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# The role of laminin-5 and its receptors in mammary epithelial cell branching morphogenesis

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## SUMMARY

**In vivo, normal mammary epithelial cells utilize hemidesmosome attachment devices to adhere to stroma. However, analyses of a potential role for hemidesmosomes and their components in mammary epithelial tissue morphogenesis have never been attempted. MCF-10A cells are a spontaneously immortalized line derived from mammary epithelium and possess a number of characteristics of normal mammary epithelial cells including expression of hemidesmosomal associated proteins such as the two bullous pemphigoid antigens, α6β4 integrin and its ligand laminin-5. More importantly, MCF-10A cells readily assemble mature hemidesmosomes when plated onto uncoated substrates. When maintained on matrigel, like their normal breast epithelial cell counterparts, MCF-10A cells undergo a branching morphogenesis and assemble hemidesmosomes at sites of cell-matrigel interaction. Function blocking antibodies specific for human laminin-5 and the α subunits of its two known receptors (α3β1 and α6β4 integrin) not only inhibit hemidesmosome assembly by MCF-10A cells but also impede branching morphogenesis induced by matrigel. Our results imply that the hemidesmosome, in particular those subunits comprising its laminin-5/integrin 'backbone', play an important role in morphogenetic events. We discuss these results in light of recent evidence that hemidesmosomes are sites involved in signal transduction.**

Key words: Hemidesmosome, Integrin, Laminin

## INTRODUCTION

Extracellular matrix plays a crucial role in determining the morphogenesis of a number of epithelial tissue types (Hay, 1993). One of the most dramatic examples of this phenomenon is the regulation of mammary epithelium phenotype by elements of basement membranes derived from the Engelbreth-Holm-Swarm tumor (matrigel) (Bissell and Ram, 1989; Barcellos-Hoff et al., 1989; Blum et al., 1989; Lin and Bissell, 1993). Indeed, mouse mammary epithelial cells assemble into structures remarkably similar to alveoli of lactating mammary glands and produce milk proteins when maintained in matrigel (reviewed by Lin and Bissell, 1993).

Compared with the rodent system, analyses of morphogenesis of human mammary epithelial cells have progressed more slowly, in part because of difficulties in maintaining cultures of primary human cells. This problem has been partially alleviated by the development of media for the culture of primary human mammary epithelial (HMEC) cells although establishment of primary cultures remains problematic (Stampfer, 1985; Bergstraesser and Weitzman, 1993). One alternative is the use of continuous human mammary epithelial cell lines such as MCF-10A (Soule et al., 1990). Indeed, a model for the study of mammary epithelial cell morphogenesis using MCF-10A cells has recently been described (Howlett et al., 1995).

It has now been shown that laminin-1 is the matrix component of matrigel which regulates morphogenesis as well as milk protein expression of mouse mammary epithelial cells in vitro (Streuli et al., 1995). Furthermore, the domain responsible for such regulation resides in the so-called E3 fragment of laminin-1 and is located towards the carboxy terminus of the α1 subunit of the heterotrimer (Streuli et al., 1995). Laminin-1, via its cell surface receptors, is believed to establish polarity of mammary epithelial cells, a process which is an essential prerequisite to cell differentiation (Streuli et al., 1995). However, following polarization, it is hypothesized that epithelial cells modulate their own microenvironment by producing additional basement membrane components (Bissell and Ram, 1989). The latter could include a number of laminins since laminin-1 is only one of several laminin isoforms which occur in intact basement membranes (Timpl and Brown, 1994). For example, laminin-5 is widely distributed in the basement membranes of epithelial tissues, including the mammary gland, as we show here (Verrando et al., 1987; Rousselle et al., 1991; Carter et al., 1991; Kallunki et al., 1992; Timpl and Brown, 1994). Do these endogenously secreted basement membrane elements play a role in mammary epithelial morphogenesis? To answer this question, we have analyzed the function of laminin-5 in an in vitro model of mammary epithelial morphogenesis using MCF-10A cells. These cells undergo branching morphogenesis i.e. assemble a highly anastomosed multicellular network, when cultured on matrigel. We show that matrigel-induced differentiation of MCF-10A cells is inhibited by function blocking laminin-5 antibodies as well as antibodies against two distinct laminin-5 receptors. Since laminin-5 is a component of certain cell-matrix junctions called

hemidesmosomes and MCF-10A cells assemble hemidesmosomes in vitro, we discuss the possibility of signaling events transduced by these complex morphological entities.

## MATERIALS AND METHODS

### Cell culture

MCF-10A cells were obtained from the American Tissue Culture Collection (Rockville, MD) and were maintained in a 1:1 mix of DME and Ham's F12 media supplemented with 5% equine serum, 0.01 mg/ml insulin, 20 ng/ml EGF, 100 ng/ml cholera toxin, and 500 ng/ml hydrocortisone. SCC12 cells were maintained in a serum free growth medium (Medium 154; Cascade Biologics, Inc., Portland, OR).

For our morphogenesis assays, matrigel was purchased from Collaborative Biomedical Products (Bedford, MA) and was coated onto plastic dishes at approximately 15 mg/ml. The dishes were subsequently incubated at 37°C for 30 minutes prior to addition of cells. In some instances, cells were mixed with liquid matrigel at 4°C. The cell/matrigel mix was then pipetted onto plastic and allowed to gel at 37°C.

### Antibodies

GB3, a mouse monoclonal antibody which recognizes the γ chain of human laminin-5, was obtained from Harlan Bioproducts for Science, Inc., Indianapolis, IN (Verrando et al., 1987; Matsui et al., 1995). Mouse monoclonal antibody, clone 17, specific for the β chain of laminin-5 was purchased from Transduction Laboratories (Lexington, KY). Dr William Carter, Fred Hutchinson Cancer Research Center generously provided C2-9, a function blocking mouse monoclonal antibody specific for the α3 chain of laminin-5 and P1E1, a non-function blocking antibody which also recognizes the α3 chain of human laminin-5 (Xia et al., 1996). We used P1E1 as a control IgG in some of our antibody inhibition studies. The rabbit serum J17, against BP180 and the mouse monoclonal antibody 10C5, against BP230, have been described elsewhere (Hopkinson et al., 1992; Hopkinson and Jones, 1994). GoH3, a rat monoclonal which recognizes the α6 integrin subunit, was purchased from Immunotech (Westbrook, ME). P1B5 and 3E1, mouse monoclonal antibodies which recognize the α3 integrin and β4 integrin subunits, respectively, were purchased from Gibco BRL (Gaithersburg, MD). Rabbit sera 6945 and 6845, against β4 integrin and the 'light' chain of the α6 integrin subunit, respectively, were kindly provided by Dr Vito Quaranta, Scripps Institute (Tamura et al., 1990).

### Immunofluorescence

MCF-10A cells, maintained on glass coverslips, were either permeabilized in acetone at –20°C for 2 minutes and air dried thoroughly, or, for integrin localization, were first fixed for 5 minutes in 3.7% formaldehyde, washed thoroughly in PBS, and then permeabilized in acetone at –20°C for 2 minutes prior to air drying. Cells maintained in matrigel were prepared for immunofluorescence analyses by first fixing them for 10 minutes in 3.7% formaldehyde. After washing thoroughly in PBS, they were permeabilized with 0.5% Triton X-100 in PBS at 4°C for 10 minutes and then washed once again in PBS. Preparations were incubated with primary antibody diluted in PBS at 37°C in a humid chamber for 1 hour, washed 3 times in PBS, and incubated with an appropriate fluorochrome-conjugated secondary antibody for a further 1 hour at 37°C.

For frozen tissue sections, normal human breast tissue from a reduction mammoplasty was received from the Cooperative Human Tissue Network (Columbus, OH). Tissue was snap frozen in liquid nitrogen and embedded in Tissue-Tek OCT compound (Miles Laboratory, Elkhart, IN). Sections (10 μm) of the frozen tissue were prepared and mounted on poly-L-lysine coated microscope slides. Sections were fixed for 5 minutes in –20°C acetone, air-dried thoroughly, and stained for immunofluorescence as above.

Fluorescence specimens were visualized using a Zeiss LSM10 laser scanning confocal microscope (Zeiss Inc., Thornwood, NY). Images were stored on Sony optical discs and printed on a Tektronix printer (Tektronix, Wilsonville, OR).

### Protein preparations, SDS-PAGE and western immunoblotting

Confluent cell cultures were solubilized in sample buffer consisting of 8 M urea, 1% sodium dodecyl sulfate (SDS) in 10 mM Tris-HCl, pH 6.8, and 15% β-mercaptoethanol. DNA was sheared by sonication using a 50 W Ultrasonic Processor (Vibracell Sonics and Materials Inc., Danbury, CT). Matrix of MCF-10A cells was prepared according to Gospodarowicz (1984) and solubilized in sample buffer. Proteins were separated by SDS-PAGE, transferred to nitrocellulose and processed for immunoblotting according to the method of Zackroff et al. (1984).

### Immunoprecipitation

Subconfluent dishes of MCF-10A cells were radiolabeled overnight with 50 μCi/ml of $^{35}$S-PRO-MIX cell label (Amersham Corp., Arlington Heights, IL). Conditioned medium of the labeled MCF-10A cells was collected and then pre-cleared by incubation with Protein G-Sepharose beads (Gibco BRL, Gaithersburg, MD) for one hour at 4°C. After centrifugation, monoclonal antibodies were added to the supernatant and the mix was then incubated for 1 hour at 4°C. Protein G-Sepharose beads were added and the tubes incubated for an additional hour at 4°C. Beads were collected by centrifugation and washed 5 times in TBS (10 mM Tris-HCl, pH 7.4, 145 mM NaCl and 1 mM PMSF) containing 1% Triton X-100. Proteins eluted from the beads in sample buffer were processed for SDS-PAGE/autoradiography as well as immunoblotting.

### Electron microscopy

Cells maintained on tissue culture plastic or on matrigel were fixed for a minimum of 30 minutes in 2% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2. After being washed three times in 0.1 M sodium cacodylate buffer, cells were post-fixed in 1% $OsO_4$ containing 0.8% potassium ferricyanide. Preparations were subsequently stained with uranyl acetate, dehydrated in ethanol, and embedded in Epon-Araldite resin (Tousimis Corp., Rockville, MD). Thin sections of embedded material were stained with lead nitrate and sodium citrate and viewed at 60 kV in a JEOL 100CX electron microscope (JEOL USA, Peabody, MA).

## RESULTS

### MCF-10A cells express laminin-5 as well as hemidesmosomal proteins

MCF-10A cells were maintained on glass coverslips for 24 hours and then processed for immunofluorescence microscopy using monoclonal antibodies against laminin-5. The latter stain in a leopard spot pattern along sites of cell-substrate association as determined by confocal laser scan microscopy (Fig. 1A). Laminin-5 antibody reactivity also occurs along areas of the glass coverslip where there are no apparent cells, suggesting that the MCF-10A cells leave behind 'trails' of laminin-5 as they migrate over their substrate. Since MCF-10A cells are derived from human mammary glands, we also determined whether laminin-5 is a component of breast epithelial basement membranes. Indeed, basement membranes encircling groups of breast epithelial cells show strong reactivity with laminin-5

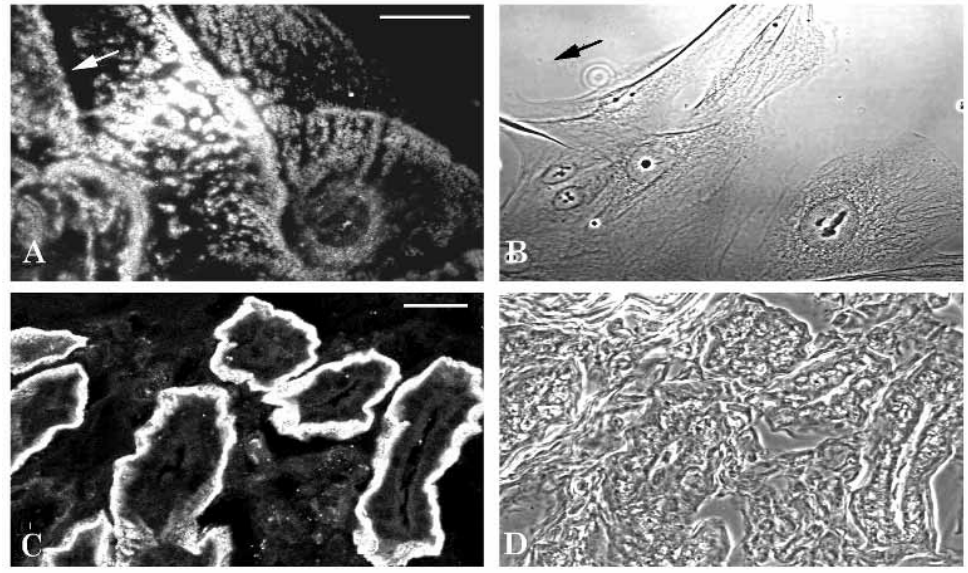


**Fig. 1.** Laminin-5 is expressed by MCF-10A cells and in human breast tissue. MCF-10A cells were cultured on glass coverslips and processed for indirect immunofluorescence microscopy using the laminin-5 monoclonal antibody (GB3) (A). The cells were viewed by confocal microscopy, the plane of focus being close to the cell-substrate interface. The laminin-5 antibodies stain in a typical leopard spot pattern. The GB3 antibodies also stain areas where there are no apparent cells (arrow). (C) A cryosection of human breast tissue from a reduction mammoplasty was processed for immunofluorescence with GB3 antibodies. These stain the basement membrane zones of islands of epithelial cells. (B and D) Phase contrast images. Bars, 10 μm.

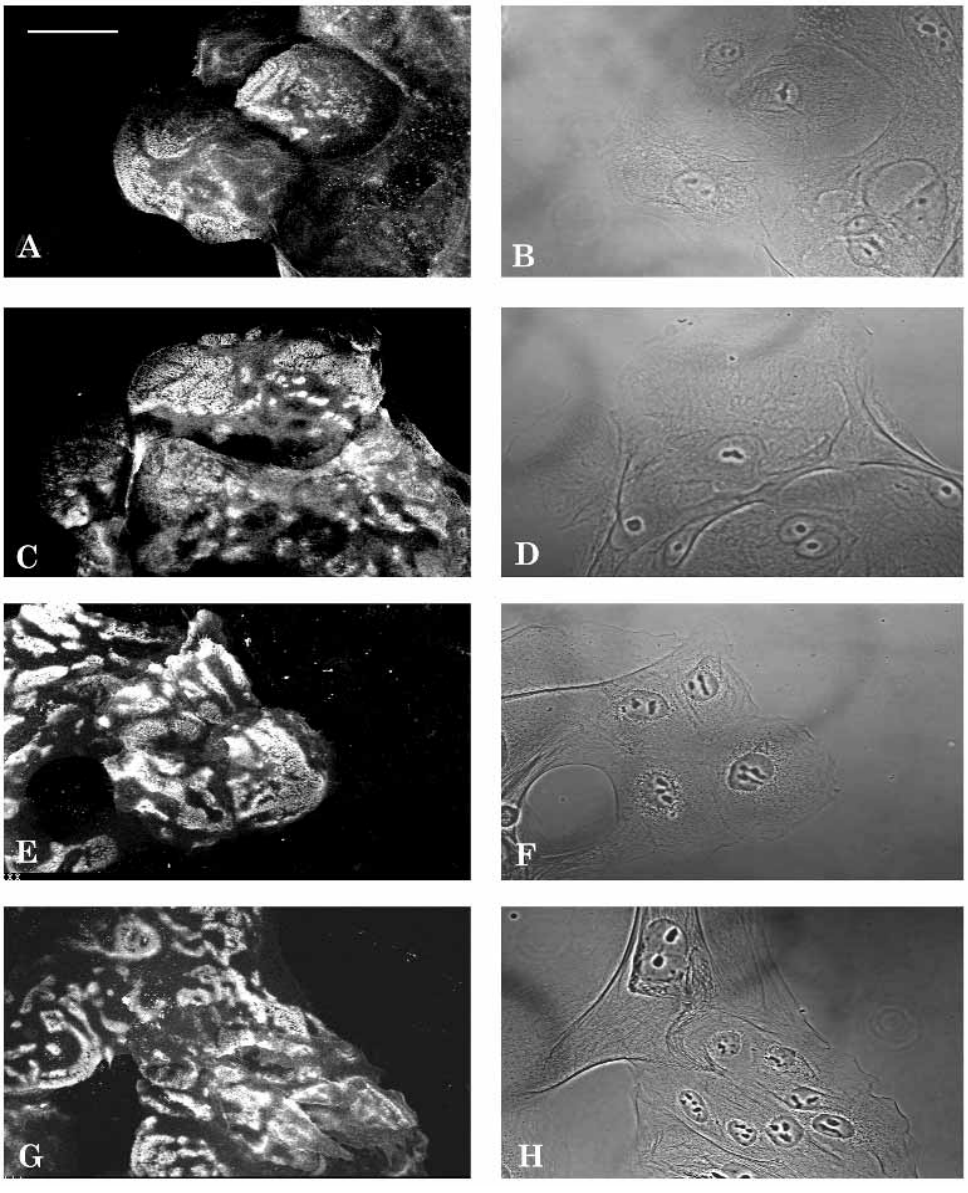


**Fig. 2.** Hemidesmosomal proteins are expressed by MCF-10A cells as shown by indirect immunofluorescence. MCF-10A cells, maintained on glass coverslips were processed for indirect immunofluorescence microscopy using antibodies specific for BP180 (J17) (A), BP230 (10C5) (C), β4 integrin (3E1) (E), and α6 integrin (GoH3) (G). In all cases the antibodies generate a patchy, leopard spot stain along the region of cell-coverslip interaction. (B,D,F,H) Phase contrast images of the cells. Bar, 10 μm.

antibodies in cryosections of mammary tissue material (Fig. 1C).

In addition to laminin-5, MCF-10A cells, processed for indirect immunofluorescence microscopy, are recognized by antibodies against major components of hemidesmosomes including both bullous pemphigoid antigens (BP180, BP230) as well as the β4 and α6 integrin subunits (Jones et al., 1994; Green and Jones, 1996) (Fig. 2). All of these antibodies generate similar leopard spot staining patterns along the basal aspect of the adherent cells (Fig. 2). This pattern is comparable to that generated by laminin-5 antibodies (Fig. 1A). However, unlike laminin-5, there is an absence of hemidesmosome protein in areas of the glass coverslips devoid of cells (Fig. 2).

Electron microscopic analyses of MCF-10A cells reveals that they assemble hemidesmosome-like structures where they abut their substrates (Fig. 3). These structures possess all of the morphological features of hemidesmosomes observed in mammary epithelial cells in situ i.e. they have triangular shaped, trilayered cytoplasmic plaques (Fig. 3; Jones et al., 1994; Bergstraesser et al., 1995).

To confirm that MCF-10A cells express hemidesmosome components, we have analyzed cell extracts by immunoblotting using antibodies directed against BP180 and BP230, and antisera against β4 integrin and the 'light' chain of α6 integrin (Fig. 4A). These antibodies recognize species of 180, 230, 200 and 30 kDa, respectively (Fig. 4A, lanes 1,3,5 and 7). Furthermore, the MCF-10A hemidesmosomal proteins co-migrate with their epidermal equivalents present in extracts of SCC12 cells (Fig. 4A, lanes 2,4,6 and 8).

## MCF-10A cells produce a laminin-5 rich matrix and secrete soluble laminin-5

We have analyzed both the matrix deposited onto substrates by MCF-10A cells as well as MCF-10A conditioned medium for the presence of laminin-5 using a combination of immunoblotting and immunoprecipitation. MCF-10A matrix was prepared according to the procedure of Gospodarowicz (1984). This matrix contains four prominent polypeptides of 155, 135, 100 and 80 kDa and is rich in subunits of laminin-5 as shown by immunoblotting using a monoclonal antibody which recognizes the β2 135 kDa laminin-5 subunit (Fig. 4B). In addition, the 155, 135 and 100 kDa species present in MCF-10A matrix co-migrate with the major polypeptides immunoprecipitated from MCF-10A conditioned medium by two laminin-5 monoclonal antibodies (GB3 and C2-9) (Fig. 4C, lanes 1 and 3). The 135 kDa polypeptides immunopreciptated from MCF-10A conditioned medium by both these anti-laminin-5 monoclonal antibodies are recognized by the β2 chain antibody in immunoblots (Fig. 4C, lanes 2 and 4).

## MCF-10A cells undergo branching morphogenesis when plated on matrigel

When MCF-10A cells are embedded into liquid matrigel, which is then allowed to gel, they remain as discrete cellular aggregates ('acini') for 7 days or more regardless of cell concentration (Howlett et al., 1995). In contrast, MCF-10A cells form an interconnected set of tube-like structures, one day after being plated at a concentration of $2.5\times10^4$ cells/cm$^2$ on top of matrigel (Fig. 5A). These are similar to the networks of HMECs observed in matrigel and collagen I gels (Bergstraesser et al., 1996; Berdichevsky et al., 1994).

The ability of MCF-10A cells to assemble into tube-like arrays is cell concentration dependent. At cell concentrations of $1.25\times10^4$/cm$^2$ or below the MCF-10A cells remain as small aggregates on the matrigel (Fig. 5B). Indeed, they remain in similar aggregates even at 7 days following plating (result not shown).

The tube-like multicellular aggregates of MCF-10A cells in matrigel were processed for confocal immunofluorescence microscopy using antibodies against laminin-5, α6 integrin and α3 integrin (Fig. 6). Both laminin-5 and α6 integrin are concentrated along the edges of the MCF-10A tubes where the cells abut matrigel (Fig. 6A,B). α3 integrin is localized at the

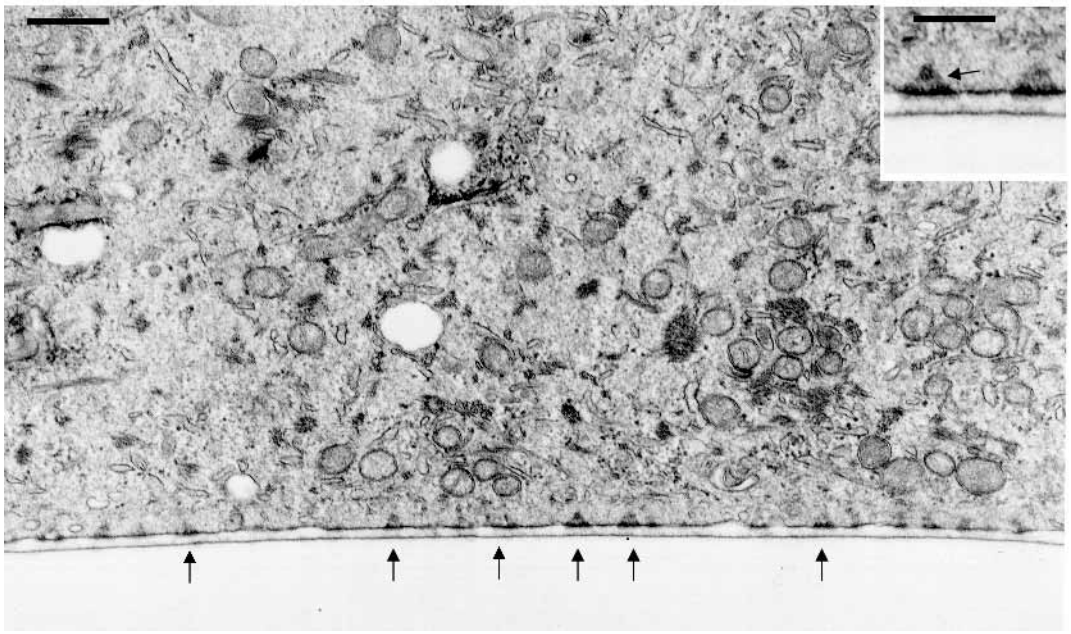

**Fig. 3.** MCF-10A cells assemble hemidesmosomes when maintained in vitro. This electron micrograph shows a cross section of MCF-10A cells. Arrows indicate numerous electron dense hemidesmosome structures. These possess tripartite cytoplasmic plaques (inset, arrow). Bar, 500 nm (inset, 250 nm).

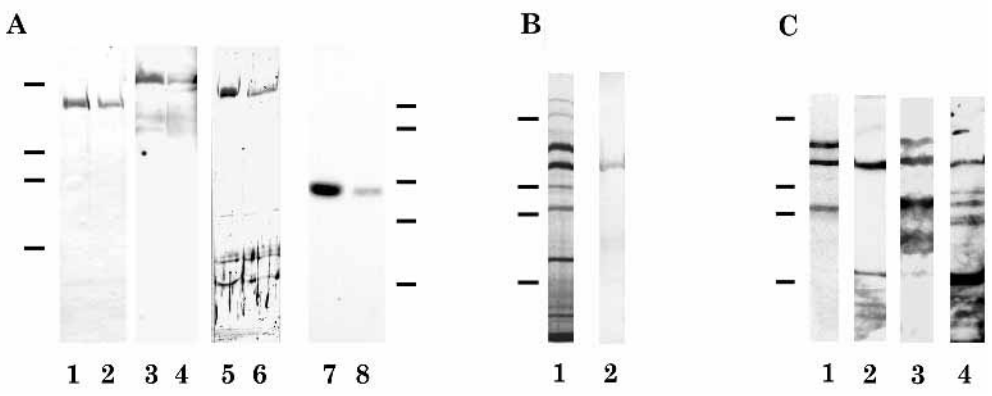


**Fig. 4.** (A) Hemidesmosomal proteins are expressed by MCF-10A cells as shown by immunoblotting. MCF-10A cell extracts (lanes 1,3,5 and 7) and extracts of SCC12 cells, a keratinocyte line (lanes 2,4,6 and 8) were separated by SDS-PAGE on either 6% (lanes 1-6) or 15% (lanes 7,8) polyacrylamide gels, transferred to nitrocellulose, and immunoblotted with antibodies against BP180 (J17, lanes 1,2), BP230 (10C5, lanes 3,4), β4 integrin (6945, lanes 5,6), or the 'light' chain of α6 integrin (6845, lanes 7,8). (B) MCF-10A cells deposit laminin-5 on their substrate. MCF-10A matrix was collected according to the method of Gospodarowicz (1984), processed for SDS-PAGE on a 6% gel, and either silver stained (lane 1) or transferred to nitrocellulose and immunoblotted with a monoclonal antibody (clone 17) against the β chain of laminin-5 (lane 2). In the silver stained preparation, there are prominent polypeptides at 150, 135 and 100 kDa representing the α, β and the γ chains of laminin-5 (lane 1). The 135 kDa protein in this preparation is recognized by the clone 17 antibody (lane 2). (C) MCF-10A secrete laminin-5 into their medium. The medium conditioned by radio-labeled MCF-10A cells was processed for immunoprecipitation using two monoclonal laminin-5 antibodies (GB3, lanes 1,2; C2-9, lanes 3,4). The immunoprecipitated proteins were analyzed by SDS-PAGE/autoradiography (lanes 1 and 3) or prepared for immunoblotting using clone 17 monoclonal antibody against the β chain of laminin-5 (lanes 2 and 4). The laminin-5 antibodies precipitate three major polypeptides of 150, 135 and 100 kDa (lanes 1,3). The 135 kDa protein is recognized by the clone 17 antibody (lanes 2,4). Note that there is some breakdown of the laminin-5 in the C2-9 antibody precipitate (lane 2). This may explain the ladder of proteins recognized by the clone 17 antibody in lane 4. The low molecular mass reactive species in lanes 2 and 4 are due to cross reactivity of the secondary antibody anti-mouse IgG with the immunprecipitated mouse IgG. Bars on the left side of A, B, and C indicate molecular mass standards of 200, 116, 97.4, and 66 kDa. Bars on the right side of A indicate standards of 66, 45, 31, 21.5 and 14.5 kDa. Each lane of the gels was loaded with approximately 10 μg of protein.

latter sites although it is also present at areas of cell-cell contact (Fig. 6C). An IgG control fails to stain the cell population in Fig. 6D.

### Antibody inhibition of MCF-10A morphogenesis

We next used an immunological approach to assess the potential role of laminin-5 and its receptors (the integrins α6β4 and α3β1) in matrigel induced branching morphogenesis of MCF-10A cells. For these studies MCF-10A cells were incubated for 15 minutes at 37°C in medium containing either control IgG (50 μg/ml) or in function blocking antibodies directed against α6 integrin (GoH3 at 50 μg/ml), α3 integrin (P1B5 diluted 1:20) and laminin-5 (C2-9 diluted 1:5) (Fig. 7). The cells in the antibody containing medium were plated onto matrigel coated surfaces at $2.5\times10^4/cm^2$. After 24 hours the cells incubated in control IgG had formed long interconnected tubes whereas there was an obvious inhibition of branching morphogenesis in cultures which had been incubated in the α3 and α6 integrin antibodies as well as those cells incubated with the laminin-5 antibodies (Fig. 7).

We also fixed and processed the antibody treated cells for electron microscopy. We analyzed at least twenty MCF-10A cells in contact with matrigel under each experimental condition (Fig. 8). MCF-10A cells plated onto matrigel in the presence of control IgG assemble hemidesmosomes at sites of cell-matrigel association (Fig. 8A). The latter appear as electron dense structures with extracellular sub-basal dense plates which indicate formation of 'mature' hemidesmosomes (Fig. 8A, inset). In contrast, no hemidesmosomes were observed along regions of cell-matrigel interaction in cultures incubated in function blocking α3 integrin, α6 integrin and laminin-5 antibodies (Fig. 8B-D).

### Conclusions

In this study we have shown that MCF-10A cells, an immortalized mammary epithelial cell line, like HMECs, derived from reduction mammoplasties, undergo a branching morphogenesis when maintained on matrigel (Bergstraesser et al., 1996). This phenomenon is highly dependent on cell concentration. We have never observed the formation of tubular arrays when MCF-10A cells are plated onto matrigel at concentrations below $1.25\times10^4 cells/cm^2$. Just a twofold increase

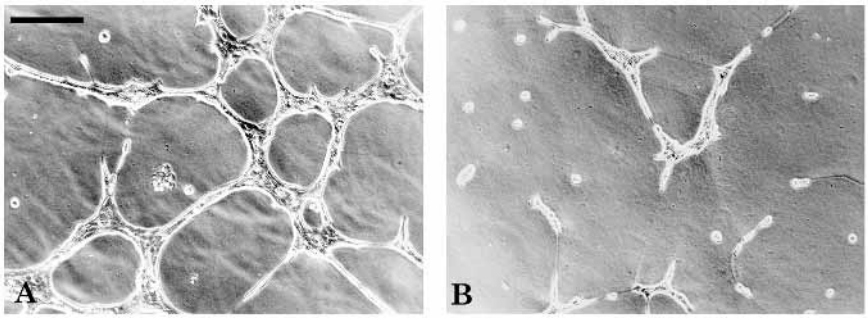


**Fig. 5.** MCF-10A cells undergo branching morphogenesis on matrigel in a cell concentration dependent manner. $2.5\times10^4/cm^2$ (A) and $1.25\times10^4/cm^2$ (B) MCF-10A cells were plated onto matrigel which had been used to coat 35 mm dishes. At 24 hours following plating, the cells in A have undergone a branching morphogenesis while the cells in B appear in small aggregates. Bar, 500 μm.

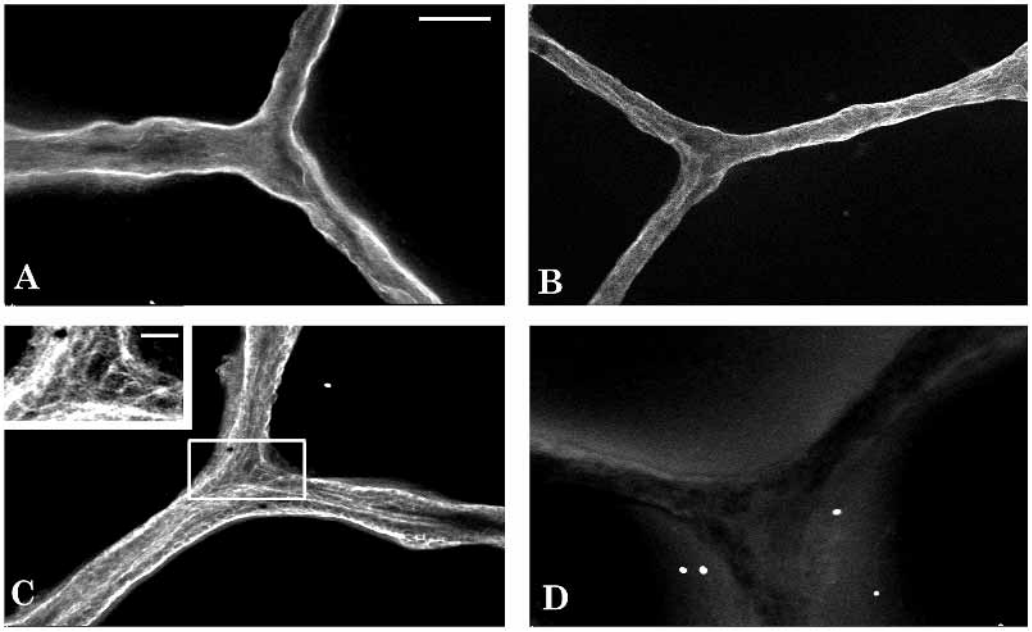


**Fig. 6.** Laminin-5 and its receptors are expressed by MCF-10A cells undergoing morphogenesis on matrigel. MCF-10A cells maintained on matrigel for 24 hours were processed for indirect confocal immunofluorescence with monoclonal antibodies recognizing laminin-5 (GB3, A), α6 integrin (GoH3, B), α3 integrin (P1B5, C), or an IgG control (D). Note that the antibodies in A,B and C show staining along regions of cell-matrigel interaction. The inset in C is a higher magnification of the boxed area and reveals that α3 integrin occurs at sites of cell-cell as well as cell-matrigel interaction. Bars: (A), 100 μm; (C), 25 μm.

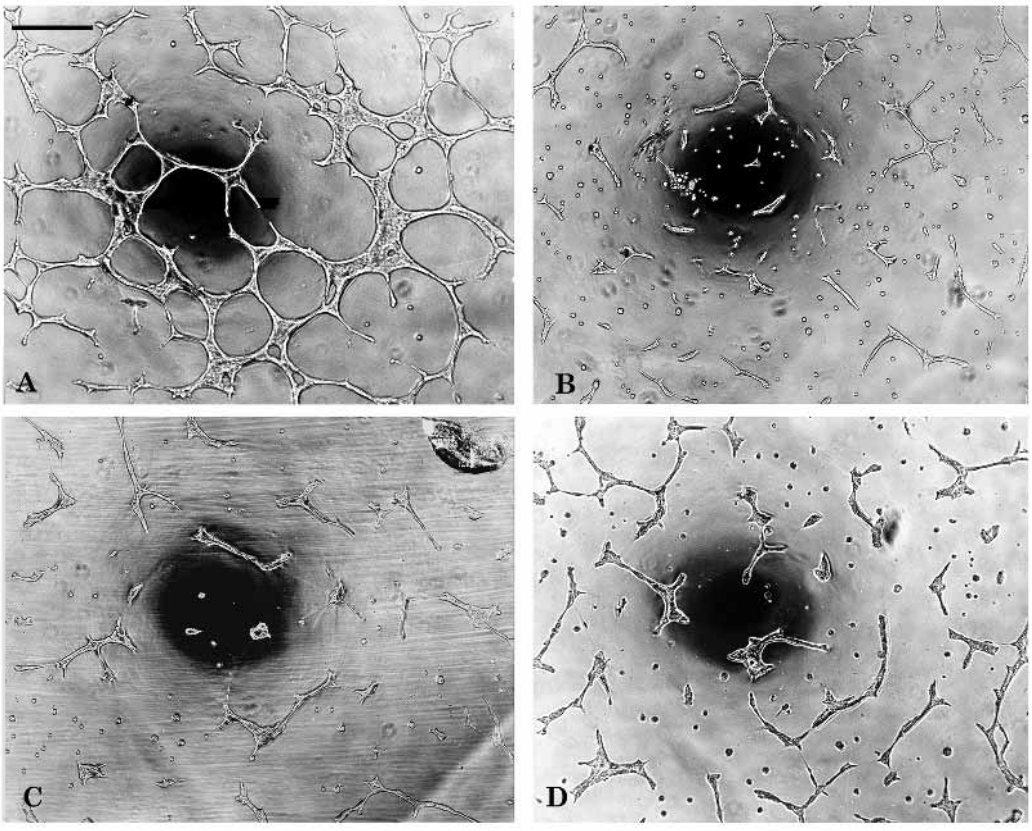


**Fig. 7.** Branching morphogenesis of MCF-10A cells on matrigel is inhibited by antibodies against laminin-5, α6 integrin and α3 integrin. MCF-10A cells were plated onto matrigel in the presence of control IgG (A), or antibodies which block the function of α6 integrin (GoH3, B), α3 integrin (P1B5, C), or laminin-5 (C2-9, D). After 24 hours, the cells in A appear organized into a highly branched array, while those incubated with blocking antibodies remain either as single cells or in small multicellular clusters (B,C and D). The large dark circle in each of the micrographs is an optical artifact. Bar, 100 μm.

in this cell number is enough to trigger a matrigel induced branching morphogenesis of the MCF-10A cells. Indeed, we find it remarkable that within 1 day of plating onto matrigel, MCF-10A cells assemble into an anastomosing network, organized into a branching pattern much like that seen in vivo in postpubertal mammary glands (Daniel and Silberstein, 1987). This type of pattern has been observed by Berdichevsky et al. (1994) when the human mammary cell line HB-2 is maintained in collagen type I gels.

HMECs assemble hemidesmosomes in vivo (Watson et al., 1988). In vitro they are also capable of forming hemidesmosomes, although this generally takes up to 14 days following plating on tissue culture substrates (Bergstraesser et al., 1995). Like HMECs in vivo, MCF-10A cells express the major components of hemidesmosomes as determined by immunoflu-

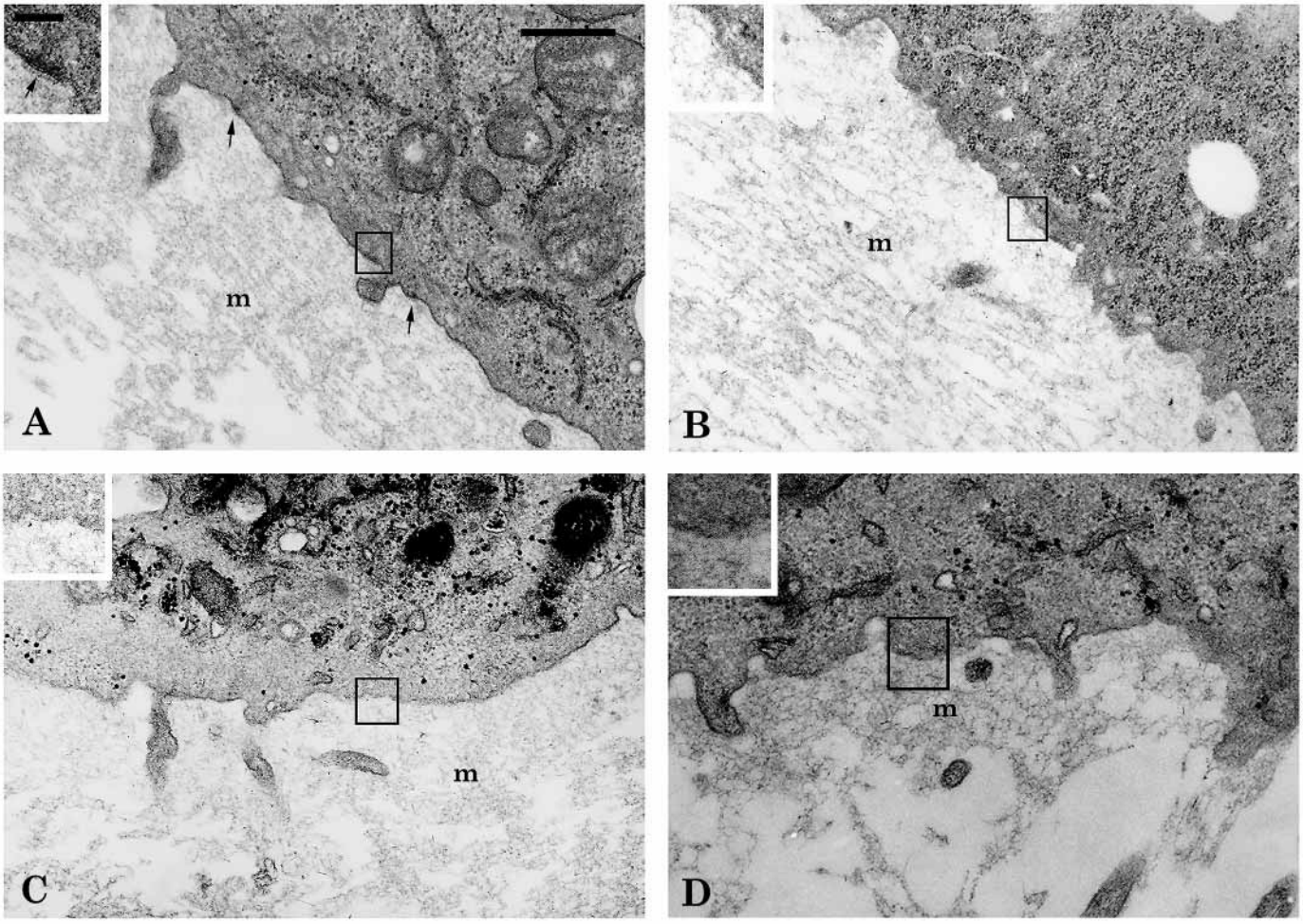


**Fig. 8.** MCF-10A cells assemble hemidesmosomes on matrigel but this is inhibited by integrin and laminin-5 antibodies. MCF-10A cells were plated onto matrigel in the presence of control IgG (A), or antibodies which block the function of α6 integrin (GoH3, B), α3 integrin (P1B5, C), or laminin-5 (C2-9, D). After 24 hours the cells on matrigel were fixed for electron microscopy. Note that in A there are three hemidesmosome-like structures along the region of cell-matrigel interaction (arrows). One of these (in the box) is shown at higher power in the inset. It possesses an electron dense cytoplasmic plaque and also a sub-basal dense plate. There are no obvious hemidesmosomes in cells in contact with matrigel in B-D (higher power views of these regions are shown in the insets). m, matrigel. Bars: (A), 500 nm; (inset), 60 nm.

orescence, immunoblotting and immunoprecipitation. Moreover, MCF-10A cells readily assemble hemidesmosome-like structures within 24 hours after plating onto uncoated glass coverslips i.e. much faster than their normal counterparts. The speed of hemidesmosome appearance in MCF-10A cells was the more surprising since earlier work had suggested that MCF-10A cells were unable to assemble bona fide hemidesmosomes in vitro (Tait et al., 1990).

When maintained on matrigel, MCF-10A cells assemble hemidesmosomes at sites of cell-matrigel interaction. Consistent with this, a hemidesmosome associated matrix component and its receptor, namely laminin-5 and α6β4 integrin, are distributed at sites of MCF-10A cell-matrigel interaction. Such observations triggered our interest in the potential role of hemidesmosome components in branching morphogenesis of MCF-10A cells. Since it is already established that laminin-5 and α6β4 integrin heterodimer are essential for hemidesmosome assembly, we have been able to assay the role of hemidesmosomes in branching morphogenesis of MCF-10A cells by using antibodies which inhibit both the activities of laminin-5 and α6β4 integrin (Jones et al., 1991; Kurpakus et al., 1991; Spinardi et al., 1995; van der Neut et al., 1996; Georges-Labouesse et al., 1996; Baker et al., 1996).

Function blocking antibodies directed against laminin-5 not only prevent hemidesmosome assembly in MCF-10A cells maintained on matrigel but also significantly inhibit branching morphogenesis. Similarly, antibody GoH3, which blocks α6 integrin function, inhibits both hemidesmosome formation and MCF-10A morphogenesis. Since the α6 integrin subunit is known to preferentially bind β4 integrin in cells which coexpress both of its β1 and β4 integrin partners, as is the case in MCF-10A cells, the inhibitory effects of GoH3 antibodies on MCF-10A cells likely impact the function of the hemidesmosome-associated α6β4 integrin heterodimer (Giancotti et al., 1992; S. E. Baker and J. C. R. Jones, unpublished observations).

Indeed, we assume that matrigel, or more specifically its laminin-1 component, provides an initial framework for MCF-10A attachment and triggers a series of morphogenetic events (Streuli et al., 1995). This includes secretion of laminin-5

which then induces the MCF-10A cells to nucleate the assembly of their own hemidesmosomes, a process requiring laminin-5/α6β4 integrin interaction. We suggest that the formation of the latter complex is necessary to complete branching morphogenesis.

The idea that hemidesmosomes may be involved in morphogenetic events is supported indirectly by recent reports which indicate that hemidesmosomes are sites of signal transduction (Maniero et al., 1995, 1996). For example, the β4 subunit of the α6β4 hemidesmosome associated integrin possesses an unusually long cytoplasmic tail which is associated with one or more protein kinases (Tamura et al., 1990; Maniero et al., 1995). The latter are believed to be involved in a matrix induced cascade of phosphorylation events resulting in phosphorylation not only of the β4 integrin subunit but also of a recently identified protein of 80 kDa (Xia et al., 1996; Maniero et al., 1995).

Laminin-5 and α6 antibodies are not exclusive in their abilities to block morphogenesis of MCF-10A cells in matrigel. A function perturbing α3 integrin antibody, P1B5, is also capable of inhibiting matrigel induced branching morphogenesis of MCF-10A cells. The α3β1 integrin heterodimer is not a component of the hemidesmosome but, like α6β4 integrin is a receptor for laminin-5 (Carter et al., 1990, 1991). In in vitro assays, it has been shown that cell interaction with laminin-5 is initiated by the α3β1 integrin heterodimer (Carter et al., 1991). Subsequently laminin-5 appears to 'switch' receptors and binds to the α6β4 integrin as a prelude to hemidesmosome assembly (Carter et al., 1990, 1991; Spinardi et al., 1995; Xia et al., 1996). Thus one explanation for the morphogenetic impact of the α3 integrin blocking antibody is that P1B5 inhibits the interaction of cells with their own laminin-5. However, we cannot discount that α3 integrin is involved in cell binding to the laminin-1 component of matrigel (Streuli et al., 1995). Of course, P1B5 may inhibit both laminin-1 and laminin-5 interactions of the MCF-10A cells.

In summary, we have identified a model system and a continuous cell line, MCF-10A, for the study of the role of hemidesmosome matrix and integrin components in tissue morphogenesis. In this model, matrigel provides a three-dimensional environment which triggers a series of cellular morphogenetic events, involving the assembly of hemidesmosomes and expression of hemidesmosome matrix and integrin components, in MCF-10A cells. Indeed, it is becoming clear that the hemidesmosome is not simply a spot weld to tether cells to connective tissue but, through the functional properties of its components, the hemidesmosome can have a profound impact on the differentiation and organization of epithelia at the tissue level.

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## REFERENCES

**Baker, S. E., Hopkinson, S. B., Fitchmun, M., Andreason, G. L., Frasier, F., Plopper, G., Quaranta, V. and Jones, J. C. R.** (1996). Laminin-5 and hemidesmosomes: role of the α3 chain subunit in hemidesmosome stability and assembly. *J. Cell Sci.* **109**, 2509-2520.

**Barcellos-Hoff, M. H., Aggeler, J., Ram, T. G., and Bissell, M. J.** (1989). Functional differentiation and alveolar morphogenesis of primary mammary cultures on reconstituted basement membrane. *Development* **105**, 223-235.

**Berdichevsky, F., Alford, D., D'Souza, B. and Taylor-Papadimitriou, J.** (1994). Branching morphogenesis of human mammary epithelial cells in collagen gels. *J. Cell Sci.* **107**, 3557-3568.

**Bergstraesser, L. M. and Weitzman, S. A.** (1993). Culture of normal and malignant primary human mammary epithelial cells in a physiological manner simulates *in vivo* growth patterns and allows discrimination of cell type. *Cancer Res.* **53**, 2644-2654.

**Bergstraesser, L. B., Srinvasan, G., Jones, J. C. R., Stahl, S. and Weitzman, S. A.** (1995). Expression of hemidesmosomes and component proteins is lost by invasive breast cancer cells. *Am. J. Pathol.* **147**, 1823-1839.

**Bergstraesser, L. M., Sherer, S., Panos, R. and Weitzman, S. A.** (1996). Stimulation and inhibition of human mammary epithelial cell duct morphogenesis in vitro. *Proc. Assoc. Am. Phys.* **108**, 140-154.

**Bissell, M. J. and Ram, T. G**. (1989). Regulation of functional cytodifferentiation and histogenesis in mammary epithelial cells: role of the extracellular matrix. *Environ. Health Perspect.* **80**, 61-70.

**Blum, J. L., Zeigler, M. E. and Wicha, M. S.** (1989). Regulation of mammary differentiation by the extracellular matrix. *Environ. Health Perspect.* **80**, 71-83.

**Carter, W. G., Kaur, P., Gil, S. F., Gahr, P. J. and Wayner, E. A.** (1990). Distinct functions for integrins α3β1 in focal adhesions and α6β4 /bullous pemphigoid antigen in a new stable anchoring contact (SAC) of keratinocytes: relation to hemidesmosomes. *J. Cell Biol.* **111**, 3141-3154.

**Carter, W. G., Ryan, M. C. and Gahr, P. J.** (1991). Epiligrin, a new cell adhesion ligand for integrin α3β1 in epithelial basement membranes. *Cell* **65**, 599-610.

**Daniel, C. W. and Silberstein, G. B.** (1987). Postnatal development of the rodent mammary gland. In *The Mammary Gland: Development, Regulation and Function* (ed. M. C. Neville and C. W. Daniel), pp. 3-36. Plenum Publishing Corp., New York.

**Georges-Labouesse, E., Messaddeq, N., Yehia, G., Cadalbert, Dierich, A. and Le Meur, M.** (1996). Absence of integrin α6 leads to epidermolysis bullosa and neonatal death in mice. *Nature Genet.* **13**, 370-373.

**Giancotti, F. G., Stepp, M. A., Suzuke, S., Engvall, E. and Ruoslahti, E.** (1992). Proteolytic processing of endogenous and recombinant β4 integrin subunit. *J. Cell Biol.* **118**, 951-959.

**Gospodarowicz, D.** (1984). In *Methods for Preparation of Media Supplements and Substrata*, vol 1 (ed. D. W. Barnes, D. A. Sirbasku and G. H. Stao), pp. 275-293. Alan R. Liss, New York.

**Green, K. J. and Jones, J. C. R**. (1996). Desmosomes and hemidesmosomes: structure and function of molecular components. *FASEB. J.* **10**, 871-881.

**Hay, E. D.** (1993). Extracellular matrix alters epithelial differentiation. *Curr. Opin. Cell Biol.* **5**, 1029-1035.

**Hopkinson, S. B., Riddelle, K. S. and Jones J. C. R.** (1992). Cytoplasmic domain of the 180-kD bullous pemphigoid antigen, a hemidesmomal component: molecular and cell biologic characterization. *J. Invest. Dermatol.* **99**, 264-270.

**Hopkinson, S. B. and Jones, J. C. R.** (1994). Identification of a second protein product of the gene encoding a human epidermal autoantigen. *Biochem. J.* **300**, 851-857.

**Howlett, A. R., Bailey, N., Damsky, C., Petersen, O. W. and Bissell, M. J.** (1995). Cellular growth and survival are mediated by β1 integrins in normal human breast epithelium but not in breast carcinoma. *J. Cell Sci.* **108**, 1945-1957.

**Jones, J. C. R., Kurpakus, M. A., Cooper, H. M. and Quaranta, V.** (1991). A function for the integrin α6β4 in the hemidesmosome. *Cell Reg.* **2**, 427-438.

**Jones, J. C. R., Asmuth, J., Baker, S. E., Langhofer, M., Roth, S. I. and Hopkinson, S. B.** (1994). Hemidesmosomes: extracellular matrix/intermediate filament connectors. *Exp. Cell Res.* **213**, 1-11.

**Kallunki, P., Saino, K., Eddy, R., Byers, M., Kallunki, T., Sariola, H., Beck, K., Hirvonen, H., Shows, T. B. and Tryggvason, K.** (1992). A truncated laminin chain homologous to the B2 chain: structure, spatial expression and chromosomal assignment. *J. Cell Biol.* **109**, 3377-3390.

**Kurpakus, M. A., Quaranta, V. and Jones J. C. R.** (1991). Surface relocation of α6β4 integrins and assembly of hemidesmosomes in an *in vitro* model of wound healing. *J. Cell Biol.* **115**, 1737-1750.

**Lin, C. Q. and Bissell, M. J.** (1993). Multi-faceted regulation of cell differentiation by extracellular matrix. *FASEB J.* **7**, 737-743.

**Maniero, F., Pepe, A., Wary, K. K., Spinardi, L., Mohammadi, M., Schlessinger, J. and Giancotti, F. G.** (1995). Signal transduction by the α6β4 integrin: distinct subunit sites mediate recruitment of Shc/Grb2 and association with the cytoskeleton of hemidesmosomes. *EMBO J.* **14**, 4470-4481.

**Mainiero, F., Pepe, A., Yeon, M., Ren, Y. and Giancotti, F. G.** (1996). The intracellular functions of α6β4 integrin are regulated by EGF. *J. Cell Biol.* **134**, 241-253.

**Matsui, C., Nelson, C. F., Hernandez, G. T., Herron, G. S., Bauer, E. A. and Hoeffler, W. K.** (1995). γ2 chain of laminin-5 is recognized by monoclonal antibody GB3. *J. Invest. Dermatol.* **105**, 648-652.

**Rousselle, P., Lunstrum, G. P., Keene, D. R. and Burgeson, R. E**. (1991). Kalinin: an epithelium-specific basement membrane adhesion molecule that is a component of anchoring filaments. *J. Cell Biol.* **114**, 567-576.

**Soule, H. D., Maloney, T. N., Wolman, S. R., Pererson, W. D. Jr, Brenz, R., McGrath, C. M., Russo, J., Pauley, R. J., Jones, R. F. and Brooks, S. C.** (1990). Isolation and characterization of a spontaneously immortalized human breast epithelial cell line MCF-10. *Cancer Res.* **50**, 6075-6086.

**Spinardi, L., Einheber, S., Cullen, T., Milner, T. A. and Giancotti, F. G.** (1995). A recombinant tail-less integrin β4 subunit disrupts hemidesmosomes, but does not suppress α6β4-mediated cell adhesion to laminins. *J. Cell Biol.* **129**, 473-487.

**Stampfer, M. R.** (1985). Isolation and growth of human mammary epithelial cells. *J. Tiss. Cult. Meth.* **9**, 107-115.

**Streuli, C. H., Schmidhauser, C., Bailey, N., Yurchenco, P., Skubitz, A. P. N., Roskelley, C. D. and Bissell, M. J.** (1995). Laminin mediates tissue-specific gene expression in mammary epithelia. *J. Cell Biol.* **129**, 591-603.

**Tait, L., Soule, H. D. and Russo, J.** (1990). Ultrastructural and immunocytochemical characterization of an immortalized human breast epithelial cell line MCF-10. *Cancer Res.* **50**, 6087-6094.

**Tamura, R. N., Rozzo, C., Starr, L., Chambers, J., Reichardt, L. F., Cooper, H. M. and Quaranta, V.** (1990). Epithelial integrin α6β4: complete primary structure of α6 and variant forms of β4. *J. Cell Biol.* **111**, 1593-1604.

**Timpl, R. and Brown, J. C.** (1994). The Laminins. *Matrix Biol.* **14**, 275-281.

**van der Neut, R., Krimpenfort, P., Calafat, J., Niessen, C. M. and Sonnenberg, A.** (1996). Epithelial detachment due to absence of hemidesmosomes in integrin β4 null mice. *Nature Genet.* **13**, 366-369.

**Verrando, P., Hsi, B. L., Yeh, C. J. G., Pisani, A., Serieys, N. and Ortonne, J. P.** (1987). Monoclonal antibody GB3, a new probe for the study of human basement membranes and hemidesmosomes. *Exp. Cell Res.* **93**, 862-869.

**Watson, R. J., Eyden, B. P., Howell, A. and Sellwood, R. A.** (1988). Ultrastructrual observations on the basal lamina in the normal human breast. *J. Anat.* **156**, 1-10.

**Xia, Y., Gill, S. G. and Carter, W. G.** (1996). Anchorage mediated by integrin α6β4 to laminin 5 (epiligrin) regulates tyrosine phosphorylation of a membrane associated 80-kD protein. *J. Cell Biol.* **132**, 727-740.

**Zackroff, R. V., Goldman, A. E., Jones, J. C. R., Steinert, P. M. and Goldman, R. D.** (1984). Isolation and characterization of keratin-like proteins from cultured cells with fibroblastic morphology. *J. Cell Biol.* **98**, 1231-1237.

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