombinant G5 protein in western immunoblots (see below) was removed and the splenocytes were fused with SP2 mouse myeloma cells using polyethylene glycol according to standard procedures (Harlow and Lane, 1988). The fused cells were grown in RPMI 1640 (Sigma Chemical Co., St Louis, MO) with 10% heat-inactivated fetal bovine serum (Cascade Biologics, Portland, OR), HAT selection supplement (Sigma), 1 mM sodium pyruvate, 10 U/ml antibiotic/antimycotic and 20 mM L-glutamine (Gibco BRL, Gaithersburg, MD). Hybridoma cells producing G5 domain antibody were identified by western blotting using recombinant G5 protein. Cells were cloned three times and the cloned cells were then used to generate a high titer ascites fluid (Desmos, Inc., San Diego, CA). 3D5, a monoclonal antibody specific to the human laminin γ2 subunit, was generated using MCF-10A cell matrix as antigen. Briefly, female BALB/C mice were immunized with MCF-10A matrix protein preparations. Hybridoma cells were produced as above and antibody production was characterized by western blotting. Cells were cloned three times and the cloned cells used to produce a high titer ascites fluid (Desmos, Inc., San Diego, CA).
Preparation of recombinant α3 G5 protein

A region of the laminin α3 subunit comprising most of the G5 domain and spanning nucleotides 1561-1713 was prepared by PCR using the 5’ primer 5’-CTGAAGAAAGGAGGTCA TGTC and the 3’ primer 5’- GGGTCGAGA TA TAGTAAGAAGT from cDNA derived from MCF-10A. This region was cloned into a PET32b expression vector (Novagen, Inc., Madison, WI) adjacent to a His-tag region, and introduced into DE3αeta by electroporation. Vector containing the G5 domain insert was selected for with ampicillin antibiotic (Fisher Scientific, Pittsburgh, PA). Cultures of bacteria containing the appropriate vector were induced with 1 mM IPTG (Sigma), and introduced into DE3αeta.

Sections were cut perpendicular to the substratum and viewed on a Zeiss Inc., Thornwood, NY), digital images were obtained with a Nikon Optiphot microscope (Nikon Inc., Melville, NY). Tissue specimens were viewed on an LSM 410 laser-scanning confocal microscope (Zeiss Inc., Thornwood, NY). Images were captured on MO optical disks (Sony, Montvale, NJ) and printed on a Tektronix printer (Tektronix, Wilsonville, OR). Tissue specimens were prepared for immunofluorescence microscopy as detailed previously (Klatte et al., 1989). They were viewed and photographed with a Nikon Optiphot microscope (Nikon Inc., Melville, NY).

Electron microscopy

MCF-10A cells maintained on tissue culture plastic were fixed in 1% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2, for 30 minutes. Fixed preparations were washed three times in 0.1 M sodium cacodylate buffer, pH 7.2, and post-fixed for 4 hour in 1% osmium tetroxide containing 0.8% potassium ferrocyanide at 4°C. Samples were then stained with uranyl acetate, dehydrated in ethanol and embedded in Epon-Araldite resin (Tousimis Corp., Rockville, MD). Sections were cut perpendicular to the substratum and viewed on a JEOL 1220 electron microscope at 60 kV (Riddelle et al., 1991).

Motility assays

For laminin antibody-blocking studies, LN5-rich matrix was prepared as above in a tissue culture dish and pre-incubated for 30 minutes with the appropriate antibody at 37°C in PBS. SCC12 cells were plated onto the treated matrix in medium containing 20 mM Hepes for 2 hours at 37°C (Gospodarowicz, 1984; Langhofer, 1993). For integrin antibody-blocking studies, SCC12 cells were first plated onto untreated LN5 for 1.5 hours, then integrin-specific monoclonal antibodies were added to the culture medium and the cells were maintained at 37°C for an additional 30 minutes. At 2 hours after plating, the cells were viewed by phase-contrast microscopy using an Axiovert 25 inverted microscope system (Zeiss) and the field was photographed every 30 minutes over a 3 hour period. The location of each cell was translated to numerical coordinates using the National Institutes of Health Image program (Bethesda, MD). Motility for each cell was calculated as displacement in μm from the starting point to the ending point. An average of 40 cells was monitored for each trial. Antibodies in the blocking studies were used at a concentration of 25-100 μg/ml, depending on the instructions of the suppliers.

RESULTS

Preparation of a monoclonal antibody probe specific for unprocessed α3 laminin subunit

Previous data from our laboratory and others have indicated that the α3 subunit of LN5 undergoes extracellular proteolytic cleavage (Goldfinger et al., 1998; Marinkovich et al., 1992). Following cleavage, we have shown that LN5 switches function from a matrix molecule, which supports cell motility, to one that induces establishment of matrix anchorage devices called hemidesmosomes (Goldfinger et al., 1998). During the course of the latter studies, we localized an enzymatic cleavage site to the G domain of the α3 molecule. We did so by showing that a mouse antiserum, termed Cta3, directed against a recombinant protein whose sequence corresponds to a region within the G5 subdomain of the C-terminal end of the α3 subunit, only recognizes unprocessed, full-length α3 subunit (Goldfinger et al., 1998). This antiserum fails to react with proteolytically cleaved 160 kDa α3 laminin subunit (Goldfinger et al., 1998). For the current study, we prepared monoclonal antibodies against the same recombinant G5 fragment. We have used one such monoclonal antibody preparation (12C4) to monitor the fate of the unprocessed α3 subunit under various experimental conditions.

To confirm the specificity of the 12C4 antibody, we analyzed matrix and whole cell extracts of confluent cultures of two human epithelial cell types (pp126 and MCF-10A). In a previous report we showed that the matrix of pp126 cells contains primarily unprocessed α3 subunit, while the matrix of MCF-10A cells contains primarily processed α3 chain (Goldfinger et al., 1998). In addition, we chose MCF-10A cells for our studies since PCR analyses, using primers for all the currently known human laminin subunits, as well as immunochemical studies, suggest that LN5 is the major laminin isoform expressed by this particular cell type and that these cells do not secrete laminin-6 or laminin-7 (W. Scholz and J. C. R. Jones, unpublished observations). The latter laminin isoforms, like LN5, are those that are known to contain an α3 subunit (Champliaud et al., 1997).

A species of 190 kDa is observed in immunoblots of pp126 cell extracellular matrix and whole cell extract preparations probed with 12C4 antibody (Fig. 1). In contrast, the 12C4 antibody fails to recognize any obvious polypeptide in MCF-
10A matrix, although it shows reactivity with a 190 kDa species in MCF-10A whole cell extracts (Fig. 1). As a control antibody for these studies we used the α3 chain monoclonal antibody 10B5, which binds both unprocessed and processed α3 subunits (Goldfinger et al., 1998). 10B5 antibodies recognize a 190 kDa species in immunoblots of extracellular matrix and whole cell extracts derived from pp126 cells and show reactivity with a 160 kDa polypeptide in the extracellular matrix and whole cell extract of MCF-10A cells (Fig. 1). In addition, the 10B5 antibodies recognize a 190 kDa species in MCF-10A whole cell extracts. These results confirm our previous data that pp126 cells secrete but do not process their α3 LN5 subunit whereas the α3 chain subunit of MCF-10A cells is converted from 190 to 160 kDa following secretion. In addition, these data confirm that processing involves cleavage of the α3 chain within its G domain.

Fig. 1. Expression of laminin α3 isoforms in extracellular matrix and whole cell extract preparations derived from MCF-10A and pp126 cells. Extracellular matrix (ECM) and whole cell extract (WCE) preparations from MCF-10A and pp126 cells were processed for western blotting using 12C4 antibody and 10B5 antibody as indicated. 12C4 antibodies fail to recognize any polypeptides in MCF-10A ECM (lane 1), but recognize a 190 kDa protein in MCF-10A WCE (lane 5). A single species of 190 kDa is identified by 12C4 antibodies in pp126 ECM and WCE (lanes 2 and 6). 10B5 antibodies recognize polypeptides of 160 kDa in MCF-10A ECM, and 190 kDa in pp126 ECM (lanes 3 and 4). In a MCF-10A WCE preparation, 10B5 antibodies recognize both 160 and 190 kDa species (lane 7), whereas the same antibodies only identify a 190 kDa polypeptide in a pp126 WCE preparation (lane 8). The positions of marker proteins are shown.

Fig. 2. Unprocessed laminin α3 subunit is expressed in wounded, healing skin. Normal human skin tissue sections (a–c) and consecutive wounded skin sections (d–f) from 2-day post-resurfacing punch biopsies were fixed and processed for indirect immunofluorescence microscopy using a γ2 laminin subunit antibody GB3 (a,d), 12C4 antibody (b,e) and an antiserum against K17 (c,f). It should be noted that the epidermal cell sheet has partially detached from the basement membrane in d–f as a consequence of sectioning. Normal skin (a) and wounded skin (d, arrow) both show basement membrane staining with GB3 antibody. 12C4 antibodies do not show any reactivity in normal skin (b). However, 12C4 antibodies show strong reactivity in the basement membrane beneath migrating keratinocytes in healing human skin wounds (e, arrow). Anti-K17 antibodies show little cell staining in normal skin although the K17 antibodies recognize certain epidermal cells in hair follicles (c). These same antibodies show bright intracellular staining in epidermal cells in healing skin wounds (f). Bars, 200 μm (a,c–f); 100 μm (b).
Expression of unprocessed α3 laminin subunit in skin wounds in vivo

Earlier studies with LN5 derived from the extracellular matrix of pp126 and MCF-10A cells indicated that LN5 containing a α3 subunit of 160 kDa induces the formation of hemidesmosomes in epithelial cells and retards their motility, whereas LN5 protein complexes containing a 190 kDa unprocessed α3 laminin subunit promote cell motility (Goldfinger et al., 1998). These results imply that proteolytic processing of LN5 in the basement membrane may be an important regulator of cell adhesion and motility in situations where cells alternate between these behaviors, such as in wound healing. To determine whether the expression of the unprocessed α3 laminin subunit is observed during wound healing in vivo, we prepared sections of normal and wounded, healing human skin. A monoclonal antibody that recognizes the human laminin γ2 subunit, GB3, stains throughout the basement membrane in normal, unwounded skin (Fig. 2a) and in skin, 2 days post-wounding (Fig. 2d) (Matsui et al., 1995; Verrando et al., 1987). In contrast, 12C4 antibodies fail to recognize the basement membrane of the epidermis in normal skin sections (Fig. 2b), but stain the basement membrane of epidermal cells repopulating a wound (Fig. 2e). Similar staining has been observed in sections of wounded skin using an uncharacterized LN5 antibody (Lampe et al., 1998). These same cells that are recognized by 12C4 antibodies are stained by an antiserum against keratin 17 (K17), which has been shown to be present at high levels in keratinocytes migrating to cover a wound (Fig. 2f) (Paladini et al., 1996).

Localization of the unprocessed laminin α3 subunit and LN5 receptors in cultured cells

The 12C4 antibody was subsequently used to examine the localization of unprocessed α3 laminin subunit in pp126 and MCF-10A cell cultures. MCF-10A and pp126 cells grown on glass coverslips were prepared for double-label immunofluorescence using 12C4 antibodies (a,h), the LN5 polyclonal serum J18 (b,e), a monoclonal antibody against α6 integrin (d) and a monoclonal antibody against α3 integrin (g). Cells were viewed by confocal microscopy. 12C4 antibodies label throughout the extracellular matrices of pp126 cells (a) and colocalize with J18 antibodies (b). The staining generated by α6 integrin in d also shows overlap with the distribution of LN5 in (e) (arrows) although there are areas of α6 staining where there is no apparent LN5 (arrowhead) and vice versa (asterisk). In (g), the α3 integrin shows some colocalization with the matrix elements stained by 12C4 antibodies (h) (asterisks). C,f and i show phase-contrast images of the fixed and processed cells. Bar, 25 μm.
of 12C4 antibody reactivity on immunoblots of matrix derived from confluent MCF-10A cells (Fig. 1).

Double-label staining of subconfluent MCF-10A cell cultures with 12C4 antibodies and the LN5 antiserum J18 reveals several interesting features (Fig. 4a,b). The J18 antibodies stain in a typical rosette-like pattern throughout the cell culture, with some of the staining generated by these antibodies extending beyond the boundaries of the cells (Fig. 4b). The staining generated by 12C4 antibodies in the same preparations is not as extensive as that of the J18 serum although where 12C4 antibodies localize there is comparable J18 staining (Fig. 4a,b). Staining generated by the 12C4 antibodies in subconfluent cultures of MCF-10A cells can be divided into three distinct types of patterns. The 12C4 antibodies stain the trans-Golgi network in certain cells (Fig. 4a,f). We presume this staining pattern represents unprocessed and unsecreted laminin α3 subunit. In addition, in some cells, the 12C4 antibodies show intense staining in arc patterns located at the outer cell periphery at the edges of multilcellular groups (Fig. 4a,f). In some cells, staining with 12C4 antibodies is often observed in arcs parallel to the cell edge, several μm away from the cell on the surface of the glass coverslip (Fig. 4a, compare with phase-contrast image of cells in d). Close observation reveals that the tips of long cell-surface extensions of MCF-10A cells appear to terminate in these arcs of 12C4 staining (Fig. 4a,c). To assess whether these extensions are filopodia or retraction fibers, images of living MCF-10A cells, maintained on ‘indicator’ coverslips, were captured digitally every 30 minutes, and after 4 hours the cells were processed for double-label immunofluorescence using the 12C4 and J18 antibodies. The direction of movement of at least ten cells was determined from the captured images and then the staining patterns generated in and around the same cells were assessed by immunofluorescence microscopy. The results suggest that in all instances the cell extensions that terminate close to or on the arcs of 12C4 staining are retraction fibers rather than filopodia (data not shown).

We also examined the localization of integrins α6β4 and α3β1, which are receptors for LN5, in both pp126 and MCF-10A cell preparations (Carter et al., 1991; Delwel and Sonnenberg, 1996; Niessen et al., 1994). In pp126 cells, the staining generated by two different α6 integrin antibody probes (GoH3 and BQ16) occurs in small, dispersed punctate spots, and also in ‘cat-paw’ patterns, where it colocalizes with LN5 localized with J18 serum (Fig. 3d,e). The latter can be used to indicate LN5 containing an unprocessed α3 subunit since we observe little, if any, processed α3 subunit in the matrix of pp126 cells (Fig. 1; Goldfinger et al., 1998). However, there are instances where LN5 occurs in the absence of corresponding α6 integrin and vice versa (Fig. 3d,e). An α3 integrin polyclonal antibody stains both pp126 cell-cell contact sites as well as along the region of cell-substrate interaction (Fig. 3g). We have confirmed the specificity of the α3 integrin serum by immunoprecipitation (result not shown). There is occasional colocalization of α3 integrin with LN5 staining in pp126 cells (Fig. 3g,h).

In subconfluent MCF-10A cell populations, although α6 integrin codistributes with antigens recognized by LN5 antibodies in the matrix underlying cell bodies, antibody staining for α6 integrin terminates just proximal to the arcs stained by 12C4 antibody, as shown in a triple-labeled specimen (Fig. 4c). α6 integrin also localizes in a punctate staining pattern along the lengths of retraction fibers, although α6 integrin appears absent at the tips of the fibers where there...
Preparations derived from MCF-10A cell cultures that have recognize a 190 kDa polypeptide in immunoblots of matrix wound closure. Consistent with these data, 12C4 antibodies (Fig. 5a,b). This is true at all stages of wound healing prior to edges of those cells that are migrating into the wound space. scrapewound's. MCF-10A cells were grown to confluence, then scrape-wounded with a pipette tip. The cultures were lightly trypsinized and then processed for immunofluorescence using the J18 anti-LN5 serum (a). Although there is extensive staining in the matrix of the remaining cells, there is little staining in the scrape site (a). The wounded cultures were allowed to partially heal and, at 8 hours after wounding, the cells were processed for immunofluorescence microscopy using 12C4 antibodies (b,c). A low magnification view of the wound edge is shown in (b). Several cells at the edge of the cellular sheet which has migrated to ‘fill in’ a wound are shown at a higher magnification in (c). 12C4 antibodies stain strongly in the cytoplasm of many cells at this leading edge, as well as in some cells distal from the wound edge (b, wide arrow). These antibodies also label arcs in the ECM beneath the leading edge of cells at the wound front (b,c arrows). There is no apparent 12C4 staining within the matrix elsewhere in the wounded cultures. Bars, 25 μm.

Unprocessed laminin α3 subunit is present at the leading edge of MCF-10A scrape wounds. MCF-10A cells were grown to confluence, then scrape-wounded with a pipette tip. The cultures were lightly trypsinized and then processed for immunofluorescence using the J18 anti-LN5 serum (a). Although there is extensive staining in the matrix of the remaining cells, there is little staining in the scrape site (a). The wounded cultures were allowed to partially heal and, at 8 hours after wounding, the cells were processed for immunofluorescence microscopy using 12C4 antibodies (b,c). A low magnification view of the wound edge is shown in (b). Several cells at the edge of the cellular sheet which has migrated to ‘fill in’ a wound are shown at a higher magnification in (c). 12C4 antibodies stain strongly in the cytoplasm of many cells at this leading edge, as well as in some cells distal from the wound edge (b, wide arrow). These antibodies also label arcs in the ECM beneath the leading edge of cells at the wound front (b,c arrows). There is no apparent 12C4 staining within the matrix elsewhere in the wounded cultures. Bars, 25 μm.

is a concentration of 12C4 antigen (Fig. 4a,c). In addition, antibodies against α3 integrin show some colocalization with 12C4 antibodies in certain of the arc-like patterns that occur toward the edge of subconfluent MCF-10A cells (Fig. 4e,f). However, there are many instances where 12C4 staining patterns and α3 integrin localization are distinct. For example, α3 integrin is commonly found at sites of MCF-10A cell-cell contact whereas 12C4 staining is not (Fig. 4e,f).

Unprocessed laminin α3 subunit is expressed at the leading edge of actively migrating epithelial cells populating wounds in vitro

We have also followed the fate of the unprocessed α3 laminin subunit in matrix of migrating MCF-10A cells as the cells cover a scrape wound. For these studies, confluent cultures of MCF-10A cells on coverslips were wounded by a single pass with a plastic pipette tip. The wounded cultures were incubated briefly (30 seconds) with trypsin to remove cellular debris at the wound edge and in the wound space. Immediately following trypsinization, there appears to be little detectable LN5 in the wound although the matrix of the unwounded cells is brightly stained by a LN5 antiserum (Fig. 5a).

The wounded cultures were allowed to heal for 6, 12, 18 or 24 hours before being fixed and labeled with 12C4 antibodies. Scrape wounds are completely healed by 18 hours with the cells moving as a sheet over the denuded cell culture substrate. However, expression of unprocessed α3 laminin subunit is upregulated at the wound edges, as indicated by intense Golgi staining in most cells along the wound edge by 12C4 antibodies (Fig. 5a). The latter also strongly stain the leading edges of those cells that are migrating into the wound space (Fig. 5a,b). This is true at all stages of wound healing prior to wound closure. Consistent with these data, 12C4 antibodies recognize a 190 kDa polypeptide in immunoblots of matrix preparations derived from MCF-10A cell cultures that have been wounded and allowed to undergo partial healing for 8 hours (Fig. 6, lane 2). 12C4 antibodies do not recognize any species in matrix preparations derived from confluent, non-wounded MCF-10A cell cultures (Fig. 6, lane 1). It should also be noted that 12C4 recognizes a group of polypeptides in the molecular mass range 21-39 kDa in matrix derived from wounded cultures. These low molecular mass species may represent cleaved fragments of the α3 chain. In addition, although it has been reported that the γ2 chain of LN5 is cleaved by matrix metalloprotease-2, resulting in the production of a 80 kDa fragment during tissue remodeling, we find no evidence of such a fragment in the γ2 chain of LN5 in the matrix of MCF-10A cell cultures that have been wounded and allowed to heal (Fig. 6, lanes 3 and 4) (Giannelli et al., 1997). At this time we cannot explain the discrepancy in results.

We next investigated the localization of integrins α3 and α6 in MCF-10A cells populating scrape wounds. Whereas α6 integrin is polarized basally in rosette patterns in unwounded, confluent cultures of MCF-10A cells, it is found primarily at cell-cell contact sites in the leading margin of migrating MCF-10A cells that reepithelialize in vitro wounds (Fig. 7a,c). This is consistent with localization data presented by Kurpakus et al. (1991), who analyzed the organization of α6β4 integrin in the migrating epithelium of a tissue explant wound-healing model. α6 integrin is not found in any obvious codistribution with the unprocessed α3 laminin subunit (Fig. 7c,d). α6 integrin shows a basal localization in a punctate pattern in cells distal from the wound margin in the MCF-10A cultures (Fig. 7e). On the other hand, α3 integrin, which is found predominantly at regions of cell-cell interaction in confluent MCF-10A cell populations (not shown), is occasionally found colocalized with unprocessed α3 laminin subunit at the leading front of cells migrating over the MCF-10A culture wound site (Fig. 7g,h).

Fig. 5. Unprocessed laminin α3 subunit is present at the leading edge of MCF-10A scrape wounds. MCF-10A cells were grown to confluence, then scrape-wounded with a pipette tip. The cultures were lightly trypsinized and then processed for immunofluorescence using the J18 anti-LN5 serum (a). Although there is extensive staining in the matrix of the remaining cells, there is little staining in the scrape site (a). The wounded cultures were allowed to partially heal and, at 8 hours after wounding, the cells were processed for immunofluorescence microscopy using 12C4 antibodies (b,c). A low magnification view of the wound edge is shown in (b). Several cells at the edge of the cellular sheet which has migrated to ‘fill in’ a wound are shown at a higher magnification in (c). 12C4 antibodies stain strongly in the cytoplasm of many cells at this leading edge, as well as in some cells distal from the wound edge (b, wide arrow). These antibodies also label arcs in the ECM beneath the leading edge of cells at the wound front (b,c arrows). There is no apparent 12C4 staining within the matrix elsewhere in the wounded cultures. Bars, 25 μm.
The plate (Fig. 8c, compare insets in Fig. 8b,c). Cultures in that they lack a well-developed, tripartite, hemidesmosomes in cells in unwounded regions of MCF-10A to the leading edge, possess some hemidesmosome-like at the leading front of the migrating epithelial sheet (Fig. 8a). Neither mature nor immature hemidesmosomes in those cells assemble hemidesmosomes, possessing all of the structural previously, Stahl et al. (1997) showed that MCF-10A cells wound healing blotting with 12C4 antibody and antibody 3D5 against the MCF-10A cultures were subjected to SDS-PAGE and western preparations derived from unwounded (UW) and wounded (W) MCF-10A cell cultures were scrape-wounded and allowed to partially heal for 8 hours at 37°C. Matrix was prepared from Gospodarowicz (1984). Matrix preparations derived from unwounded (UW) and wounded (W) MCF-10A cultures were subjected to SDS-PAGE and western blotting with 12C4 antibody and antibody 3D5 against the γ2 laminin subunit. 12C4 antibodies do not recognize any material in matrix preparations derived from confluent MCF-10A cells (lane 1) but they identify a 190 kDa polypeptide in matrix preparations derived from healing MCF-10A cultures (lane 2). Interestingly, 12C4 antibodies also recognize a series of polypeptides migrating between 21 and 39 kDa. The 3D5 antibodies recognize 140 and 100 kDa species, representing unprocessed and processed γ2 laminin subunit, in matrix derived from both unwounded and wounded MCF-10A cell cultures (lanes 3 and 4) (Vailly et al., 1994). The positions of marker proteins (kDa) are shown.

Ultrastructural evaluation of MCF-10A cells during wound healing

Previously, Stahl et al. (1997) showed that MCF-10A cells assemble hemidesmosomes, possessing all of the structural characteristics of hemidesmosomes in breast epithelial cells in vivo. We therefore processed wounded MCF-10A cultures 8 hours post-wounding for electron microscopy. We observe neither mature nor immature hemidesmosomes in those cells at the leading front of the migrating epithelial sheet (Fig. 8a). In contrast, MCF-10A cells populating scrape wounds, distal to the leading edge, possess some hemidesmosome-like structures (Fig. 8b). These appear immature compared with hemidesmosomes in cells in unwounded regions of MCF-10A cultures in that they lack a well-developed, tripartite, cytoplasmic plaque as well as an extracellular sub-basal dense plate (Fig. 8c, compare insets in Fig. 8b,c).

The α3 laminin subunit and integrins α3 and α6 are involved in wound closure and cell migration in vitro

Our immunofluorescence results indicate a possible role for the α3 laminin subunit and LN5 receptors in epithelial wound healing. To investigate this further, we assessed the impact on wound healing of antibodies RG13, P1B5, GoH3 and P4C10, which inhibit the function of α3 laminin subunit, α3 integrin, α6 integrin and β1 integrin, respectively (Gonzales et al., 1999). In brief, scrape wounds were introduced into confluent MCF-10A cell cultures and then allowed to heal for 18 hours in the presence of the appropriate antibody. Wounds in cell populations incubated without antibody or in medium containing a function-perturbing αvβ3 integrin IgG antibody (LM609), heal completely within the experimental time frame (Fig. 9a,f). When α6 integrin-blocking antibody GoH3 is added to the wounded cultures, healing is partially inhibited, with approximately 70% wound closure in 18 hour (Fig. 9b,i). Wound healing is blocked to a markedly greater degree (41.6% closure) in the presence of α3 integrin-blocking antibody P1B5 (Fig. 9c,i). Incubation with a β1 integrin-blocking antibody, P4C10, also inhibits wound closure (34% closure) (Fig. 9e,i). When antibodies to both α3 and α6 integrins are combined, wound healing is almost completely inhibited (9.5% closure) (Fig. 9d,i). Wound healing is completely blocked (3.2% closure) when the wounded cultures are incubated in the presence of RG13, a function-blocking antibody that recognizes the globular region of the α3 laminin subunit, but GB3 antibody to the LN5 γ2 subunit has no effect on wound closure (Fig. 9g-i) (Gonzales et al., 1999).

Since the combination of GoH3 and P1B5 antibodies was more efficient than the same antibodies used singly in inhibiting MCF-10A cell culture wound healing, this raises the possibility that α6β4 and α3β1 integrins may overlap in some of their functions. To investigate this further, we analyzed the localization of α3 integrin in GoH3 antibody-treated, motile MCF-10A cells located away from the leading front of migrating cells covering a scrape wound. The α3 integrin subunit is predominantly at cell-cell contact points with little, if any, α3 integrin being localized at sites of cell-substrate interaction in unwounded confluent MCF-10A cell populations (Fig. 10a). In sharp contrast, in cells in wounded, GoH3 antibody-treated cultures, α3 integrin is present both at cell-cell contacts as well as at the basal cell surface (Fig. 10c). We also evaluated the localization of α6 integrin in similar cells treated with the α3 integrin-blocking antibody P1B5. α6 integrin in such cells shows a distribution similar to that observed in cells populating wounds in the absence of the P1B5 antibodies, i.e. α6 integrin localizes along regions of cell-cell contact as well as cell-matrix contact points (Fig. 10e).

Our wound studies indicate the possibility that LN5 containing unprocessed α3 subunit induces cell motility in an integrin-dependent manner. To demonstrate this directly, SCC12 squamous cell carcinoma cells were plated onto LN5 matrix containing an unprocessed α3 subunit in the presence of the same LN5 and integrin antibodies as above. The cells were then monitored by phase-contrast microscopy over 3 hours and their motility was assessed. SCC12 cells were chosen for this experiment because they are incapable of processing the α3 subunit of LN5, whereas MCF-10A cells do so (Goldfinger et al., 1998) (Fig. 1). SCC12 cells are motile on LN5 matrix containing an unprocessed α3 subunit over a 3 hour period with an average cell displacement of 34 μm (Goldfinger et al., 1998). This is displayed in Fig. 11 as 100% motility. In the presence of anti-α6 integrin antibody GoH3, the cells show decreased motility (approximately 52% of control), and are even less motile when incubated with antibodies that inhibit the function of either the α3 or β1 integrin subunits (32% of control and 29% of control, respectively) (Fig. 11). In contrast, antibodies against αvβ3 integrin have no apparent effect on cell motility, nor does antibody GB3 to the LN5 γ2 subunit. A mix of GoH3 and P1B5 antibodies inhibit motility by 79% compared with control.
RG13 antibodies against the laminin α3 subunit G domain also inhibit cell migration in this system (33% of control) (Fig. 11). These data indicate that epithelial cells utilize unprocessed laminin α3 subunit as a motility factor, in a manner dependent upon both α6 integrin-containing heterodimers, and to a greater extent, α3β1 integrin.

DISCUSSION

The mRNA transcript of the α3 laminin subunit gene encodes a protein with a predicted molecular mass of approximately 200 kDa (Ryan et al., 1994; Vidal et al., 1995). This is the size of the α3 subunit in the LN5-rich matrix of normal keratinocytes and various transformed oral and skin epithelial cell lines (Goldfinger et al., 1998). In contrast, the α3 subunit shows a molecular mass of 160 kDa in the matrix of MCF-10A and 804G cells (Baker et al., 1996; Goldfinger et al., 1998; Langhofer et al., 1993; Stahl et al., 1997). The cells of these latter two lines share one common feature: they can both assemble hemidesmosomes in vitro (Riddelle et al., 1991; Stahl et al., 1997). In our previous studies we showed that the 190 kDa form of the α3 subunit can be converted to a 160 kDa species by specific proteolytic cleavage using plasmin. Furthermore, we also showed that LN5 containing the larger α3 form acts as a motility factor for epithelial cells while LN5 induces cell attachment and hemidesmosome assembly following plasmin cleavage of the G domain of its α3 subunit (Goldfinger et al., 1998).

Here we have investigated the localization of the 190 kDa form of the α3 laminin subunit using a monoclonal antibody specific for the region of the G domain, which is lost from the molecule during proteolytic processing. During the course of our studies, we have shown that unprocessed laminin α3 subunit has a distinctive distribution in cultured cells. In pp126 cells, which fail to process their α3 subunit to any obvious degree, our monoclonal antibody stains throughout the matrix of the cells. In contrast, no unprocessed α3 subunit is seen in the LN5-rich matrix of confluent MCF-10A cells. However, unprocessed α3 subunit can be detected in the matrix of certain cells in subconfluent MCF-10A cell cultures. In particular, the unprocessed α3 subunit often appears to be organized into circles or arcs toward the ‘free’ outer edges of those MCF-10A cells in small multicellular groupings. We have also observed unprocessed α3 subunit in the same arc pattern outside the boundary of the MCF-10A cells. We speculate that MCF-10A cells deposit a matrix, containing unprocessed α3 subunit, toward their outermost edges. Provided the cell moves over the newly deposited laminin, then the α3 subunit is processed. However, in some instances MCF-10A cells move away from their deposits of LN5, leaving a trail of matrix containing...
There is a strong reactivity of 12C4 antibody in the matrix beneath the first layer of cells of the MCF-10A multicellular sheet, which repopulates in vitro scrape wounds. Either uncleaved α3 laminin subunit or the G4/G5 fragment is restricted to this site. The same is true in healing wounds of the epidermis, i.e. 12C4 antibody reactivity is found in the matrix of the leading tip of epidermal cells populating an in vivo wound. We suppose that as the sheet of cells moves over the wound bed, both in vitro and in vivo, the unprocessed α3 subunit is rapidly cleaved such that the majority of cells in the migrating cell population lie on an LN5-rich matrix containing processed α3 subunit. Using an LN5 α3 subunit function-perturbing antibody and MCF-10A cells, we have provided direct evidence for an important functional role for the α3 chain of LN5 in the process of wound closure, i.e. antibody RG13 inhibits closure of scrape wounds made in MCF-10A cultures. Since RG13 appears to impede the initiation of
migration of cells from the wound edge over the wound bed, we presume that RG13 has a direct, inhibitory impact on laminin heterotrimers containing unprocessed α3 subunit, which is deposited at the very leading edge of the sheet of migrating epithelial cells. Indeed, based on our data which reveal that this particular form of LN5 is a motility factor for cells, we favor an hypothesis that the LN5, containing unprocessed α3 subunit, drives the forward motion of the sheet of migrating cells that covers a wound site. Since it is highly improbable that the leading front of cells can drag the more distal cells in the sheet over the wound site, we suggest that LN5 containing unprocessed α3 subunit is more likely to initiate a cell signaling cascade, which regulates the spreading and migration of the distally located cells.

One intriguing aspect of our results is that processed α3 subunit occurs in the matrix of the cells within the actively migrating cellular sheet that covers the MCF-10A scrape wounds as well as in the matrix of the epidermal cells populating in vivo wounds. Our laboratory and others have shown previously that cells maintained on LN5 containing processed α3 subunit show reduced migration (Goldfinger et al., 1998; O’Toole et al., 1997). One might assume therefore that this particular matrix would impede epithelialization of the wound. However, wounds in our MCF-10A cell cultures and in vivo clearly heal despite the presence of processed α3 subunit in the matrix of the migrating epithelial cell sheet. We propose that the presence of two functionally distinct α3 laminin subunits in the matrix of the migrating cells reflects the somewhat conflicting processes that occur during wound healing. One of these processes leads to the migration of cells to ensure that a wound site is covered. At the same time there must be stabilization of epithelial cell-wound bed interaction (Garlick and Taichman, 1994; Martin, 1997). Because it can function both as a motility factor and as an adhesive substrate, depending on its subunit makeup, LN5 is ideally suited to function in both events and may play a key role in the balance between these two processes. In other words, at the leading edge of the sheet of cells that populate a wound, LN5 may
facilitate cell migration. In support of this notion, the high degree of motility displayed by SCC12 cells on unprocessed LN5 can be blocked with antibodies to the laminin α3 subunit G domain. In contrast, in more distal regions of the migrating cellular sheet, LN5 may play a role in stabilizing cell-substrate interaction. It may do so by ligating α6β4 integrin, which we have shown is localized along the site of cell-matrix interaction in the migrating epithelial cells. We speculate that formation of such a LN5/α6β4 complex leads to the assembly of mostly immature hemidesmosomes, as we describe at the ultrastructural level. Such immature hemidesmosomes may be akin to type II hemidesmosomes seen in gut epithelial cells (Uematsu et al., 1994). These may be more dynamic than their mature counterparts, allowing cell migration to occur while also providing stability to cell-wound bed interaction.

Our immunofluorescence analyses reveal that α3 integrin colocalizes in some instances with matrix containing the unprocessed laminin α3 subunit both in pp126 and MCF-10A cells. This suggests the possibility that α3β1 integrin may be involved in transducing the motility ‘signal’ of the unprocessed α3 laminin subunit. In this study, we have provided experimental support for this, since the α3 integrin antibody P1B5 and the β1 integrin antibody P4C10 inhibit closure of MCF-10A cell scrape wounds, as well as the motility of SCC12 cells plated onto LN5 containing an unprocessed α3 subunit. Furthermore, this idea is consistent with the work of Zhang and Kramer (1996) who presented evidence for a role of LN5 and its receptor α3β1 integrin in inducing epithelial cell motility, as well as a recent study in which it has been shown that α3 integrin-deficient mouse keratinocytes show decreased migration on a number of matrix substrates (Hodivala-Dilke et al., 1998). However, it should also be noted that both α3 and β1 integrin function-perturbing antibodies inhibit wound closure less efficiently than the α3 laminin subunit antibody RG13, implying that another integrin or non-integrin matrix receptor may be involved in the wound healing process. This integrin appears to be α6β4 integrin, since a combination of α3 integrin antibody and the α6 integrin antibody inhibits closure of MCF-10A cell wounds almost as well as the α3 laminin subunit antibody. Perhaps the GoH3 antibody prevents the assembly of the type II-like hemidesmosomes mentioned above. This may perturb adherence of the migrating epithelial cells to the wound surface, thereby inhibiting wound closure.

Our results also suggest that the roles of α3β1 and α6β4 may be somewhat interchangeable or overlapping (Xia et al., 1996). In other words, when the function of the α3 integrin subunit is inhibited in wounded cultures of MCF-10A cells, α6β4 integrin may be capable of partially mediating signals, which lead to cell motility. This could occur directly, via a α6β4 integrin association with the microfilament network of epithelial cells, as suggested by Rabinovitz and Mercurio (1997), or indirectly, by some sort of ‘cross-talk’ with another integrin heterodimer. Our motility assays provide additional support for this since the α6 integrin antibody GoH3 inhibits SCC12 cell migration on LN5 to a certain degree. In addition, when the function of α6 integrin is inhibited, the α3β1 integrin heterodimer may help stabilize cell attachment to the matrix in the wound bed. We have provided some circumstantial evidence for this since, in α6 integrin antibody-treated wounded MCF-10A cell cultures, α3 integrin is found along sites of cell-substrate interaction in migrating MCF-10A cells, where it may substitute for α6β4 integrin in maintaining cell-wound bed interaction. This would explain why blocking the function of both α3 and α6 integrins almost completely inhibits migration and closure of a MCF-10A cell scrape wound.

Despite the apparent interplay between α3β1 and α6β4 integrin in our wound studies of MCF-10A cells, we never observe colocalization of integrin heterodimers containing α6.
and α3 subunits in MCF-10A cells. This may reflect the dynamic nature of interaction of α3β1 integrin and LN5. Alternatively, interaction between α3β1 integrin and LN5, containing an unprocessed α3 subunit, may show a higher affinity than an α6β4 integrin association with the same ligand, while α6β4 integrin interaction with LN5, containing a processed α3 chain, may be of higher affinity than that between α3β1 integrin and the same LN5 isoform.

Based on the results we present here, we propose the following model to explain the role of LN5 and its integrin receptors in epithelial wound healing (Fig. 12). In the model, in a ‘resting’ stratified epithelial tissue, LN5, containing processed α3 subunit, binds to α6β4 integrin in hemidesmosomes in basal epithelial cells, while α3β1 integrin is localized to the lateral cell surfaces. Upon wounding, production of LN5 is upregulated, and/or there is a concomitant down-regulation of α3 chain processing at the wound edge. This results in deposition of unprocessed α3 laminin subunit at the leading edge of the wound. The integrin α3β1 interacts with the unprocessed α3 laminin subunit in a way that encourages cell migration over the wound bed. In the same cells, α6β4 integrin concentrates at the lateral cell surfaces. In contrast, in cells some distance away from the leading tip of epithelium, α6β4 integrin locates not only at sites of cell-cell contact but also along the basal surface of the cells, where it can bind matrix containing processed α3 subunit. We speculate that the latter interaction stabilizes the attachment of the migrating sheet of cells to the wound bed. It should be noted also that our scheme is inconsistent with a model of epidermal wound healing that was recently proposed by Hynes and co-workers (Hodivala-Dilke et al., 1998). The latter is based on a study of wound healing in α3 integrin-deficient keratinocytes and relies on the notion that in resting skin LN5 is ligated to α3β1 integrin (DiPersio et al., 1997; Hodivala-Dilke et al., 1998). However, this premise is not supported by the literature, since it has been shown by a number of groups that in normal skin LN5 shows an intimate association with α6β4 integrin in hemidesmosomes while α3β1 integrin is primarily distributed at sites of cell-cell contact (Carter et al., 1990; Jones et al., 1991; Kurpakus et al., 1991; Niessen et al., 1994; Sonnenberg et al., 1991).

Of course, the model we propose is clearly an over-

![Fig. 11. Involvement of LN5, containing an unprocessed α3 subunit, and its integrin receptors in motility. SCC12 cells were plated onto matrix, allowed to adhere for 2 hours and then monitored microscopically for 3 hours. In the case of the LN5 inhibitory antibody RG13 and the laminin γ2 chain antibody GB3, the matrix was incubated for 30 minutes in medium containing antibody prior to addition of the cells. In the case of studies involving integrin antibodies (GoH3 against α6 integrin, P1B5 against α3 integrin, P4C10 against β1 integrin and LM609 against αvβ3), the antibodies were added to the cell culture medium 1.5 hours after plating the cells onto matrix as indicated. An image of the treated cells was collected 30 minutes later and then at 30 minute intervals over approximately the next 3 hours. Cells were kept at 37°C throughout the assay and motility was calculated as the average total cell displacement from starting point to ending point. Motility is expressed as a percentage of control.](image1)

![Fig. 12. Model for functions of LN5, α3β1 and α6β4 integrins in epithelial wound closure. This model depicts our current speculations on the role of unprocessed α3 laminin subunit and integrins in epidermal wound healing. In resting skin, α6β4 integrin in hemidesmosomes is bound to processed LN5 containing a 160 kDa α3 subunit (LN5-160). When the skin is wounded, production of new LN5 is upregulated, and/or there is a downregulation of α3 chain proteolysis, resulting in an increase in the presence of LN5-190 at the wound edge. α6β4/LN5-160 ligation is disrupted at the wound edge, and α6β4 translocates from the basal to lateral cell surfaces. α3β1 integrin binds to the LN5-190 deposited at the wound edge, which encourages migration of keratinocytes over the wound bed. Distal from the wound edge, some α6β4 integrin binds to LN5-160, in partially formed hemidesmosomes. These may help to maintain stability of epithelial-basement membrane interaction without compromising the dynamic activities of the healing tissue.](image2)
simplification since, in vivo, cells have to migrate over a wound bed rich in fibronectin, collagen and various other matrix proteins, all of which are likely to play some role in regulating migration and adhesion via signal cascades involving integrin and non-integrin cell surface proteins. Nonetheless, our model raises a number of important questions with regard to the function of LN5 and its receptors in wound healing. What is the pathway by which LN5 and the αβ integrin regulate the motility machinery of an epithelial cell? How is the interplay between the αβ integrin and α6β4 integrin heterodimers governed? We are currently exploring these issues.

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REFERENCES


terminal globular region of laminin 5 α3 chain. Cell Growth Diff. 8, 979-987.


