Functional Re-expression of Laminin-5 in Laminin-γ2-deficient Human Keratinocytes Modifies Cell Morphology, Motility, and Adhesion*

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Laurent Gagnoux-Palacios§, Jœlle Vaillot, Monique Durand-Clementt, Ernst Wagner†, Jean-Paul Ortonne‡, and Guerrino Meneguzzi***

From ††U385 INSERM, Faculté de Médecine, 06107 Nice Cedex 2, France, ‡Bender AG, 1121 Vienna, Austria, and the Service de Dermatologie, Hôpital Pasteur, 06002 Nice Cedex 1, France

Herlitz junctional epidermolysis bullosa (H-J EB) is characterized by a reduced adherence of keratinocytes consequent to deficient expression of the extracellular adhesive ligand laminin-5. To complement the genetic defect causing H-J EB, we transferred an eukaryotic cassettes expressing the cDNA for the γ2 chain of laminin-5 into H-J EB keratinocytes in which the expression of the polypeptide is hampered by a homozygous mutation generating a premature termination codon. Transfection using adenovirus-polylysine-transferrin-DNA complexes resulted in a transient synthesis of the recombinant laminin γ2 chain that associated with the endogenous α3 and β3 chains to form laminin-5 molecules readily deposited on the tissue culture substrate. Furthermore, retroviral-mediated transduction of the γ2 cDNA yielded persistent expression and polarized secretion of laminin-5. The protein incorporated into the basement membrane produced by the revertant cells inoculated subcutaneously in nude mice. In these transfectants, re-expression of laminin-5 induced changes in cell morphology and reorganization of focal adhesions that assumed the shape and distribution of the counterparts detected in normal keratinocytes. These observations correlated with an enhanced cell-substrate adhesion and a reduced motility of the transfected cells. Our results demonstrate that a restored expression of laminin-5 induces a phenotypic reversion of genetically altered H-J EB keratinocytes and open new perspectives to the analysis of the mechanisms regulating adhesion of epithelial cells.

Laminins are components of the extracellular matrix that contribute to the architecture of the basement membranes and mediate cell adhesion, growth, migration, and differentiation (1, 2). These multifunctional glycoproteins are heterotrimers composed of individual α, β, or γ polypeptide chains expressed at various stages of development in focalized tissue locations (3). The tissue-specific localization of variants suggests that each laminin isomform fulfills definite functions (4).

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** To whom correspondence should be addressed: INSERM U385, U.F.R de Médecine, Av. de Valombreuse, 06107 Nice cedex 2, France. Tel.: 33-93-37-77-79; Fax: 33-93-81-14-04.

Laminin-5 is found in the basement membrane zone of the skin, trachea, alimentary tract, and other specialized squamous epithelia with secretory or protective functions (5, 6). This heterotrimeric protein is synthesized and secreted by the basal epithelial cells (5). In the skin it colocalizes with the anchoring filaments of the lamina lucida, the thread-like structures that bridge the hemidesmosomes to the lamina densa of the dermo-epidermal junction (7, 8). Two predominant forms of laminin 5 with molecular masses of 400 and 440 kDa are found in the extracellular matrix of cultured keratinocytes and provide a specific substrate for the adhesion of proliferating and migrating cells (7). The 400-kDa form consists of an α3 (165 kDa), β3 (140 kDa), and γ2 (105 kDa) chain, each encoded by a distinct gene, that are covalently linked and folded into a rod with globular domains at each end (9–11). This species is produced by extracellular degradative activity of the N-terminal domain of the 155-kDa γ2 chain comprised in the 440-kDa heterotrimer. The 440-kDa form derives from a cell-associated 460-kDa precursor as a result of a processing event that generates the α3 chain from a 200-kDa polypeptide (12). A new laminin-5 variant prominently expressed in mucosal epithelia and harboring an α3B chain isoform of 300 kDa was also recently described (13). In this novel α3B chain, two alternating EGF-like domains and two globular domains substitute the short N-terminal domain of the α3A isoform (13). In the skin, the large majority of laminin-5 is thought to resemble the fully processed form of the protein.

An indication of the crucial role of laminin-5 in cell adhesion was first provided by the observation that in cell cultures, the mAb BMW 165 induces rounding and detachment of adherent keratinocytes without affecting fibroblasts (7). It was also demonstrated that treatment of skin biopsies with antibody BMW 165 causes extensive de-epithelialization at the level of anchoring filaments (7). The importance of laminin-5 in maintaining the cohesion of the integuments was definitely proved by the identification of mutations in the genes coding for this basement membrane component in patients affected by the Herlitz syndrome (H-J EB) (Ref. 14 and references therein). This severe form of junctional epidermolysis bullosa is characterized by the detachment of the squamous epithelia from the underlying mesenchyme (15).

Human H-J EB keratinocytes presenting an abnormal expression of laminin-5 may constitute an interesting tool to dissect the contribution of this extracellular molecule to cell adhesion and migration. However, cultured H-J EB keratino-
cytes display a reduced growth rate and an altered adhesion to the tissue culture substrate that hampers propagation. Using SV40 infectious particles, we have recently immortalized a human keratinocyte cell line LS5V that grows in culture continuously but retains differentiation potential and the phenotypic character of the parental H-JEB keratinocytes, noticeably a reduced adhesion and enhanced motility characteristic of the parental H-JEB keratinocytes, notably.

We report that the recombinant laminin-2 chain DNA recovers synthesis and secretion of laminin-5 in H-JEB keratinocytes. We also provide evidence that the recombinant laminin-5 molecules are biologically active and induce reversion of the H-JEB phenotype.

**MATERIALS AND METHODS**

**Cell Culture—** Primary and SV40-immortalized (cell line LS5V) H-JEB keratinocytes, and the SV40-transformed cell line SVK14 (gift of Dr. B. Lane) were grown in a 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's F-12 medium containing 10% fetal calf serum, 1 mM sodium pyruvate, 2 mM L-glutamine, 10 mM of penicillin and streptomycin, 10 ng/ml epidermal growth factor, 100 mM cholera toxin, 440 ng/ml hydrocortisone (KSM medium) (16). When indicated, primary normal keratinocytes isolated from human foreskins were also grown in keratinocyte-defined medium (PromoCell) (16). Virus-producing and packaging cell lines psi-crip and psi-cre were grown in Dulbecco's modified Eagle's medium containing 4.5 g/liter glucose and 10% fetal bovine serum (15).

**Antibodies—** Synthesis of laminin-5 was examined using mAb GB3 (5), mAb K3 specific to the laminin-α2 chain (12), mAb BM165 (7) and polyclonal antibody (pAb) SE8 (19), specific to the laminin-α3 chain, and pAb SE144, specific to the laminin-γ2 chain (10). Reactivity of vinculin and talin were determined using mAbs RVIN-1 and 8d4 (Sigma), respectively.

**Plasmids and Retroviruses—** Plasmid pC3-γ2 contains the full-length open reading frame of the mRNA encoding the laminin-γ2 chain inserted in the expression vector pcDNA3 (Invitrogen) downstream of the human cytomegalovirus CMV immediate-early enhancer-promoter (Fig. 1). Fig. 1 also depicts the amphotrophic replication-defective retroviral vector pTGY2, in which the laminin-γ2 cDNA is cloned downstream the mouse sarcoma virus long terminal repeat (LTR) of plasmid pT5GS92, a generous gift of M. Mebius. Plasmid pT5GS92 is a construct derived from plasmid pX3 (20), where the murine leukemia virus was replaced by the myeloproliferative sarcoma virus LTR. Plasmids pCMV-β-gal (Invitrogen) and pLXSP (20), contain a Escherichia coli β-galactosidase gene driven by the CMV promoter and the mouse sarcoma virus LTR, respectively. Plasmids were amplified in E. coli XL1 blue, and purified either on Csc1-ethidium bromide equilibrium density gradients or using a QIAGEN plasmid purification kit.

**Transient Transfection Assays—** 50–80% confluent keratinocyte monolayers (2.5×10^4–5×10^4 cells) grown in 3-mm Petri dishes were transfected using either lipofection or adenovirus-transfer/polylysine-mediated gene delivery. Lipofection was performed using Lipofectamine® (Life Technologies, Inc.). 2 μg of plasmid DNA and 10 μl of Lipofectamine® were separately diluted in 100 μl of serum-reduced Opti-MEM I medium. The two solutions were combined, incubated 30 min at room temperature, diluted with 800 μl of serum-reduced Opti-MEM I medium and added to the cells. After 5 h at 37 °C, cells were rinsed, fed with KSM medium, and supplemented with 10% fetal calf serum (Life Technologies, Inc.). Transfection efficiency was evaluated 42 h after transfection by β-galactosidase staining at 4 °C in the presence of 1 μg/ml of centricon® microconcentrators system, the protein content of each sample was determined by the Bradford assay (Bio-Rad). Samples were boiled in sample buffer (60 μl Tris, pH 6.8, 1% SDS, 10% glycerol, and 5% mercaptoethanol) separated by SDS-polyacrylamide gel electrophoresis (7.5% acrylamide gels) and processed after blotting on nylon
RESULTS

 Delivery of the laminin γ2 chain cDNA into cultured H-J EB keratinocytes by an Adenovirus-Polylysine Transferrin-Reporter Complex—To verify that the genetic defect of H-J EB keratinocytes can be complemented by transfection of an ad hoc cDNA, a full-length cDNA encoding the human laminin γ2 chain under the control of the cytomegalovirus early late promoter was inserted into the eukaryotic expression vector pCDNA3 to obtain plasmid pCγ2 (Fig. 1). Plasmid pCγ2 was then expressed in the H-J EB cell line LSV5 following transient transfection mediated by an AdpL-TfpL complex (22, 23). This method was retained because we had established that 90–95% of LSV5 cells, 75% of SVK14 cells, and 30% of normal human primary keratinocytes were successfully transduced in this way (Table I). Western analysis of total extracts of LSV5 cells transfected with plasmid pCγ2 revealed that the recombinant laminin γ2 polypeptide was actively synthesized. The polypeptide migrated as a single band with an apparent molecular mass (155 kDa) identical to that of the endogenous chain produced by normal human keratinocytes. Western analysis of the culture medium conditioned by the transfectants demonstrated that the recombinant laminin γ2 chain was secreted and processed into the 105-kDa form. The ratio between the detectable amounts of the processed 105-kDa form and the unprocessed 155-kDa polypeptide appeared, however, lower than that found in medium conditioned by normal human keratinocytes (Fig. 2). Overexpressed 155-kDa recombinant laminin γ2 accumulated intracellularly (not shown).

Immunofluorescence analysis using the mAb GB3, which binds to a conformational epitope in the laminin α3β3γ2 heterotrimer (5, 29), displayed a strong intracellular reactivity with the transfected LSV5 cells (Fig. 3). Immunoreactive trails were also detected in zones of the culture substrate encompassing the cells. Taken together, these observations indicated that in transfected H-J EB cells, expression of the recombinant laminin γ2 chain results in the association of this polypeptide with the endogenous laminin α3 and β3 chains to form native laminin-5 molecules that are deposited on the culture substrate.

Retroviral-mediated Transduction of Laminin γ2 Chain to H-J EB Keratinocytes—To achieve a persistent expression of the laminin γ2 polypeptide in H-J EB keratinocytes, the full-length cDNA for the human laminin γ2 chain was introduced into LSV5 keratinocytes by retroviral-mediated gene transfer.

The cDNA was inserted into the retroviral vector pTG5192 carrying a selectable marker, the neomycin phosphotransferase gene cassette (PSVNEO), such that expression of the laminin γ2 polypeptide was driven from the promoter-enhancer sequences contained within the viral LTR (Fig. 1). High titer polyclonal viral producer cells were generated from the psi-crip amphotropic packaging cell line using standard methods based on selection in the presence of G418. Integrity of the laminin γ2 retrovirus was verified by Northern analysis to detect the expected mRNA in viral producer cells (not shown) and by analysis of laminin γ2 chain expression in transduced human keratinocyte LSV5 cells.

Expression of the transfected cDNA was first monitored by immunofluorescence using the pAb SE144, which recognizes the laminin γ2 polypeptide (10), and mAb GB3, which reacts with the native laminin-5 heterotrimers (5). In the transduced cell cultures, at least 80% of the G418-resistant cells (LSV5-R) were reactive to the antibodies. Immunoreactivity of transfectants resulted in strong cytoplasmic fluorescence and staining of the extracellular matrix deposited on the culture substrate underneath the cells, which suggested synthesis and secretion of laminin-5 (not shown).

Cell lysates and samples of culture medium conditioned by the transduced cells were then analyzed by immunoblotting. As shown in Fig. 4A, pAb SE144 detected a specific migration band of 155 kDa in the cellular extracts and an additional 105-kDa band in the corresponding medium samples. These results imply that the recombinant γ2 chain polypeptide is actively expressed and secreted by LSV5-R cells and then processed extracellularly (12). Subsequent immunoprecipitation analysis of medium conditioned by LSV5-R cells, using antibodies specific to each distinct chain of laminin-5 identified bands with an apparent molecular mass of 165, 155, 145, and 105 kDa (Fig. 4B), which correspond to the expected mass of the polypeptides that compose the 440- and 400-kDa extracellular forms of laminin-5 (12).
In light of these observations, we investigated whether LSV5-R cells maintain the polarized secretion of laminin-5 observed in normal keratinocytes and whether the molecule integrates the basement membrane zone of the dermo-epidermal junction. Cultures of LSV5-R cells were grown on cellulose acetate filters and exposed to air to obtain stratification into a multilayered epithelium (27). Immunofluorescence analysis of these artificial epithelia using mAb GB3 demonstrated that LSV5-R cells synthesize immunoreactive laminin-5 that is actively secreted in a polarized way and deposited on the culture substrate (Fig. 5A). As previously shown in similar experiments performed with normal human keratinocytes (27), laminin-5 accumulated on the inert surface and eventually diffused inside the filter.

The ability of LSV5-R cells to generate a basement membrane was then assessed in vivo. To this purpose, LSV5-R cells were injected subcutaneously in nude mice. In contact with the mouse mesenchyme, the original LSV5 cells display limited proliferation and form benign cysts presenting an outer layer of undifferentiated keratinocytes, internal layers of differentiating cells, and a core of dead corneocytes (16). These cysts are not immunoreactive with mAb GB3 (Fig. 5B). In contrast, with cysts obtained after injection of LSV5-R cells, immunofluorescence analysis using mAb GB3 revealed labeling of the basement membrane at the interface of grafted cells and the mouse mesenchyme (Fig. 5B), which indicates that laminin-5 produced by the retrovirus-transfected cells incorporates into complex supramolecular structures.

Re-expression of Laminin-5 Induces Changes in Cell Morphology—LSV5 cells expanded in vitro in the presence of serum retain the morphology of the parental H-J EB keratinocytes (16). Their shape correlates with a loose attachment to the culture substrate, which is evidenced by the presence of wide zones of disconnection of the ventral plasma membrane from the plastic tissue culture support and numerous lamellipodia in the areas with prominent cell-substrate separation (Fig. 6A). In similar culture conditions, LSV5-R cells display a more flattened morphology maintained along with passages in culture. Inspection of electron microscope sections of cultured LSV5-R keratinocytes cut perpendicular to the culture surfaces revealed that the flat shape of the cells correlated with a close apposition of the ventral plasma membrane to the culture surface (Fig. 6B). The electron-dense material detected in the adhesion sites appeared more compact in LSV5-R than in parental LSV5 cells.

The number and topography of focal contacts in LSV5-R cells were then studied using the anti-vinculin mAb (hVIN1) and by interference reflection microscopy observation (30). Compared with LSV5 cells, LSV5-R keratinocytes display a modified morphology and distribution of adhesion devices. In LSV5 cells, vinculin spots co-distributed with numerous adhesion plaques that presented as tiny darkened interference reflection micros-
copy streaks at the peripheral edge of individual cells, associated with the endings of short microfilament bundles (Fig. 7B). These adhesion sites failed to enlarge and mature into focal adhesions. In spread LSV5-R cells, focal contacts were reduced in number and had assumed a spiky shape (Fig. 7D). They also appeared larger and denser than the counterparts seen in LSV5 keratinocytes and had acquired attached stress fibers as mature focal adhesions. As expected, talin displayed the same immunoreactivity pattern as vinculin (not shown).

Enhanced Binding of LSV5-R Keratinocytes to the Culture Substrate—Expression of recombinant laminin-5 appeared to enhance the adhesion of H-J EB cells, because monolayers of LSV5 cells spontaneously dissociated from the support, whereas a dispase treatment was required to detach confluent LSV5-R cells. We therefore measured the attachment strength of LSV5-R keratinocytes using a cell binding assay providing a quantitative measure of cell-substrate adhesion (31). Cells seeded in sealed compartments were allowed to adhere 19 h and were then submitted to a mechanical force to determine their resistance to detachment from the culture substrate (26, 31). As shown in Fig. 8, at a relative centrifugal force of 500 × g, 35% of LSV5 cells were dislodged against 18% in the case of LSV5-R keratinocytes. The binding strength of LSV5-R keratinocytes was estimated to be comparable with that of normal human keratinocytes (24% of detached cells) and sensibly enhanced compared with parental H-J EB keratinocytes (40% of detached cells). The results obtained from independent experiments suggested a marked strengthening in adhesion of LSV5-R keratinocytes compared with parental LSV5 cells.

Synthesis of Endogenous Laminin-5 Reduces Cell Motility—Keratinocyte motility is influenced by extracellular matrix (32). H-J EB keratinocytes seeded on collagen type IV display hypermotility compared with normal controls (16, 25). To evaluate the migration capacity of LSV5-R keratinocytes, these cells were plated on gold particles coated with 15 μg/ml collagen IV. Compared with LSV5 and parental H-J EB keratinocytes, which moved on the tissue culture support leaving tracks behind them, LSV5-R cells demonstrated reduced motility, comparable with that of wild type normal and SV40-transformed keratinocytes (Fig. 9). Migration indices were in the range of 12.8 and 13.6 for LSV5 and H-J EB keratinocytes, respectively, and 9 for LSV5-R cells, which approximately represents a 30% reduction of cell locomotion. No appreciable difference in migration rates was noted between LSV5 and control LSV5 cells transduced with a retrovirus expressing a β-gal reporter gene (not shown). In light of these results and consistent with the observation that laminin-5 inhibits motility of normal kerati-

**FIG. 5.** Polarized localization of laminin-5 synthesized by cell line LSV5-R. LSV5-R (A) and control LSV5 (B) keratinocytes seeded on cellulose acetate filters were allowed to stratify for 7 days at the air-medium interface. Frozen sections were then reacted with mAb GB3. Immunofluorescence analysis of cysts obtained after subcutaneous injection in nude mice of LSV5-R keratinocytes. Laminin-5 synthesized by LSV5-R keratinocytes (C) localized at the basement membrane zone of the cysts. Cysts obtained after injection of parental LSV5 cells (D) are not reactive to mAb GB3.

**FIG. 6.** Morphology and cell-substratum adhesion of LSV5-R keratinocytes. Cross-section of subconfluent cultures of LSV5 (A) and LSV5-R (B) cells seeded on plastic culture substrate. In contrast to the original LSV5 cells that present a rounded shape and rare regions of cell-substrate attachment, LSV5-R keratinocytes display a flattened phenotype and a ventral plasma membrane closely apposed to the substrate. Magnification, 2500×.
We have analyzed the effect of re-expression of the extracellular ligand laminin-5 in LSV5 keratinocytes that present a defective adhesion consequent to a genetic mutation hampering expression of the laminin g2 chain (16, 17). This report provides the first evidence that delivery of DNA into keratinocytes using AdpL-TfpL complexes results in high transfection efficiency and restoration of a genetically impaired function and that transduction with an exogenous g2 chain cDNA using a retroviral vector promotes the phenotypic reversion of the diseased keratinocytes.

Here we have shown that both in transient and long term
expression experiments, the recipient cells produced a polypeptide identical in molecular mass to the 155-kDa species of laminin-5. This polypeptide associated with the endogenous laminin a3 and b3 chains and formed a heterotrimeric a3b3g2 complex secreted extracellularly that was recognized by three distinct antibodies raised against each individual chain of laminin-5 and by mAb GB3. Because chain assembly into a complex preserving the native conformation of laminin-5 is required for binding with GB3 (5, 29), GB3 recognition of the heterotrimer secreted by LSV5 cells in the culture medium and in the extracellular matrix suggests that the restored synthesis of a2 chain results in production of properly assembled laminin-5 molecules. Expression vectors have been employed by other groups to study synthesis and assembly of recombinant laminin chains (33, 34). However, the biological effect induced by the expression products of the delivered cDNAs could not be evaluated, either because the transfectants synthesized the corresponding wild type endogenous laminin chain (33) or because the cell types used for the transduction experiments do not constitutively produce the laminin species comprising the polypeptide encoded by the transfected DNA sequences (34). The novelty of our findings is that we demonstrate the effect of the restored synthesis of the laminin a2 chain on the morphology and behavior of cells re-expressing the adhesion ligand laminin-5.

Receptor-mediated endocytosis associated with adenovirus particles constitutes a method of choice to transduce epithelial cells (22, 23). In this report we show that expression vectors linked AdpL-TfpL-DNA complexes are efficiently introduced into established keratinocyte cell lines but also in primary human keratinocytes. Transfection efficiencies were superior to any other procedure described thus far (35, 36); therefore this method could constitute an important tool for examining the regulation of promoters and function of genes in epithelial cells as refractory to transfection as primary keratinocytes. As others that used CMV vectors to express laminin-5 cDNAs (34), we observed that LSV5 cells transduced with a2 chain cDNA driven by the CMV promoter yielded an elevated production of the recombinant polypeptide that incorporated into laminin-5 molecules. However, consistent with the observation that the high level delivery inherent to this transfection method may have a certain cytotoxic effect (22), the recipient cells manifested a reduced growth rate and senescence that hampered the long term analysis of the transduced phenotype.

Lines of evidence indicated that in long term expression experiments, LSV5 keratinocytes transfected with viral vectors had reverted the diseased phenotype. Restored LSV5-R keratinocytes were isolated that re-expressed and deposited laminin-5 on the tissue culture substrate and the mesenchyme of nude mice grafted with the transduced cells as observed with human keratinocytes producing functional molecules of this adhesion ligand (16, 27). LSV5-R keratinocytes also assumed an epithelial-like shape correlating with changes in the distribution of focal adhesions and a tighter adhesion of the basal plasma membrane to substrate. Laminin-5 has been identified as the major ligand for adhesion of keratinocytes via focal adhesions (37). It has been proposed that initial interaction of keratinocytes with laminin-5 results in cell attachment, spreading, and migration, whereas stable adhesion to the basement membrane of quiescent keratinocytes occurs via hemidesmosomes (38). In primary J EB keratinocytes and LSV5 cells, formation of focal adhesions appears hindered at a precursor stage in which microspikes on bundles of actin filaments are formed within the edge of the cells (16, 28). Re-expression of laminin-5 induces disassembly of most of the minute focal contacts at the boundary of LSV5-R cells and enlargement of others that nucleate and associate stress fibers in a process similar to the maturation of focal adhesion (39). We have not yet determined which regulatory components of focal adhesion are involved in the process. However preliminary indications obtained using a panel of anti-phosphotyrosine antibodies argue for quantitative changes in the phosphorylation of focal adhesion components in these cells.2

The changes in morphology of LSV5-R cells induced by re-expression of laminin-5 were concomitant with an enhanced adhesion of LSV5-R to the culture substrate, which suggests a more efficient functioning of the adhesion apparatus. Earlier biophysical studies on cultured cells suggested that optimal migration occurs at intermediate adhesion strength (40). According to these observations, re-expression of laminin-5 and consequent increase of focal adhesion stability may explain the decrease of locomotion of LSV5-R keratinocytes. Recent evidence indicates that migration of both normal and J EB keratinocytes is inhibited in vitro by the addition of exogenous laminin-5 to the culture medium that compensates the failure of J EB keratinocytes to deposit sufficient amounts of functional laminin-5 (25). Furthermore, these studies supported the idea that inhibition of migration induced by exogenous laminin-5 implices integrins a3b1 (41), which is the putative receptor of laminin-5 in focal adhesions (37, 38). Our results indicate that laminin-5 may play a pivotal role in the coordination between cell adhesion and cell motility possibly via specific proteins in focal adhesion such as vinculin. In fact, in LSV5-R cells, the reorganization of vinculin in focal adhesions in response to the re-expression of laminin-5 correlates well with the reduced locomotion of these keratinocytes and also with the finding that expression of vinculin influences cell motility in vitro (42, 43).

Laminin-5 is likely to play an essential role in the organization of hemidesmosomes, because in the skin of H-J EB patients without expression of this protein, hemidesmosomes are either rudimentary or absent (15, 17). Consistent with this observation, binding of laminin-5 to integrin a6b4 has been shown to constitute the crucial step in nucleation of hemidesmosome components (44). Accumulation of the extracellular matrix ligand at a threshold level appears also to be required for production and/or stabilization of hemidesmosomes in vitro (45). Ultrastructural observation of LSV5-R keratinocytes did not reveal formation of hemidesmosome-like adhesion structures. Because these cells synthesize all the known cellular components of hemidesmosomes (16), their inability to form defined hemidesmosomal structures may depend on the culture conditions we used but also on their restricted production of laminin-5 (Fig. 4). Furthermore, formation of hemidesmosomes may require the presence of extracellular 400-kDa species of laminin-5, which is poorly produced by LSV5-R cells. Interestingly, extracellular 400-kDa laminin-5 is abundantly produced by cancer epithelial cells that assemble mature hemidesmosomes in vitro (45–47), whereas colon cancer epithelial cells, which derive from a tissue where hemidesmosomes are not detected, secrete exclusively the 440-kDa form of laminin-5 (48).3

In summary, our results show that AdpL-TfpL complexes efficiently deliver recombinant cDNAs into keratinocytes. This method can therefore facilitate the analysis of assembly, stability, maturation, and secretion of laminin-5 using eukaryotic vectors expressing mutated cDNA constructs to complement genetically identified defects of laminin a3, b3, and g2 chains in H-J EB keratinocytes. We also demonstrate that retroviro-mediated transfer of a vector expressing the laminin g2 chain cDNA restores stable synthesis of functional laminin-5 that

2 L. Gagnoux-Palacios, unpublished results.
3 V. Oriol-Rousseau, D. Aberdam, P. Roussele, G. Meneguzzi, M. Kedingker, and P. Simon-Assmann, manuscript in preparation.
induces changes in focal adhesions and influences morphology, motility, and adhesion of the transfected H-J EB keratinocytes. These results open new perspectives in the analysis of the adhesion mechanisms of epithelial cells. To this purpose, the establishment of keratinocyte cell lines and cultures of epithelial stem cells from JEB patients with mutations in the different hemidesmosome components and laminin-5 are in progress in our laboratory.

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