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Modifications in the basement membrane supramolecular structure of type IV collagen and laminin 5 organization facilitates skin derivative formation

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Abstract

In intimate contact with epithelial tissue is the extracellular matrix that forms a highly specialized condensed layer known as the basement membrane. The is supramolecular structure involves type IV collagen and laminin 5 is known to provide physical support for the epithelial tissue overlying it. In this study we examine other proposed role(s) of these molecular structures and their receptors during skin development using the mammary gland as a model. The pattern of expression of these molecules during skin formation was examined using immunohistochemistry, utilizing collagen IV, laminin 5 and β4 or α6 integrin antibodies. The dissected mammary glands were also examined by transmission electron microscopy. Our results suggest that these supramolecular structures play important roles in skin derivative development, more specifically mammary gland formation, of these roles; they ease their resistance to skin derivatives down growth (invasion) into the under laying tissue.

Key words: Mammary gland, basement membrane, mammogenesis, developing skin.

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Introduction

In close contact with epithelial tissue the extracellular matrix forms a highly specialized condensed layer known as the basement membrane. Basement membrane supramolecular organization is determined principally but not solely by type IV collagen, laminin, nidogen (entactin), fibronectin, tenascin and perlecan. Interactions of the basement membrane molecules with their related cells are mediated by cell surface adhesion receptors, of which integrins are the main type.

Collagen IV is the principle type of collagen that forms the insoluble scaffolding of the basement membrane network. It is synthesised by both stromal and epithelial cells and seems to have both a stiffening as well as a flexibility role within the basement membrane [1]. Other types of collagen can be found at the epidermal basement membrane either associating with fibril surfaces (including type VI, IX, XII, and type XIV collagen), or as transmembranous proteins (including types XIII and XVII collagen). Defects in, or absence of, collagen VII has been shown to be the primary cause of dystrophic epidermolysis bullosa [2], an inherited skin blistering disorder. Similarly, a mutation in the collagen VII gene has recently been found to cause another skin disorder known as epidermolysis bullosa pruriginosa [3-5]. Collagen VII was reported to be missing during human foetal development from the tips of the developing skin appendageal buds [6], although it is present in the basement membrane of the skin where no appendage development has taken place. Collagen IV, however, completely surrounds the developing appendage and is continuous within the epidermis [6].

In mammals, 12 laminin isoforms have been described so far, however it appears that not all possible combinations are achievable due to assembly restrictions [see reviews by [7,8]]. For example, the γ2 chain has never been reported to combine with the β1 chain [see [7]]. Laminins can be further divided into 4 subfamilies according to the length of their N-terminal domain and the number of amino acids within it.

Laminin isoforms are tissue- as well as differentiation specific; for example in mature mammalian skin the basement membrane contains laminin 5 (α3β3γ2) [9], laminin 1 (α1β1γ1) [10], laminin 2 (α2β1γ1) [11], and laminin 10 (α5β1γ1) [11]. During skin development these isoforms appear and disappear according to the stage of development.Defects in the three chains of laminin 5 have been identified as the cause of junctional epidermolysis bullosa [12-19], and mutation in genes encoding the laminin 1 isoform produced a phenotype of junctional epidermolysis bullosa, see review [8].

Hayashi and colleagues have further shown that during hair development, laminin 1 is absent from the distal end of the growing hair follicle, but is present in the basement membrane underlying the skin and around the hair follicle neck [10]. In a different study, laminin 5 was shown to have a similar pattern [9]. The laminin 10-null mouse at E16.5 was reported to contain fewer hair germ cells than the control, and when fragments of skin from control E16.5 embryos were transplanted in the dorsal side of E16.5 laminin 10 null mice, the hair germ cells failed to grow and subsequently there was a complete regression of the hair follicle [20].

These studies suggest that laminin 1 and 5 probably contribute to the physical integrity of the skin and only "give way" at some sites when needed, demonstrated during appendage formation. In contrast, laminin 10 probably plays an opposing role, i.e. encouraging and supporting appendage growth.

Integrins are the major receptors for extracellular matrix molecules. They have been the subject of a large body of scientific research since they were discovered more than 20 years ago as a family of cell surface receptors [21].

The integrin α6β4 is the major integrin receptor in the skin at steady state and is found in the epidermal basal layer [22,23]. This integrin dimer connects the basal cells, through specialized cell-substrate attachment junctions known as hemidesmosomes, of which it is an integral part, to the basement membrane molecules laminin 1 and laminin 5 [1]. Absence of either α6 or β4 causes a severe skin blistering condition [24,25], that results from lack of functional hemidesmosomes.

Postnatal mammary gland basement membrane contains most of the laminin subunits: α1, α3, α5, β1, β2, β3, γ1 and γ2 (26). This suggests that the mammary gland basement membrane should contain; laminin 1 (α1β1γ1), 5 (α3β3γ2), and 10/11 (α5β1γ1 / α5β2γ1) [26]. Also mammary gland basement membrane has collagen IV [27]. The major integrin receptors in the mammary gland are α2β1, α3β1, α6β1, also α6β4 [see [28] and references therein).

Currently, there is considerable evidence for different roles played by the extra cellular matrix (ECM) proteins (laminin and collagen) and their cell surface receptors the integrins at the different mammary gland developmental stages.

Most of what is known about the role of ECM proteins, and in particular the basement membrane during mammary gland development, has been obtained from experiments on post-embryonic mammary glands. One of the very few experiments on expression of ECM proteins and their receptors at the basement membrane of embryonic mammary glands showed that basement membrane laminin 5 expression coincided with the basal keratinocyte expression of β4 integrins [29]. Both were present at the basement membrane around the mammary buds and epidermis at both E12 and E15. At E17, laminin 5 was almost absent from the developing mammary ducts and β4 was very much reduced, while both were still present at the basement membrane underlying the epidermis. This is analogous to the pattern of laminin 1 expression in developing hair follicles [10] and supports the suggestion that laminins are involved in mammary duct development (30). They also demonstrate the importance of laminins for epithelial invasion, through which mammary gland development is affected.

The role of the laminin-binding integrins during embryonic mammary gland development appears to be complicated and is currently incompletely understood. For example, mice lacking α3 or α6 or β4 integrins die at term or shortly after birth [24,25,31], so the potential effect on mammary gland development after birth is not known. However, examination of the E17 mammary gland of either α3-, α6- or β4-null mice revealed that they had normal mammary glands (similar to the wild type) (28). In a pioneering experiment, mammary rudiments of α3- or α6-null mice at E17 were transplanted into mammary fat pads of syngeneic hosts [28] (the experiment was not done with β4 nulls as lack of α6 also resulted in complete absence of β4 expression). The results showed that transplanted mammary rudiments of either genotype developed and functioned almost as normal mammary gland. Transplanted mammary rudiments of α6-null mice showed normal localisation of laminin 1 expression in the basement membrane, however, from electron microscope evidence as well as from the punctuate expression pattern of laminin 5 seen by immunofluorescence, it seems that the mammary glands in these mice suffer from abnormal hemidesmosome formation [28]. However this also shows that neither α3 nor α6 integrins are vital for mammary gland development.

From the evidence available it appears that laminins are crucial for epithelial invasion, however it is still unclear which types of laminins are involved in such mechanisms. There is also some ambiguity about which type of integrins interact with the different laminin(s). For this study the hypothesis was that laminin 5 could be involved in the initiation, while laminin 10 is responsible for the maintenance, of the mammary bud downward growth into the dermis. We also propose that α6β4 acts as the major receptor for laminin 5, and α3β1 is the major receptor for laminin 10 during mammogenesis. The main aim of this study was therefore to investigate this hypothesis by examining the expression pattern of selected basement membrane components, to see if there were significant

differences in the local ECM composition that might affect the progression of mammary gland prenatal development.

Materials and Methods

Animals

For the experiments described in this study, a total of 546 CD1 mouse embryos were studied: (E12=24, E12.5=56, E13=26, E13.5=31, E14=46, E14.5=88, E15.5= 37, E16=12, E16.5=43, E17.5=80 and E18.5=103).

Generating embryos of defined ages

As mouse embryonic development is a very rapid process, it was important to study embryos of as similar developmental age as possible. Two different mating techniques were compared. One was based on a short (about 2hr) and defined time for mating, and the second was based on the more widely used technique where the animals were left together overnight to mate and pregnancies determined by the presence of a vaginal plug at E 0.5.

Dissection

Pregnant mice were euthanased by CO_2 suffocation followed by dislocation of the neck. Individual embryos were collected and transferred into a glass Petri dish coated with Sylgard® 184 Kit, silicone elastomer (Sigma, UK). This provides a stable surface for pinning down the embryos in order to secure them for dissection. Under the dissection microscope submerged under DMEM, the embryo was pinned outstretched using insect pins into the Sylgard®. Gradually the pins were moved closer to the body as the limbs and tail were trimmed. By adjusting the angle of the incident lights and tilting the embryo sideways, the mammary glands were identified according to their location and shape.

About 2-4mm^2 fragments of the skin (depending on the age of the embryo), containing one or a maximum two adjacent mammary glands, e.g. No.4 & 5, were dissected out.

Immunofluorescence

Skin fragments containing mammary glands were snap frozen in liquid nitrogen immersed in Tissue-Tek® (Agar, UK) within an appropriate size foil cup. Serial frozen sections (10µm) were cut in a cryostat at -20º C and collected on pre-coated slides (BDH, UK).

The DakoCytomation EnVision® Dual Link System Peroxidase kit (DAKO, UK) was used for immunohistochemistry, following the manufacturer's recommended procedures. In this method, after washing in PBS the slides were incubated in the appropriate concentration of each antibody (collagen IV, laminin5 and β4 or α6 integrins; see Table 1 for details of antibodies used). overnight at -4° C, followed by two washes in PBS, each for 5 min. After that, the peroxidase-labelled polymer (DAKO, UK) was applied for 30 min followed by 5 min in PBS. Sections were then covered with substrate chromogen for 10 min, washed in running tap water, then counterstained with haematoxylin for 10-15 seconds. After counterstaining in both techniques, the slides were processed through a series of de-hydration steps and mounted.

Examination procedures

Samples were examined by fluorescent or bright field microscopy using a Zeiss Axioskop, fitted with a c olour AxioCam digital camera which was used for collecting images (using objective lenses X4, X16, X25 and X40).

Table 1. Ddetails the primary antibodies used in this study. mAb: Monoclonal antibody pAb: Polyclonal antibody

346-11A	Integrin β IV/mAb	1: 200	Pharmingen, USA/ 09491D
GoH3	Integrin α VI /mAb	1: 200	Serotec Ltd, UK/ MCA699
CD104	Collagen IV/pAb	1: 200	CHEMICON, Temecula, CA, USA./ AB756P
1110T	Laminin 5 (α3)/mAb	1: 500	(Gift) T. Sasaki, Philadelphia USA

Results

Examination of ultra-thin sections under the electron microscope showed a continuous and well developed basement membrane around the mammary gland at all main embryonic stages i.e. the bud, peg and the sheath stages (data not shown). This was then followed by immunofluorescence analysis of the expression pattern of selected basement membrane components, i.e. collagen IV, integrins α6 and β4 and laminin 5.

Expression pattern of Collagen IV

In frozen section of developing mammary gland, immunofluorescence investigations show that collagen IV is expressed in the skin basement membrane at E12.5, E15.5, E16.5 and E18.5. When the mammary gland was still at the bud stage (at E12.5), collagen IV was present in the basement membrane underlying the ectoderm and around the mammary bud; it was also within the mesenchymal cells underneath the ectoderm and those around the mammary bud (Fig 1A). As mammary gland development progressed, the advanced peg stage of mammary gland development (at E15.5) showed much more intense collagen IV staining than the earlier bud stage, especially

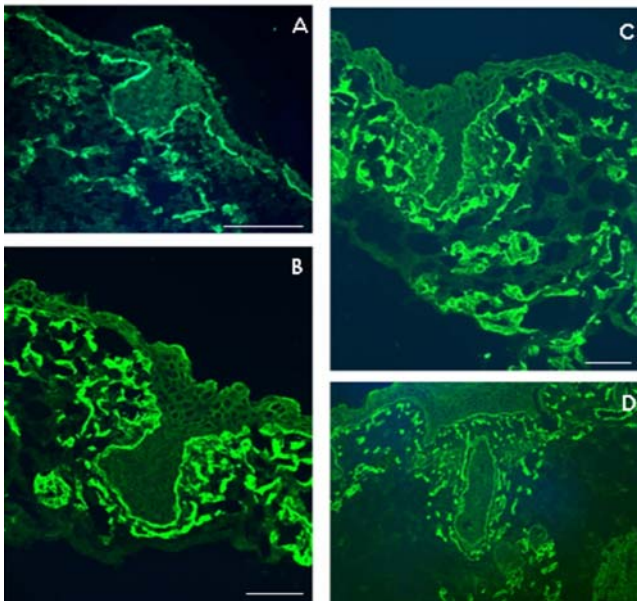

Figure 1. *The expression pattern of collagen IV (AB756P, CHEMICON) visualized by immunofluorescence in frozen sections of the embryonic mammary gland at (A) Bud stage, (B) Advanced peg stage, (C) sprouting stage and (D) sheath stage. Collagen IV is abundant in all stages in the mammary mesenchyme, the fat pad precursor and the basement membrane surrounding the downward growing mammary gland.*
Scale bar = 100μm

within the mammary mesenchyme and the fat pad precursor. This persisted through the subsequent sprouting (E16.5) and sheath stages (E18.5) of development (Fig 1C and D, respectively).

Expression pattern of α6 and β4 integrins

Using antibody GoH3, α6 integrin was detected during the early stages (the bud and peg stages) of prenatal mammary gland development, α6 integrin was expressed by basal cells at the dermal-epidermal junction (Fig 2A). At the sheath stage of mammary development, however, α6 integrin was not detected in basal cells of the mammary main ducts, although it was retained by the basal cells of the embryonic epidermis and those of the nipple sheath (Fig 2B).

The expression pattern of β4 integrin was also examined using antibody 346-11A in frozen sections of mammary gland of mice at different embryonic days of development (E12.5, E14.5, E16.5 and E18.5). It was observed that from early stages of mammary gland development, β4 integrin is absent from the growing tip of the mammary bud. However, it was present at the dermal-epidermal junction of the basal cells in the adjacent ectoderm and the distal part of the mammary bud close to the ectoderm (Fig. 2C). The intensity of β4 expression declined from

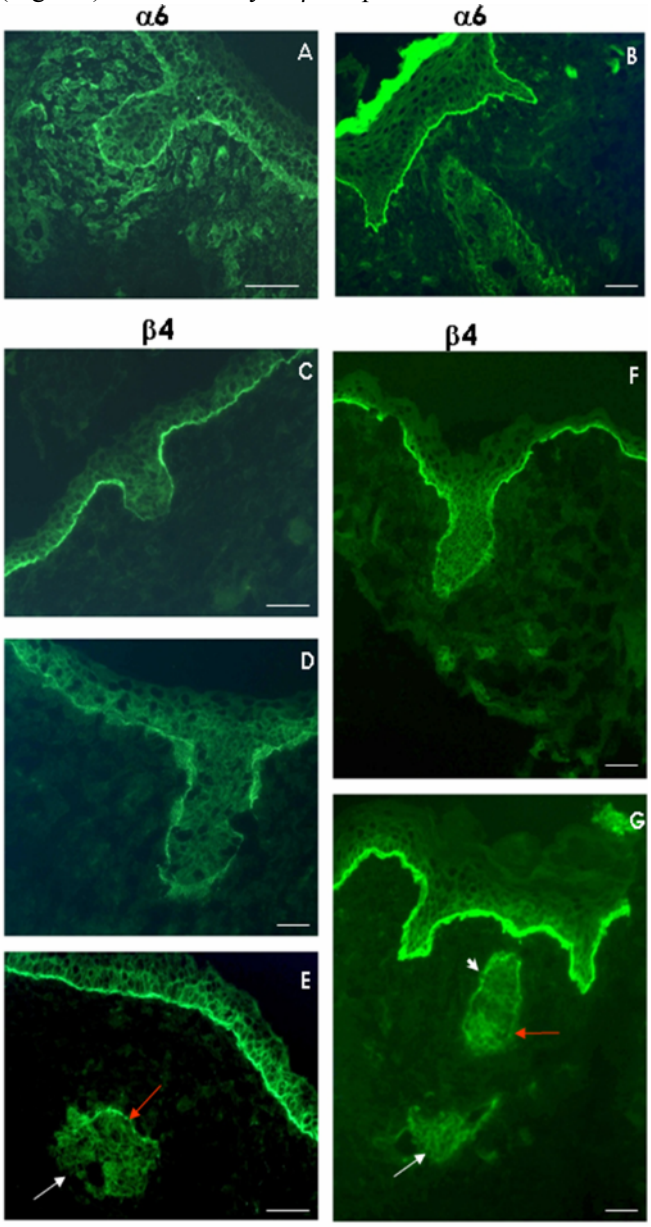

Figure 2. *Micrographs show the expression pattern of α6 (A and B) and β4 (C to G) integrin subunits in frozen sections of embryonic mammary glands at different stages of development. (A) Expression of α6 in the peg stage, (B) α6 in the sheath stage, (C) β4 in the bud stage, (D) Section through the middle of the mammary peg showing that the peg is completely enveloped by β4 staining, (E) Expression of β4 in the growing tip of the mammary peg; note that the top part (red arrow) is positive while the downward growing tip is negative (white arrow), this section is three sections away from the section shown in (D) in which the whole mammary section was enveloped by positive staining for β4. (F) Expression of β4 in the sprouting stage, note that the intensity of staining is declining from the origin at the epidermis to the deeper into*

the mesenchyme part of the developing duct. Two serial-sections further away, in which the advancing tip was seen, significant reduction, then loss in β4 expression was seen (data not shown), (G) expression of β4 in the sheath stage, note that the intensity of staining is reduced around the lower part of the main duct (red arrow) in comparison to the intensity in the upper part (arrow head) of the duct or the dermis-epidermal junction. β4 staining was also absent from around the deep part of the duct (white arrow). Scale bar = 50μm

the ectoderm downwards and completely disappeared in the tip of the mammary bud. This suggests an altered attachment of the epithelial cells to the ECM at the downward growing tip of the mammary bud, while attachment mechanism at the connection of the mammary bud to the ectoderm appeared to be unchanged.

At the peg stage, a similar pattern to that seen during the bud stage was also observed. β4 integrin-positive staining was seen at the basal aspect of the developing epidermis and around the mammary neck and body (Fig 2D), whilst β4 expression was absent from a small part of the downward growing tip of the mammary peg (Fig 2E white arrow). This appears to indicate the advancing edge of the mammary body, and was only seen in the rare sections that pass through these very restricted and localized patches.

In the early sprouting stage, β4 expression maintained a similar pattern to that seen during the peg and bud stages. Expression of β4 in the dermal-epidermal junction zone extended across the newly forming nipple sheath (Fig 2F), but the growing end of the mammary duct was negative.

At the sheath stage of prenatal mammary gland development, β4 was absent from the dermal-epidermal junction around the mammary ducts. Weak staining could be detected in the upper (distal) segment while the lower (deeper) segments were clearly negative. On other hand positive staining for β4 was maintained at a high level in the embryonic basement membrane zone of the epidermis including that of the nipple sheath (Fig 2G).

Expression pattern of Laminin 5

An antibody to laminin 5, 1110T, which recognizes the α3 subunit, was used to examine the expression pattern of laminin 5 during prenatal mammary gland development. Frozen sections of mammary glands at different stages of development were obtained from mouse embryos at E13, E15, E16 and E18. Mammary glands at the late bud stage and early peg stage of E13 mice show very similar patterns consisting of highly positive staining with laminin 5 antibody around the newly developing mammary neck and most of the mammary body (Fig 3 A). The most proximal region of the body however was significantly low in the expression of laminin 5. This region of reduced expression could be easily missed depending on the plane of the section.

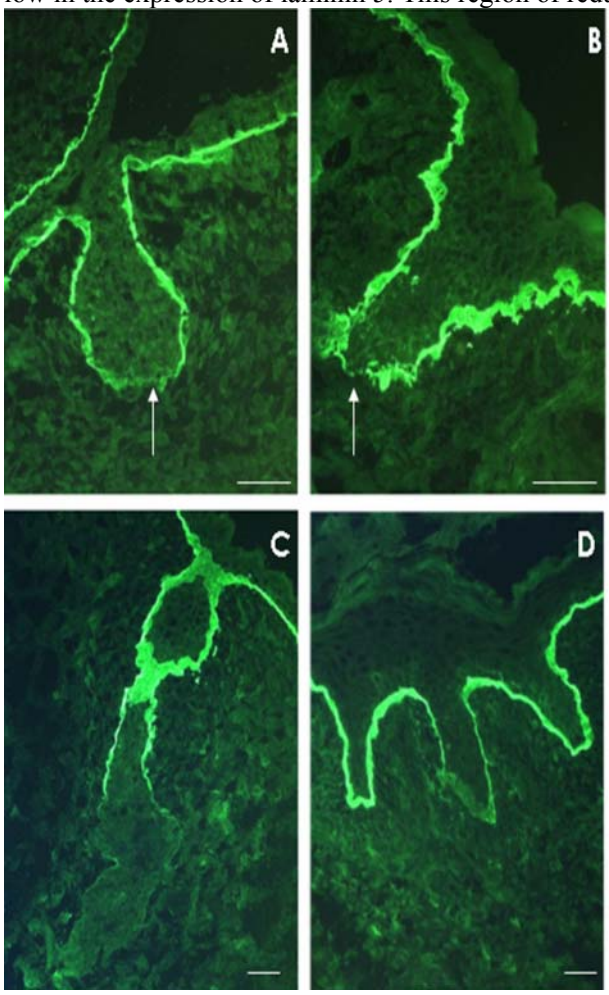

Figure 3. *Micrographs showing the expression of laminin 5 in frozen sections, (A) Early mammary peg stage of E13 mouse: white arrow points to the tip of the mammary body at which expression of laminin 5 is less intense. The tip is identified as the deepest part of the mammary structure, based on serial sectioning of the mammary gland. (B) Early sprouting stage showing similar pattern to that seen in (A). Arrow points to a tip of the growing mammary duct. (C) Advanced sprouting stage; note that the lower half of the main duct is negative while the upper is positive. (D) Mammary gland at the sheath stage; note that the main duct is still expressing laminin 5.*
Scale bar = 50μm

Later on during the sheath stage at E18, laminin 5 positive reactivity was retained by the basement membrane of the embryonic epidermis, around the nipple sheath and most of the upper (close to the epidermis) part of the mammary gland main duct (Fig 3D). However, none of the small

ducts showed positive reactivity for laminin 5 at this stage.

Discussion

Prenatal mammogenesis does not disrupt collagen IV

Our detailed immunofluorescence studies using antibody for collagen IV showed that during prenatal development collagen IV is present throughout the basement membrane of the mouse skin and around the developing mammary glands at all of the different stages. Such a pattern is similar to that seen during human foetal hair development [6] and mouse hair development [32]. These results are not entirely surprising as collagen IV can stimulate motility in normal and tumour cells in vitro [33], and should not therefore have an inhibitory effect in the downward growth. Mammary glands of virgin mice also express collagen IV in all ducts including the terminal end bud [30]. The only conflicting report is one saying that in prepubertal mammary glands of heifers, collagen IV is primarily in the basement membrane of mature ducts and less in other parts of the gland [34]. The conclusion would be that the tissue reorganization of prenatal mammary gland development does not require, or depend upon, disruption or interruption of the basement membrane collagen IV.

Integrins and prenatal mammogenesis

Immunofluorescence examination of α6 and β4 integrins showed that α6 was present during the mammary bud and peg stages and was lost from the main duct at the sheath stage. In contrast, β4 integrin was absent from advancing tip of the mammary gland at all of the prenatal mammogenesis stages, yet it was present around the rest of the developing mammary gland.

The α6 integrin expression pattern suggests that α6 is most likely required for prenatal mammary gland development at the early stages (bud and peg stages) when it completely envelops the advancing mammary bud and peg.

However, this was not the case in later stages (at least the sheath stage), since it was seen in the basement membrane zone of the epidermis while being absent from that of the mammary duct. However, the fact that α6-null mice show normal mammary glands at E17 (no distortion or delay), and also developed normally when transplanted into fat pads of syngeneic hosts [28], remains a mystery.

The expression pattern of β4 integrin, provides further understanding of the structural development of mammary gland. As a gap in the expression of β4 integrin was identified at the tip of the growing mammary gland bud and peg stage. This gap indicates differences in the biochemistry of cell-substrate junction, which probably occurs to allow easier penetration of the basement membrane and promotes mammary invasion at that specific site. The β4 integrin was missing from only a very restricted small part of the mammary gland; at its' advancing tip. This observation could be easily missed, and may explain the report by Nanba and colleagues [29].

Laminin 5 shows a similar distribution to β4 integrin

Although laminin 5 was present at the junction of the basal cells with the basement membrane and around the developing mammary bud and peg, gaps in staining were also seen in the proximal (deepest) part. Our serial sections suggests that this may be the growing tips of the mammary bud and pegs, as these gaps are also in the deepest parts. At later stages the gaps became much more apparent when the mammary ducts had grown deep into the mesenchyme during the advanced sprouting and sheath stages. This result again is contrary to Nanba and colleagues [29], reporting that laminin 5 is in the basement membrane completely enclosing the mammary bud and peg. Yet it is in agreement with the recently reports of laminin 5 expression during mouse hair follicle development [32,35], as laminin 5 was seen around the developing hair follicle except in the deep invading part of the hair follicle.

Our results show that the expression pattern of β4 integrin is similar to that of laminin 5, but different from that of α6 integrin. The β4- and laminin 5- negative patterns suggest that the tip of the growing mammary gland may have a basement membrane with a different biochemical composition from that in the rest of the mammary tree. Thus β4 with α6 integrin may bind to laminin 5 in the whole mammary gland except at the growing end where an alternative integrin isotype, most likely β1 as suggested by some preliminary observations (not shown), joins α6 and maybe binds to a different ligand, such as laminin 10 or laminin 1 or collagen. Both laminin 10 and 1 have been found in the basement membrane surrounding the developing hair follicle [20,32], in a similar expression pattern to that of α6 found in this study.

In conclusion, it appears that cells at the growing tip of the developing mammary gland and hair follicles behave differently from cells further back in the duct. A different composition of the basal lamina at the growing tip may be significant as it may facilitate invasive epithelial behaviour for downward growth, either mechanically (if this composition of basement membrane is more deformable) or indirectly via an alteration in signal transduction pathways.

At the beginning of this study we hypothesized that laminin 5 is involved in initiation of mammary gland development whilst laminin 10 is needed for maintaining the mammary bud down-growth into the dermis, and that α6β4 is the major receptor for laminin 5 while α3β1 is the major receptor for laminin 10. Although laminin 10 was

not investigated due to the lack of specific anti-laminin 10 antibodies that can be used on mouse tissue, the results described here partially support this hypothesis. From the expression pattern it appears that α6β4 is most likely the receptor for laminin 5 around the developing mammary gland, however, further work is needed regarding the specific type of laminin(s) and its/their receptor(s) at the advancing tip of the mammary structure.

References

1. Clark RAF, Tonnesen MG. Integrins in skin biology and pathophysiology. In: Freinkel RK, Woodly DT, editors. The Biology Of The Skin: The Parthenon Publishing Group; 2001. p. 333-352.
2. Uitto J, Pulkkinen L, Christiano AM. Molecular basis of the dystrophic and junctional forms of epidermolysis bullosa: mutations in the type VII collagen and kalinin (laminin 5) genes. J Invest Dermatol 1994; 103 (5 Suppl): 39S-46S.
3. Mellerio JE, Ashton GH, Mohammedi R, Lyon CC, Kirby B, Harman KE, et al. Allelic heterogeneity of dominant and recessive COL7A1 mutations underlying epidermolysis bullosa pruriginosa. J Invest Dermatol 1999; 112(6): 984-987.
4. McGrath JA, Schofield OM, Eady RA. Epidermolysis bullosa pruriginosa: dystrophic epidermolysis bullosa with distinctive clinicopathological features. Br J Dermatol 1994; 130(5): 617-625.
5. Jiang W, Bu D, Yang Y, Zhu X. A novel splice site mutation in collagen type VII gene in a Chinese family with dominant dystrophic epidermolysis bullosa pruriginosa. Acta Derm Venereol 2002; 82 (3): 187-191.
6. Karelina TV, Bannikov GA, Eisen AZ. Basement membrane zone remodeling during appendageal development in human fetal skin. The absence of type VII collagen is associated with gelatinase-A (MMP2) activity. J Invest Dermatol 2000; 114 (2): 371-375.
7. Colognato H, Yurchenco PD. Form and function: the laminin family of heterotrimers. Dev Dyn 2000; 218 (2): 213-234.
8. Aumailley M, Smyth N. The role of laminins in basement membrane function. J Anat 1998; 193 (Pt 1): 1-21.
9. Nanba D, Hieda Y, Nakanishi Y. Remodeling of desmosomal and hemidesmosomal adhesion systems during early morphogenesis of mouse pelage hair follicles. J Invest Dermatol 2000; 114 (1): 171-177.
10. Hayashi K, Mochizuki M, Nomizu M, Uchinuma E, Yamashina S, Kadoya Y. Inhibition of hair follicle growth by a laminin-1 G-domain peptide, RKRLQVQLSIRT, in an organ culture of isolated vibrissa rudiment. J Invest Dermatol 2002; 118 (4): 712-718.
11. Schuler F, Sorokin LM. Expression of laminin isoforms in mouse myogenic cells in vitro and in vivo. J Cell Sci 1995; 108 (Pt 12): 3795-3805.
12. Aberdam D, Galliano MF, Vailly J, Pulkkinen L, Bonifas J, Christiano AM, et al. Herlitz's junctional epidermolysis bullosa is linked to mutations in the gene (LAMC2) for the gamma 2 subunit of nicein/kalinin (LAMININ-5). Nat Genet 1994; 6 (3): 299-304.
13. Baudoin C, Miquel C, Blanchet-Bardon C, Gambini C, Meneguzzi G, Ortonne JP. Herlitz junctional epidermolysis bullosa keratinocytes display heterogeneous defects of nicein/kalinin gene expression. J Clin Invest 1994; 93(2): 862-869.
14. Baudoin C, Miquel C, Gagnoux-Palacios L, Pulkkinen L, Christiano AM, Uitto J, et al. A novel homozygous nonsense mutation in the LAMC2 gene in patients with the Herlitz junctional epidermolysis bullosa. Hum Mol Genet 1994; 3(10): 1909-1910.
15. Pulkkinen L, Christiano AM, Gerecke D, Wagman DW, Burgeson RE, Pittelkow MR, et al. A homozygous nonsense mutation in the beta 3 chain gene of laminin 5 (LAMB3) in Herlitz junctional epidermolysis bullosa. Genomics 1994; 24 (2): 357-360.
16. Pulkkinen L, Christiano AM, Airenne T, Haakana H, Tryggvason K, Uitto J. Mutations in the gamma 2 chain gene (LAMC2) of kalinin/laminin 5 in the junctional forms of epidermolysis bullosa. Nat Genet 1994; 6 (3): 293-7.
17. Kivirikko S, McGrath JA, Baudoin C, Aberdam D, Ciatti S, Dunnill MG, et al. A homozygous nonsense mutation in the alpha 3 chain gene of laminin 5 (LAMA3) in lethal (Herlitz) junctional epidermolysis bullosa. Hum Mol Genet 1995; 4(5): 959-962.
18. Vailly J, Pulkkinen L, Miquel C, Christiano AM, Gerecke D, Burgeson RE, et al. Identification of a homozygous one-basepair deletion in exon 14 of the LAMB3 gene in a patient with Herlitz junctional epidermolysis bullosa and prenatal diagnosis in a family at risk for recurrence. J Invest Dermatol 1995; 104(4): 462-466.
19. Vailly J, Pulkkinen L, Christiano AM, Tryggvason K, Uitto J, Ortonne JP, et al. Identification of a homozygous exon-skipping mutation in the LAMC2 gene in a patient with Herlitz's junctional epidermolysis bullosa. J Invest Dermatol 1995; 104(3): 434-437.
20. Li J, Tzu J, Chen Y, Zhang YP, Nguyen NT, Gao J, et al. Laminin-10 is crucial for hair morphogenesis. Embo J 2003; 22(10): 2400-2410.
21. Hynes RO. Integrins: a family of cell surface receptors. Cell 1987; 48(4): 549-554.
22. Sonnenberg A, Calafat J, Janssen H, Daams H, van der Raaij-Helmer LM, Falcioni R, et al. Integrin alpha 6/beta 4 complex is located in hemidesmosomes, suggesting a major role in epidermal cell-basement membrane adhesion. J Cell Biol 1991; 113(4): 907-917.
23. Watt FM, Hertle MD. Keratinocyte integrins. In: Leigh IM, Lane EB, Watt FM, editors. The Keratinocyte Handbook. Cambridge, Uk: Cambridge University Press; 1994. p. 153-164.
24. Georges-Labouesse E, Messaddeq N, Yehia G, Cadalbert L, Dierich A, Le Meur M. Absence of integrin al-

pha 6 leads to epidermolysis bullosa and neonatal death in mice. Nat Genet 1996; 13(3): 370-373.
25. Niessen CM, van der Raaij-Helmer MH, Hulsman EH, van der Neut R, Jonkman MF, Sonnenberg A. Deficiency of the integrin beta 4 subunit in junctional epidermolysis bullosa with pyloric atresia: consequences for hemidesmosome formation and adhesion properties. J Cell Sci 1996; 109 (Pt 7): 1695-1706.
26. Prince JM, Klinowska TC, Marshman E, Lowe ET, Mayer U, Miner J, et al. Cell-matrix interactions during development and apoptosis of the mouse mammary gland in vivo. Dev Dyn 2002; 223 (4): 497-516.
27. Monaghan P, Warburton MJ, Perusinghe N, Rudland PS. topographical arrangement of basement membrane proteins in lactating rat mammary gland: Comparison of the distribution of type IV collagen, laminin, fibronectin, and Thy-1 at the ultrastructural level. Proc Natl Acad Sci USA 1983; 80: 3344-3348.
28. Klinowska TC, Alexander CM, Georges-Labouesse E, Van der Neut R, Kreidberg JA, Jones CJ, et al. Epithelial development and differentiation in the mammary gland is not dependent on alpha 3 or alpha 6 integrin subunits. Dev Biol 2001; 233(2): 449-467.
29. Nanba D, Nakanishi Y, Hieda Y. Changes in adhesive properties of epithelial cells during early morphogenesis of the mammary gland. Dev Growth Differ 2001; 43 (5): 535-544.
30. Klinowska TC, Soriano JV, Edwards GM, Oliver JM, Valentijn AJ, Montesano R, et al. Laminin and beta1 integrins are crucial for normal mammary gland development in the mouse. Dev Biol 1999; 215(1): 13-32.
31. DiPersio CM, Hodivala-Dilke KM, Jaenisch R, Kreidberg JA, Hynes RO. alpha3beta1 Integrin is required for normal development of the epidermal basement membrane. J Cell Biol 1997; 137(3): 729-742.
32. Chuang YH, Dean D, Allen J, Dawber R, Wojnarowska F. Comparison between the expression of basement membrane zone antigens of human interfollicular epidermis and anagen hair follicle using indirect immunofluorescence. British Journal of dermatology. 2003; 149: 274-281.
33. Aznavoorian SA, Stracke ML, Krutzsch H, Schiffmann E, Liotta LA. J Cell Biol. 1990; 110: 1427-1438.
34. 34. Berry SDK, Howard RD, Akers RM. Mammary localization and abundance of laminin, fibronectin, and collagen IV proteins in prepubertal heifers. j. Dairy. Sci. 2003; 2003(86): 2864-2874.
35. Joubeh S, Mori O, Owaribe K, Hashimoto T. Immunofluorescence analysis of the basement membrane zone component in human anagen hair follicles. Exp Dermatol 2003; 12: 365-370.