Association of the tetraspanin CD151 with the laminin-binding integrins α3β1, α6β1, α6β4 and α7β1 in cells in culture and in vivo

Sterk, L. M. T., Geuijen, C. A. W., van den Berg, J. G., Claessen, N., Weening, J. J. and Sonnenberg, A. *J. Cell Sci*. **115**, 1161-1173.

In the printed version of this article, Fig. 10 was incorrect. The correct figure is shown below.

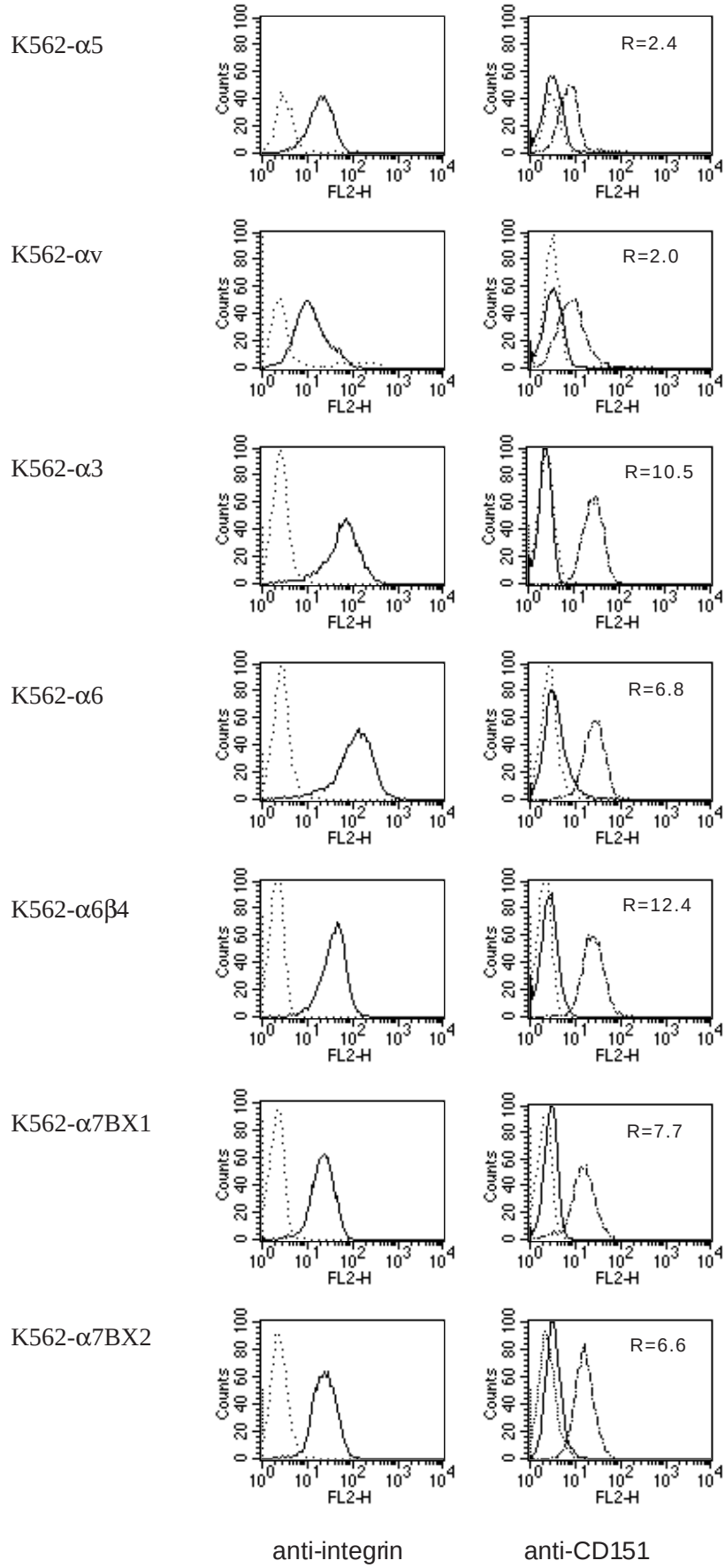

Fig. 10. Expression of laminin-binding integrins on transfected K562 cells increases CD151 cell surface expression. Flow cytometric analysis of wild-type K562 cells endogenously expressing α5β1 and transfected K562 also expressing αvβ1, α3β1, α6β1, α6β4, α7X1β1 or α7X2β1. Solid lines in the left panels indicate staining with anti-integrin antibodies: anti-α5 mAb Sam-1 (K562 cells), anti-αv mAb 13C2 (K562-αv), anti-α3 mAb J143 (K562-α3), anti-α6 mAb GoH3 (K562-α6), anti-β4 mAb 439-9B (K562-α6β4) and anti-α7 mAb CA25 (K562-α7BX1 and K562-α7BX2). Staining of cells with anti-CD151 antibodies is shown in the right panels. Solid lines represent staining of cells with mAb TS151R and broken lines with mAb P48. A negative control (dotted line) with secondary goat anti-mouse or rat IgG alone is shown in each panel. R represents the ratio of the mean fluorescence intensity of cells stained with mAb P48 and with secondary antibody alone. A three- to sixfold increase of CD151 expression is observed in transfected cells that ectopically express lamininbinding integrins. The TS151R epitope is masked in all cells expressing one of the laminin-binding integrins.

Association of the tetraspanin CD151 with the laminin-binding integrins α3β1, α6β1, α6β4 and α7β1 in cells in culture and in vivo

Lotus M. T. Sterk[1,2], Cecile A. W. Geuijen[1], José G. van den Berg[3], Nike Claessen[3], Jan J. Weening[3] and Arnoud Sonnenberg[1]

[1]Division of Cell Biology and [2]Division of Pathology, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands
[3]Division of Pathology, Academic Medical Center, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands
Author of correspondence (e-mail: asonn@nki.nl)

Accepted 21 December 2001
Journal of Cell Science 115, 1161-1173 (2002) © The Company of Biologists Ltd

Summary

CD151 is a cell surface protein that belongs to the tetraspanin superfamily. It forms complexes with the laminin-binding integrins α3β1, α6β1 and α6β4 and is co-distributed with these integrins in many tissues at sites of cell-matrix interactions. In this study we show that CD151 can also form stable complexes with the laminin-binding integrin α7β1. The strength of this interaction is comparable to that between CD151 and α3β1. Complexes of α3β1, α6β1 and α7β1 with CD151 are equally well formed with all splice variants of the α3, α6 and α7 subunits, and complex formation is not affected by mutations that prevent the cleavage of the integrin α6 subunit. Like the expression of α3β1 and α6β1, expression of α7β1 in K562 cells results in increased levels of CD151 at its surface. Two non-integrin laminin receptors, dystroglycan and the polypeptide on which the Lutheran blood group antigens are expressed, are also often colocalized with CD151, but no association with CD151-α3β1 complexes was found with biochemical analysis.

The anti-CD151 antibody TS151R detects an epitope at a site at which CD151 interacts with integrins, and therefore it cannot react with CD151 when it is bound to an integrin. Comparison of the staining patterns produced by TS151R with that by of an anti-CD151 antibody recognizing an epitope outside the binding site (P48) revealed that most tissues expressing one or more laminin-binding integrins reacted with P48 but not with TS151R. However, smooth muscle cells that express α7β1 and renal tubular epithelial cells that express α6β1 were stained equally well by TS151R and P48. These results suggest that the interactions between CD151 and laminin-binding integrins are subject to cell-type-specific regulation.

Key words: Tetraspanin, Laminin receptor, Integrin, Dystroglycan, Lutheran blood group antigens

Introduction

CD151 is a transmembrane molecule that has been recently characterized as a member of the evolutionary conserved tetraspanin superfamily (Wright and Tomlinson, 1994; Maecker et al., 1997). Tetraspanin molecules, so called because they cross the plasma membrane four times, have two extracellular loops and three cytoplasmic domains including the C- and N-terminus. Many interactions among tetraspanins and between tetraspanins, integrins and other molecules have been described (Berditchevski et al., 1996; Mannion et al., 1996; Hasegawa et al., 1998; Fitter et al., 1999; Sincock et al., 1999). Hence it is thought that one function of tetraspanins is to facilitate the coupling of signaling pathways by assembling and connecting specific cell surface proteins into large molecular complexes.

On the basis of the stability of the formed complexes, interactions of tetraspanins can be classified into three categories (Claas et al., 2001). CD151-α3β1 complexes represent the exceptionally strong, highly stable and selective level 1 association. Most interactions between tetraspanins and integrins, for example, the association of CD151 with α6β4, are less strong and considered to be level 2; they are numerous, fairly stable and selective. CD151-α6β1 interactions are either level 1 or level 2, probably depending on the cell type in which these molecules are expressed. Level 3 interactions, which occur mostly between tetraspanins and molecules other than tetraspanins or integrins, are indirect, numerous and are the least stable of the interactions. The level 1 interaction of CD151 with α3 occurs between the large extracellular loop of CD151 and the extracellular membrane proximal region of the α3 subunit (Yauch et al., 2000). A CD151 epitope recognized by the mAb TS151R is located at this site of interaction (Serru et al., 1999; Yauch et al., 2000). Recently, it was shown that the binding site on CD151 for tetraspanins is different from the one involved in the binding to integrins (Berditchevski et al., 2001).

Integrins are heterodimeric cell surface receptors consisting of a non-covalently associated α and β subunit, which link the extracellular matrix to the cytoskeleton (Hynes, 1992; Schwartz et al., 1995; van der Flier and Sonnenberg, 2001). The integrins α3β1, α6β1 and α7β1 bind preferentially to laminin and contain sequential and structural homologies. During maturation, the α3, α6 and α7 integrin subunits are post-translationally cleaved into a heavy and a light chain.

Furthermore, the mRNAs for these integrin subunits can be alternatively spliced, leading to extracellular and cytoplasmic variants (Collo and Quaranta, 1993; Hogervorst et al., 1993; de Melker et al., 1997). The ligand binding and signaling function of these integrin variants is potentially different, which during development may lead to changes in function when variants are switched (Ziober et al., 1993; Thorsteindóttir et al., 1995; Schober et al., 2000).

While much is known about the function and biochemical properties of integrins, the exact function of the associated tetraspanins is still elusive but is gradually becoming unraveled. Previously, we have shown that CD151 is a component of hemidesmosomes, specialized structures in the basal epidermal cell layer that link intermediate filaments to the extracellular matrix (Sterk et al., 2000). Others have shown the involvement of CD151 in cell adhesion (Hasegawa et al., 1998; Fitter et al., 1999), (tumor) cell migration and metastasizing (Yáñez-Mó et al., 1998; Yauch et al., 1998; Sincock et al., 1999; Sugiura and Berditchevski, 1999; Berditchevski and Odintsova, 1999; Testa et al., 1999), vesicular transport of integrins (Sincock et al., 1999), signaling (Berditchevski et al., 1997; Yauch et al., 1998; Sugiura and Berditchevski, 1999; Berditchevski and Odintsova, 1999; Zhang et al., 2001), cell polarization (Yáñez-Mó et al., 2001), wound healing (Peñas et al., 2000) and neurite outgrowth (Stipp and Hemler, 2000).

In vivo, CD151 is expressed by a large variety of cells of epithelial and mesenchymal origin. The tissue distribution of CD151 shows a striking overlap with that of the combined patterns of expression of α3β1, α6β1, α6β4 and α7β1 - all laminin-binding integrins (Sincock et al., 1997). This suggests that CD151 preferentially interacts with the laminin-binding integrins.

Laminin is a basement membrane component that, by interaction with its cell surface receptors, influences cell shape, movement and differentiation (Colognato and Yurchenko, 2000). Besides representing a ligand for integrins, laminin also interacts with the recently discovered Lutheran (LU) molecule expressed on erythrocytes (El Nemer et al., 1998; Parsons et al., 2001; Udani et al., 1998) and with dystroglycan (DG), an integral component of the dystrophin glycoprotein complex (DGC) originally detected in skeletal muscle and subsequently in epithelia (Ibraghimov-Breskovnaya et al., 1992; Durbeej and Campbell, 1999).

In the present study we investigated the expression of various tetraspanins and laminin receptors in tissues that strongly express one or more of the laminin-binding integrins, such as kidney (α3β1 in glomerular cells; α6β1 in tubular cells), skin (α3β1 and α6β4) and muscle (α7β1). At sites of contacts between cells and the matrix, CD151 is the only one of the tetraspanins tested that is always colocalized with the laminin-binding integrins. The intimate relation between CD151 and the laminin-binding integrins is also stressed by their high stoichiometric interaction and the increase of CD151 cell surface expression in K562 cells transfected with one of these integrins. By immunoprecipitation it was demonstrated for the first time that CD151 is associated with α7β1, and that their firm association is comparable to that of the association between α3β1 and CD151. Cytoplasmic or extracellular splice variants or mutations preventing cleavage of the integrin α subunit were found to have no influence on the formation of integrin-CD151 complexes. Although there is a considerable overlap in expression between the laminin receptors from the three different receptor families and CD151, no interaction between CD151 and either LU or DG could be identified in vitro. Finally, we provide evidence that the same site on CD151 interacts with all the different laminin-binding integrins. Furthermore, the exposure of a CD151 epitope involved in this binding is cell type specific and may vary depending on the (patho)physiological status of the tissues involved.

Materials and Methods

Antibodies

Mouse mAbs J8H against the extracellular domain of the human α6 integrin subunit and 29A3 against the cytoplasmic domain of the human α3A subunit have been described previously (Hogervorst et al., 1993; de Melker et al., 1997). The hybridomas producing the mouse mAbs J143, recognizing an extracellular epitope on the human α3 integrin subunit (Kantor et al., 1987) and TS2/16 against β1 (Hemler et al., 1984), were obtained from the American Type Culture Collection (Rockville, MD). Sam-1 is a mouse mAb against α5 (Keizer et al., 1987), and GoH3 is a rat mAb against α6 (Sonnenberg et al., 1987). The mouse mAbs CA25 and 13C2 against α7 and αv were generously provided by R. H. Kramer (University of California San Francisco, San Francisco, CA) and M. Horton (Imperial Cancer Research Fund, London, UK), respectively. Rat mAb 439-9B against β4 (Falcioni et al., 1988) was purchased from Pharmingen (San Diego, CA). The rabbit polyclonal antibodies against the cytoplasmic domains of α3A (DiPersio et al., 1995), αv (Defilippi et al., 1991) and α7B were kind gifts from M. DiPersio (Albany Medical College, Albany, NY), G. Tarone (Università di Torino, Torino, Italy) and U. Mayer (University of Manchester, Manchester, UK), respectively. Mouse mAbs against α-dystroglycan were obtained from Novocastra (Newcastle, UK). Polyclonal antibody AP83 against β-dystroglycan was kindly provided by K. P. Campbell and M. Durbeej (Howard Hughes Medical Institute, Iowa, USA). Mouse mAb LU4F2 (workshop 70597) against Lutheran blood group antigens was a kind gift from E. van der Schoot (Central Laboratory for the Blood Transfusion Service, Amsterdam, The Netherlands). The mouse mAbs P48 and P49, also known as 11B1.G4 and 14A2.H1, respectively, were clustered as CD151 in the VI International Leukocyte Typing Workshop (Ashman et al., 1997; Sincock et al., 1997).

The other mouse mAbs against CD151 were all gifts: 5C11 from M. E. Hemler (Dana-Farber Cancer Institute, Boston, MA), 8C3 from K. Sekiguchi (Osaka University, Osaka, Japan), Sfa-1 from H. Hasegawa (Hasegawa et al., 1996) (Ehime University, Ehime, Japan), LIA1/1 from F. Sánchez-Madrid (Hospital de la Princesa, Madrid, Spain), IIG5 from M. Humphries (The University of Manchester, Manchester, UK) and TS151 and TS151R (Yauch et al., 2000; Serru et al., 1999) from E. Rubenstein (Hôpital Paul Brousse, Villejuif Cedex, France). The mouse mAbs MEM62 (CD9; (Hořejší and Vlček, 1991)), MEM53 (CD53 (Angelisová et al., 1994)), M38 (CD81 (Imai and Yoshie, 1993)) and C33 (CD82 (Imai and Yoshie, 1993)), were generously provided by V. Hořejší (Institute of Molecular Genetics, Prague, Czech Republic). F. Berditchevsky (The University of Birmingham, Birmingham, UK) kindly supplied the mouse mAb 6H1 (CD63 (Berditchevsky et al., 1995)). The sheep anti-mouse and donkey anti-rabbit horseradish-peroxidase-coupled secondary antibodies were purchased from Amersham (Harlington Heights, IL). FITC- or Texas Red-conjugated goat anti-mouse, goat anti-rat or goat anti-rabbit secondary antibodies were obtained from Rockland (Gilbertsville, PA). The FITC- and TRITC-conjugated goat anti-mouse isotype specific antibodies were obtained from Nordic Immunochemical Laboratory (Tilburg, The Netherlands). Phycoerythrin-conjugated goat anti-mouse or anti-rat antibodies were obtained from Jackson laboratories (Westgrove, PA).

Generation of retroviral expression constructs and stable cellular transduction

The full-length c-DNA encoding the X1 and X2 splice variants of the extracellular domains of human α7B were a kind gift from D. Mielenz and K. von dem Mark (University of Erlangen, Erlangen, Germany). α7BX1 and -X2 c-DNA was released from pUC-18 or pcDNA3 respectively, by digesting with *Eco*RI. The resulting fragments were ligated into the retroviral LZRS-IRES-zeo expression vector, a modified LZRS retroviral vector conferring resistance to zeocin (Kinsella and Nolan, 1996; van Leeuwen et al., 1997), to give an LZRS-IRES-zeo-α7BX1 or -α7BX2 vector. These constructs were then introduced into the Phoenix packaging cells (Kinsella and Nolan, 1996) by the calcium phosphate precipitation method and virus containing supernatant was collected. K562 cells were infected with the recombinant virus by the DOTAP method (Boehringer Mannheim Corp.). After incubation for 8 hours at 37°C, infected cells were grown in medium as described below for the wild-type K562 cells. Cells expressing α7 at their surface were analyzed and sorted using FACScan® flow cytometer (Becton Dickinson, Mountain View, CA).

Cell lines

The human erythroleukemic cell line K562 was maintained in RPMI-1640 supplemented with 10% heat-inactivated fetal calf serum (Life Technologies, Paisly, UK), 100 U/ml penicillin and 100 μg/ml streptomycin (both from Life Technologies). K562 cells stably expressing α3β1, α6β1 and α6β4 were established as described previously (Delwel et al., 1993; Niessen et al., 1994). An established cell line of human glomerular visceral epithelial cells (Krishnamurti et al., 1996) was cultured in medium made up of 1 volume of each Dulbecco's modified Eagle's medium (DMEM) (ICN, Costa Mesa, CA) and Hams F-10 (Life Technologies), supplemented with 5% Nu serum (Becton Dickinson, Bedford, MA), 25 ng/ml prostaglandin E_1, 0.5 nM tri-iodothyronine, 10 nM sodium selenite, 5 μg/ml transferrin, 50 nM hydrocortisone, 5 μg/ml insulin (all hormones from Sigma, St. Louis, MO), 100 U/ml penicillin and 100 μg/ml streptomycin. Human proximal tubular epithelial cells (PTEC) also known as HK-2 cells are HPV 16-immortalized cells purchased from the American Type Culture Collection. Cells were grown in conditioned medium consisting of 1 volume of DMEM and 1 volume Ham F-12, supplemented with 5% heat-inactivated FCS, 100 U/ml penicillin and 100 μg/ml streptomycin, 2 mM L-glutamine, 5 μg/ml insulin, 5 μg/ml transferrin, 5 ng/ml sodium selenite, 20 ng/ml tri-iodothyronine, 5 ng/ml hydrocortisone, 5 ng/ml prostaglandin E_1 and 5 ng/ml epidermal growth factor (all from Sigma, St. Louis, MO). Cells were grown at 37°C in a humidified, 5% CO_2 atmosphere.

Immunoprecipitation of ^{125}I-labeled cells

Transfected K562 cells, stably expressing α3A, α3B, α6A, α6B or $\alpha6A^{RGGD}$ mutant, were surface labeled with ^{125}I by the lactoperoxidase method as previously described (Sonnenberg et al., 1987). Cells were solubilized in lysis buffer containing 1% (w/v) CHAPS (Sigma), 5 mM $MgCl_2$, 25 mM Hepes, pH 7.5, 150 mM NaCl and proteinase inhibitors (1 mM phenylmethanesulphonyl fluoride, 10 μg/ml soybean trypsin inhibitor and 10 μg/ml leupeptin). Lysates were clarified at 20,000 ***g*** and precipitated with protein A-sepharose beads CL-4B (Amersham Pharmacia Biotech Inc., Uppsala, Sweden), which had previously been sequentially incubated with rabbit anti-mouse IgG and the precipitating antibody. After incubation for 1.5 hour at 4°C, the beads carrying the immune complexes were washed and treated with sodium dodecyl sulfate (SDS) sample buffer. Precipitated proteins were analyzed by SDS-polyacrylamide gel electrophoresis.

Immunoblotting

Wild-type and transfected K562 cells stably expressing αvβ1, α3β1, α6β1, α6β4, α7X1β1 or α7X2β1, and podocytes and PTEC were lysed in 1% (w/v) CHAPS buffer with proteinase inhibitors. Alternatively, cells were lysed in 1% (v/v) Nonidet P-40, 50 mM Tris-HCl, pH 7.5, 150 mM NaCl and 1 mM EDTA. Lysates were incubated for 1 hour at 4°C with Gamma Bind-G Sepharose beads or protein A-Sepharose beads (Amersham Pharmacia Biotech, Uppsala, Sweden), previously incubated overnight at 4°C with the precipitating antibodies. The beads carrying the immune complexes were washed three times with lysis buffer and two times with PBS at 4°C. The immune complexes were eluted by the addition of sample buffer, heated at 95°C or 65°C and separated by SDS-PAGE under reducing (2% β-mercaptoethanol) or non-reducing conditions. After electrophoresis, gels were electrophoretically transferred to a PVDF membrane (Immobilon-P, Bedford). Blots were stained with Coomassie blue to indicate the markers, destained in 45% methanol, 5% acetic acid in demineralized water and blocked for 30 minutes with 5% dry milk in TBST-buffer (10 mM Tris, pH 7.5, 150 mM NaCl, 0.3% Tween-20). Subsequently blots were incubated for 1 hour with primary antibodies in 0.5% dry milk in TBST. Primary antibodies were mAbs 29A3 against α3 (neat supernatant) or 8C3 against CD151 (1:1,000), or polyclonal antibodies against α3 (1:100), α7B (1:100), αv (1:100) or β-dystroglycan (1:100). After washing three times with TBST/0.5% dry milk, blots were incubated for an additional hour with secondary sheep anti-mouse, donkey anti-rabbit or goat anti-rat Ig-horseradish-peroxidase-coupled, diluted 1:5,000 or 1:1,000 respectively, in 0.5% dry milk in TBST. After washing three times with TBST, proteins were visualized by enhanced chemiluminescence (ECL, Amersham Pharmacia Biotech.), as described by the manufacturer.

Analyses of integrin and CD151 surface expression

The surface expression of integrin subunits and CD151 on wild-type and transfected K562 cells was assessed by flow cytometry. 5×10^5 cells were washed three times with PBS containing 2% FCS (PBS/FCS), followed by incubation with the primary antibody or antibodies for 1hour. The cells were washed three times with PBS/FCS and then incubated with phycoerythrin-conjugated goat anti-mouse or anti-rat antibodies for another hour. After another three washes with PBS/FCS, cells were resuspended in PBSA and analyzed with a FACScan® flow cytometer.

Immunohistochemistry and immunofluorescence of tissue sections

Cryostat sections (3 μm thick) from human kidney, skin, smooth, striated or heart muscle were fixed in acetone for 10 minutes at 4°C. For immunoperoxidase staining, endogenous peroxidase was blocked by 0.1% NaN3 and 0.3% H_2O_2 in PBS for 10 minutes. For blockage, PBS containing 2% BSA (PBSA) was used for 10 minutes, followed by overnight incubation with the primary antibody at 4°C. Anti-rabbit or anti-mouse horseradish-peroxidase-conjugated secondary antibodies (PowerVision, Technologies Co., Daly City, CA) were added for 30 minutes and visualized by hydrogen peroxide and 3′,3′-amino-9-ethyl carbazole (Sigma Chemical Co.). Sections were counterstained with hematoxylin and mounted in Depex (Britisch Drug House Chemicals, Poole, UK). In between steps, sections were washed three times with PBS. For double-labeled immunofluorescence analyses, sections were blocked in PBSA for 30 minutes and incubated with the primary antibody diluted in PBSA overnight at 4°C. The sections were washed three times with PBSA and then, depending on the primary antibodies used, incubated with different combinations of goat anti-mouse, goat anti-rat or goat anti-rabbit FITC- or Texas-Red-conjugated antibodies for 45 minutes. After washing twice with PBS, sections were mounted in Vectashield (Vector Laboratories Inc., Burlingame, CA) and viewed under a Leica TCS NT confocal laser-scanning microscope equipped with an Ar/Kr

laser (Leica Microsystems, Heidelberg, Germany). Unless otherwise mentioned, both procedures were performed at room temperature.

Results

Co-distribution of CD151 and the various laminin receptors in human kidney, skin and different kinds of muscle

Interactions between tetraspanins and integrins have been defined in biochemical assays, but there is limited information on the associations of these proteins in tissues. The tetraspanin CD151 has been shown to form tight complexes with α3β1 (Yauch et al., 1998), but in addition it may also associate with other laminin-binding integrins (Fitter et al., 1999; Sincock et al., 1999; Sterk et al., 2001). To assess its distribution relative to that of laminin-binding integrins in vivo, we determined the expression in tissues that express different laminin binding integrins; kidney (glomeruli: α3β1; tubuli: α6β1), skin (α3β1 and α6β4) and muscle (α7β1). In these tissues the distribution of other tetraspanins and of the laminin receptors DG and LU was also assessed.

Analysis of kidney tissues by immunoperoxidase staining, using mAb P48, revealed strong expression of CD151 in glomeruli, at the basal surface of tubular epithelial cells and in vascular smooth muscle cells (Fig. 1A). Double immunofluorescence analysis revealed a striking colocalization with α3β1 (Fig. 2A) and DG (Fig. 2B) within glomeruli in a predominant, continuous GBM-bound pattern. Along the GBM, DG was only partially co-distributed with α3β1 (Fig. 2C), whereas LU was absent from the glomerulus but strongly expressed in the subendothelial matrix and along the basal side of the tubules, colocalized with α6β1 (Fig. 2D). Like CD9 and CD63, the LU molecule is also focally distributed throughout the cytoplasm of tubular epithelial cells (not shown). Transport of LU to the apical or basolateral side of polarized cells has been described before (El Nemer et al., 1999). This, together with the presence of LU at the site of cell-cell contacts between keratinocytes (see below), suggests that LU is not merely a laminin receptor but has other functions as well. The tetraspanins CD9 (not shown), CD63 (Fig. 2E) and CD81 (Fig. 2F) are present in glomerular cells but are not colocalized with α3β1 along the GBM. Glomerular cells do not express CD53 or CD82, which however are present on a subset of the distal tubules together with CD9 and CD81, the latter being weakly expressed by all tubular cells (not shown).

Immunolocalization of CD151 in skin revealed strong reactivity at the basolateral surface of basal keratinocytes (Fig. 1B), where the integrins α3β1 and α6β4 are also concentrated (Sterk et al., 2000). Only very small amounts of DG are present at the dermo-epidermal junction, whereas LU is focally expressed on all basal keratinocyte surfaces (not shown). The tetraspanins CD9 and CD81 are not only present in the basal cell layer but also in the more superficial epidermal cell layers. They are absent from the dermo-epidermal junction but are concentrated at inter-keratinocytic contacts (not shown). CD63 is only expressed by melanocytes present between the basal keratinocytes (not shown).

Skeletal, cardiac and smooth muscle cells all express CD151 at their plasma membrane (Fig. 1C, Fig. 9A-C). Additionally, CD151 is present at the costamers in skeletal and cardiac muscle and the intercalated discs in the heart. In these cells, it is colocalized with α7Bβ1 (Fig. 3A,C) and DG (not shown). Besides CD151, smooth muscle cells express the tetraspanins CD9 and CD81 and some CD53 and CD82 (not shown). Small amounts of CD63 and CD81 are present at the sarcolemma of skeletal and heart muscle and at the intercalated discs. LU is not expressed in myocytes (not shown). In peripheral nerves, also present in the smooth muscle sections, α6β4, α7β1, LU, CD9, CD63, CD81 and CD151 are all localized at the endo- and perineurium (not shown). In summary, CD151 is extensively colocalized with various laminin receptors, both integrins and non-integrins, at sites of cell-matrix interactions.

α3 and α6 are coprecipitated with CD151

To determine whether CD151 is also able to associate with the integrin α3 and α6 subunits, K562 cells stably transfected with α3 or α6 were ^{125}I-surface-labeled and lysed in 1% CHAPS. As shown in Fig. 4, antibodies against α3 precipitated α3β1 and antibodies against α6, α6β1 from lysates of α3- and α6 transfected K562 cells, respectively. The transfected integrins were also present in precipitates prepared with antibodies

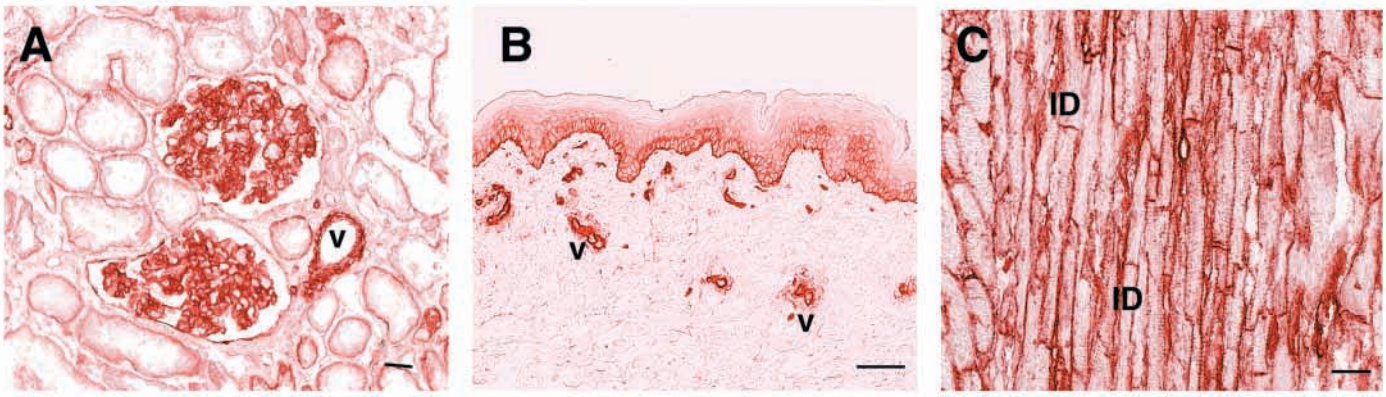

Fig. 1. Immunohistochemical distribution of CD151 in kidney, skin and heart. Cryostat sections of human kidney (A), skin (B) and heart tissue (C) were stained by immunoperoxidase reaction with anti-CD151 (P48). Intense staining is seen in the glomeruli and the epithelia of kidney tubules (A), keratinocytes of the skin (B) and in vascular smooth (A,B) and cardiac muscle cells (C). Note that CD151 is localized on the basolateral surface of epithelia and at the sarcolemma of cardiac myocytes. It is also detected at costameres and intercalated discs (ID). v, vessel. Bars, 50 (A), 100 (B) and 10 μm (C).

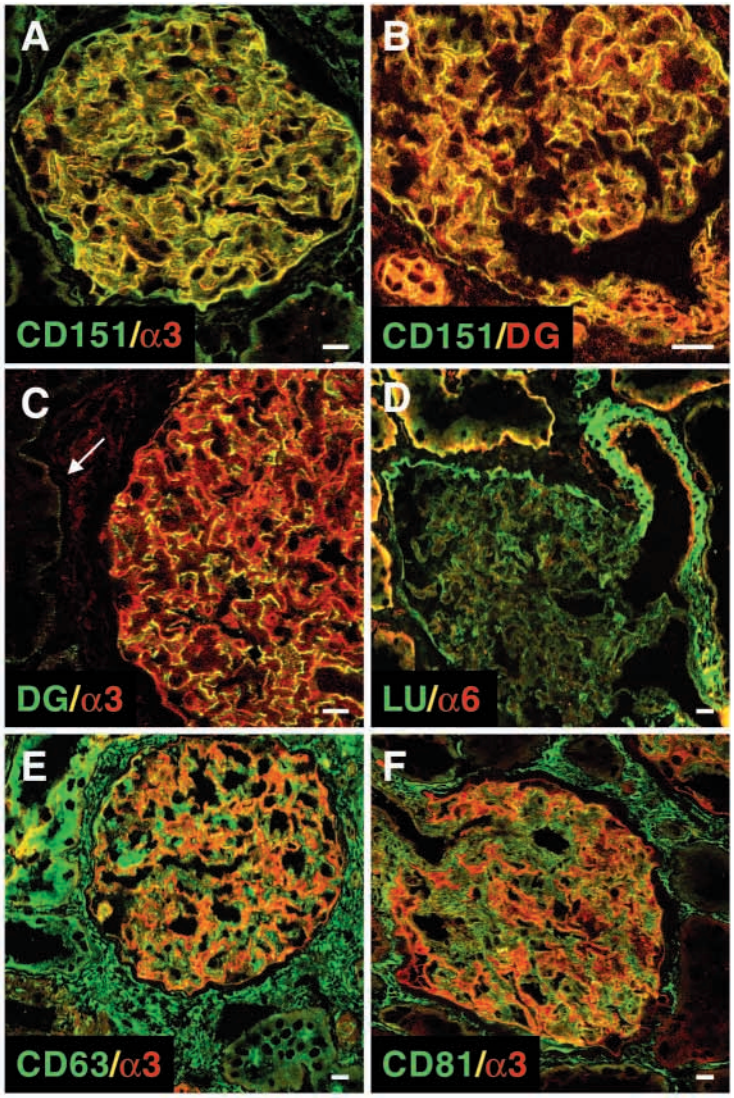

Fig. 2. Colocalization of CD151 with laminin receptors α3β1 and dystroglycan in the glomerulus and with α6β1, dystroglycan and the Lutheran molecule in tubular epithelial cells. Cryostat sections of human kidney were processed for double immunofluorescence confocal microscopy using P48 against CD151 (A,B), polyclonal antibodies against α3 (A,C,E,F), polyclonal (AP83) and monoclonal antibodies against dystroglycan (DG) (B,C, respectively), LU4F2 against the Lutheran blood group molecule (LU) (D), GoH3 against α6 (D), 6H1 against CD63 (E) and M38 against CD81 (F). Composite images were generated by superimposition of the green and red signals, with areas of overlap appearing as yellow. In the glomerulus, CD151 is co-distributed with laminin-receptors α3β1 and DG but not with LU. The distribution pattern of α3β1 is mainly restricted to the GBM side, where it completely overlaps with that of CD151 and partly with that of DG. CD151 is also present in the cytoplasm of cells and at cell surfaces other than the GBM side. Although prominently present in the cytoplasm of glomerular cells, CD63 and CD81 show hardly any overlap with α3β1 at the GBM side. In tubular epithelial cells, CD151 is colocalized with α6β1, DG (arrow) and LU. CD81 is only present at a low level, and CD63 is mainly present throughout the cytoplasm. Note the prominent staining with anti-LU in the subendothelial matrix and with α6β1 present on the endothelial lining. Bar, 10 μm.

against CD63, CD81 and CD151. However, although large amounts of α3β1 and α6β1 were coprecipitated with CD151, only small amounts were coprecipitated with CD63 or CD81. This difference is not due to differences in the level of expression of the proteins, as all three tetraspanins proved to be present at substantial levels in K562 cells, as shown by FACS analysis (Yauch et al., 1998) (L.M.T.H. and A.S., unpublished). Rather, it is the result of the high stoichiometric binding of CD151 to the laminin-binding integrins, which indicates the unique and specific role of CD151 in the formation of these stable complexes. Furthermore, immunoprecipitates of CD63 and CD151 from the two transfected K562 cell lines, contained a protein of the size of CD81 (23 kDa). Because it is known that tetraspanins can interact with other tetraspanins, we suspect that CD81 is coprecipitated because it is associated with CD63 and CD151, and not, as in the case of CD151, with integrins. CD63, which migrates as a smear between 45-60 kDa on gels, could not be detected in the various immunoprecipitations, probably because it is labeled only poorly with ^{125}I. As anticipated, similar amounts of the α3 and α6 subunits were coprecipitated with CD151 from lysates of K562 cells transfected with the cytoplasmic A or B variants of α3 or α6 (not shown). Thus, the formation of CD151-α3β1 or

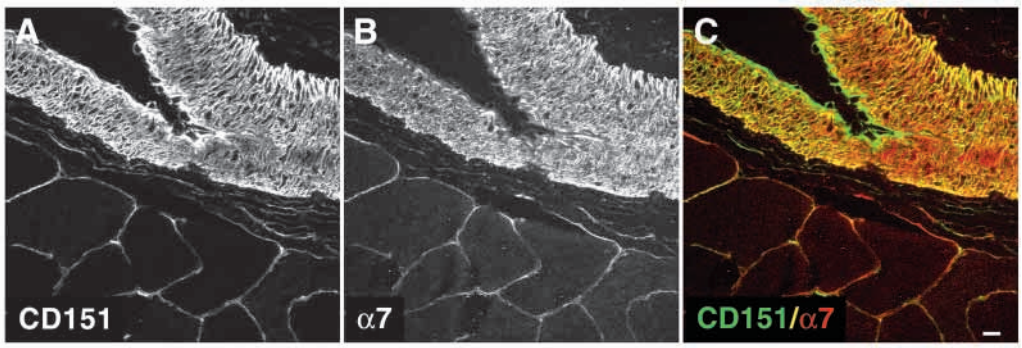

Fig. 3. Colocalization of CD151 with the laminin receptor α7β1 in smooth and skeletal muscle. Cryostat sections of human smooth and striated muscle were processed for double immunofluorescence confocal microscopy using P48 against CD151 (A) and polyclonal antibodies against α7B (B). The composite image (C) was generated by superimposition of the green and red signals, with areas of overlap appearing as yellow. The distribution pattern of CD151 and α7B completely overlap both at the sarcolemma of striated skeletal muscle (lower half) and on smooth muscle cells present in vessel walls (upper half). Note the prominent presence of CD151 in endothelial cells and the small amounts of CD151 in the stroma. Bar, 10 μm.

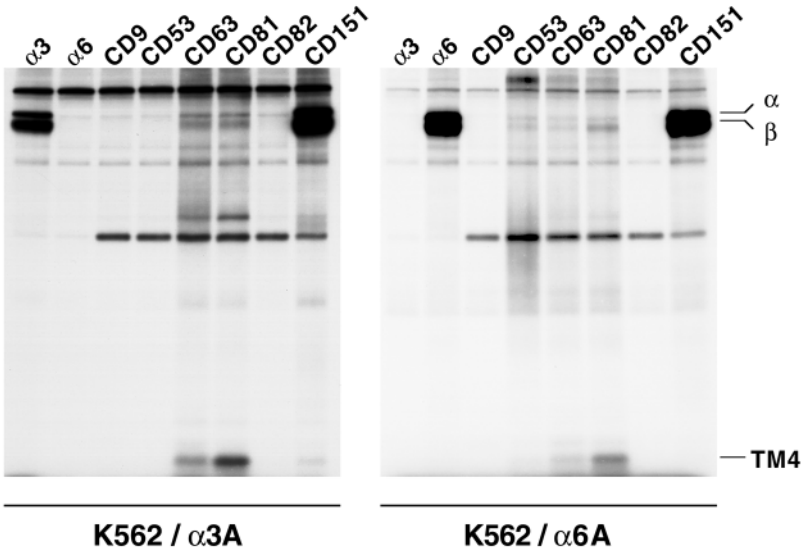

Fig. 4. Association of CD151 with α3β1 and α6β1 in transfected K562 cells. Transfected K562 cells stably expressing α3β1 or α6β1 were ^{125}I-surface-labeled, extracted with 1% CHAPS and lysates were subjected to immunoprecipitation with antibodies. Antibodies used were the anti-integrin antibodies J143 against the α3- and J8H against the α6 subunit and the anti-tetraspanin antibodies MEM62 against CD9, MEM53 against CD53, 6H1 against CD63, M38 against CD81, C33 against CD82 and 5C11 against CD151. Both α3β1 and α6β1 are present in immunoprecipitates prepared with antibodies against CD63, CD81 and CD151. However, the amount of integrin that is coprecipitated with CD151 is much greater than with the other tetraspanins, reflecting the stability and selectivity of the CD151-integrin binding. The formation of multimolecular complexes is reflected by the additional presence of CD81 (TM4) precipitated by antibodies against CD63 and CD151.

CD151-α6β1 complexes is not influenced by the nature of the splice variants in the integrin molecules.

CD151 stably associates with the two extracellular variants (X1 and X2) of α7B

Association of CD151 with either splice variant of the extracellular domain X1 or X2 of the laminin receptor α7β1 was investigated in coimmunoprecipitation experiments; the strength of the interaction was also tested by using different lysis buffers. CHAPS is considered to be a mild detergent in which weak interactions between different molecules are not disrupted, whereas Nonidet P-40 is more stringent leaving only stable complexes intact. As shown in Fig. 5 (upper panel), α7 is coprecipitated with CD151 from K562 cells stably expressing either the X1 or X2 splice variant, and vice versa CD151 is co-precipitated with α7 (lower panel). The α7 subunit and CD151 were precipitated in comparable amounts irrespective of the lysis buffer used. Thus, CD151 not only associates with the α3β1 and α6β1 integrins but also with α7β1, the strength of this interaction being comparable with that with α3β1.

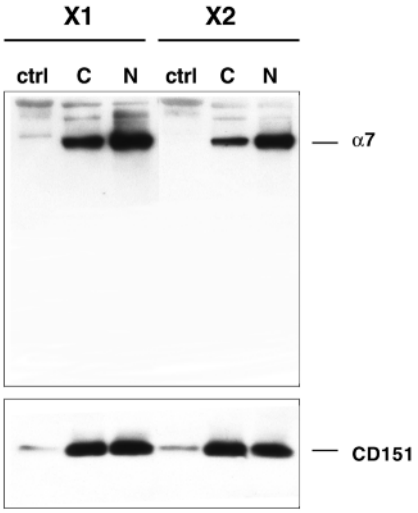

Fig. 5. CD151 is strongly associated with both splice variants (X1 and X2) of α7Bβ1 in transfected K562 cells. K562 cells stably expressing the α7β1 extracellular splice variant X1 or X2 were lysed in 1% CHAPS (C) or 1% Nonidet P-40 (N). Lysates were subjected to immunoprecipitation with mAb P48 against CD151 (upper panel) or with polyclonal antibodies against α7B (lower panel). The presence of α7B or CD151 in the immune complexes was detected by immunoblotting with anti-α7B or anti-CD151 (mAb 8C3) antibodies. No immunoreactive bands corresponding to α7B were detected in control (ctrl) immunoprecipitates (upper panel). As a further control (ctrl), the presence of CD151 in total lysates is shown (lower panel). Note that α7B is coprecipitated with CD151 and vice versa, and that this is independent of the extracellular splice variant or the lysis buffer used.

No influence of extracellular cleavage from the integrin α subunit on CD151 binding

The extracellular domain of the integrin α3, α6 and α7 subunits is cleaved during biosynthesis into a heavy and a light chain. This has been shown to be required for proper inside-out signaling by α6β1 in K562 cells but has no influence on ligand binding (Delwel et al., 1996). When the RKKR sequence (amino acids 876-879) is mutated to RGGR, cleavage into a heavy and a light chain cannot occur. For the formation of stable complexes of tetraspanins and integrins, their extracellular domains are essential (Yauch et al., 2000). To determine whether cleavage influences the binding of integrins to CD151, K562 cells were transfected with the α6A^{RGGR}-mutant (uncleaved). Cells were ^{125}I-surface-labeled, lysed in 1% CHAPS and the lysates precipitated with antibodies against α5, α6, β1, CD81 or CD151. As shown in Fig. 6, α6β1 could be precipitated by antibodies against α6 or β1 as well as by antibodies against CD81 or CD151. Consistent with previous results (Fig. 4), the amount of α6 precipitated by anti-CD81 is much smaller than that precipitated by anti-CD151. The α5β1 integrin that is endogenously present on K562 cells is only precipitated by antibodies against α5 or β1, not by antibodies against either of the two tetraspanins, CD81 or CD151.

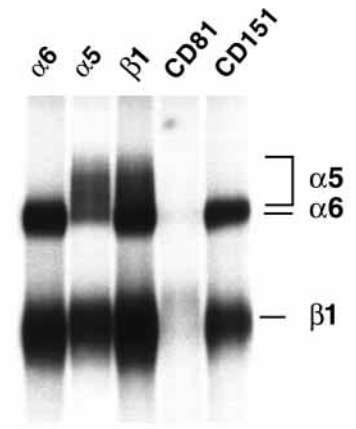

Fig. 6. The absence of cleavage of the integrin α6 subunit on the association with CD151 in transfected K562 cells has no effect. Transfected K562 cells, stably expressing α6A^{RGGR}β1 (uncleaved α6A), were ^{125}I-surface-labeled, lysed in 1% CHAPS and immunoprecipitated with anti-integrin subunit or anti-tetraspanin antibodies. Antibodies used for immunoprecipitation were J8H directed against α6, Sam-1 against α5, TS2/16 against β1, M38 against CD81 and 5C11 against CD151. Note that α6A^{RGGR}β1 is coprecipitated with CD151 and to a lesser extent with CD81. The integrin α5β1 is not coprecipitated with tetraspanins; the typical smear around 150 kDa representing α5 is not detected in the precipitates obtained with antibodies against these molecules.

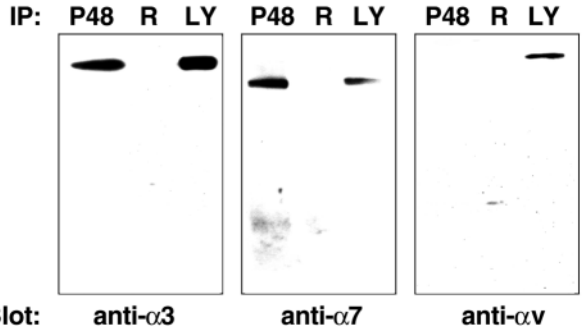

Fig. 7. Monoclonal antibody TS151R does not recognize CD151 when it is bound to α3β1 or α7β1. Transfected K562 cells stably expressing α3β1 (left), α7β1 (middle) or αvβ1 (right) were lysed in 1% Nonidet P-40 and subjected to immunoprecipitation with the mAbs P48 or TS151R, followed by immunoblotting with the indicated polyclonal antibodies against α3, α7 or αv. Immunoblot analysis of the lysates (LY) show the level of expression of each integrin.

Availability of the CD151 epitope recognized by antibody TS151R in vitro and in vivo

TS151R is a monoclonal antibody directed against an epitope on CD151 that is located at the binding site for α3, whereas P48 recognizes an epitope outside this binding site (Serru et al., 1999; Yauch et al., 2000). To investigate whether the CD151 epitope recognized by TS151R is also involved in the binding to α7β1, lysates of K562 cells stably expressing the integrin α7β1 were subjected to immunoprecipitation with mAb TS151R, and the presence of α7 was tested by immunoblotting. Immunoprecipitations prepared from lysates of K562 cells that were transfected with cDNAs encoding the α3- or αv-subunits served as positive and negative controls, respectively. As shown in Fig. 7, the α3 and α7 integrins were only precipitated by P48 and not by TS151R. The αvβ1 integrin was not precipitated by either of the antibodies. Thus, the TS151R epitope is not only masked when CD151 is associated with α3β1 but also when it is bound to α7β1.

Next we compared the distribution of the CD151 epitope recognized by TS151R with that by P48 (Table 1). Kidney sections incubated with TS151R (Fig. 8A,C) show hardly any fluorescent staining in the glomerular cells that express α3β1 (Fig. 8B,C), whereas in the tubuli expressing α6β1, staining is comparable to that of mAb P48. In the skin, where CD151 is not only associated with α3β1 but also with α6β4 (Fig. 8E,F,H,I), the staining by TS151R (Fig. 8G,I) is weak compared with that by mAb P48 (Fig. 8D,F). Staining of the different muscle tissues expressing α7β1 varied: all three muscle tissues were strongly stained by P48 (Fig. 1C, Fig. 9A,B), smooth muscle cells were also strongly stained by TS151R (Fig. 9C), cardiomyocytes only weakly at the intercalated discs (Fig. 8J,L), and striated muscle cells were not stained at all by this antibody (Fig. 9D). Staining of cardiomyocytes for α7 expression is shown in Fig. 8K,L. For further comparison and the possible recognition of CD151 epitopes involved in the binding of integrins, all the above tissues were incubated with a panel of other antibodies against CD151. This resulted in staining patterns comparable to those produced by P48 (not shown). Thus, binding of CD151 to laminin-binding integrins leads to masking of the TS151R epitope in many, but not all, types of cells in vivo.

Table 1. Differences in the recognition of CD151 by P48 compared to TS151R

Tissue	Laminin-binding integrin	Reactivity with antibody	
		P48	TS151R
Kidney			
Glomerulus	α3β1	+++	±
Tubules	α6β1	++	++
Skin	α3β1	+++	–
	α6β4	+++	±
Muscle			
Smooth	α7β1	+++	+++
Striated	α7β1	++	–
Heart	α7β1	+++	±

Reactivity of various tissues with CD151-specific antibodies; +++, very strong staining; ++, strong staining; ±, weak staining; –, no staining.

Level of CD151 cell surface expression is integrin dependent

Previously it has been shown that expression of α3β1 in K562 cells results in an increase in the surface expression of CD151 (Yauch et al., 1998). To determine whether this ability of α3β1 to induce CD151 expression is a general property of laminin-binding integrins, the surface expression of CD151 was assessed by flow cytometry (FACScan) of transfected K562 cells stained with mAb P48. As shown in Fig. 10, the expression of transfected αv had little or no effect on the expression of CD151. However, expression of α3β1, α6β1, α6β4, α7X1β1 or α7X2β1 in K562 cells increased the expression of CD151 three- to sixfold. Staining of the cells with TS151R revealed that the CD151 epitope, which is

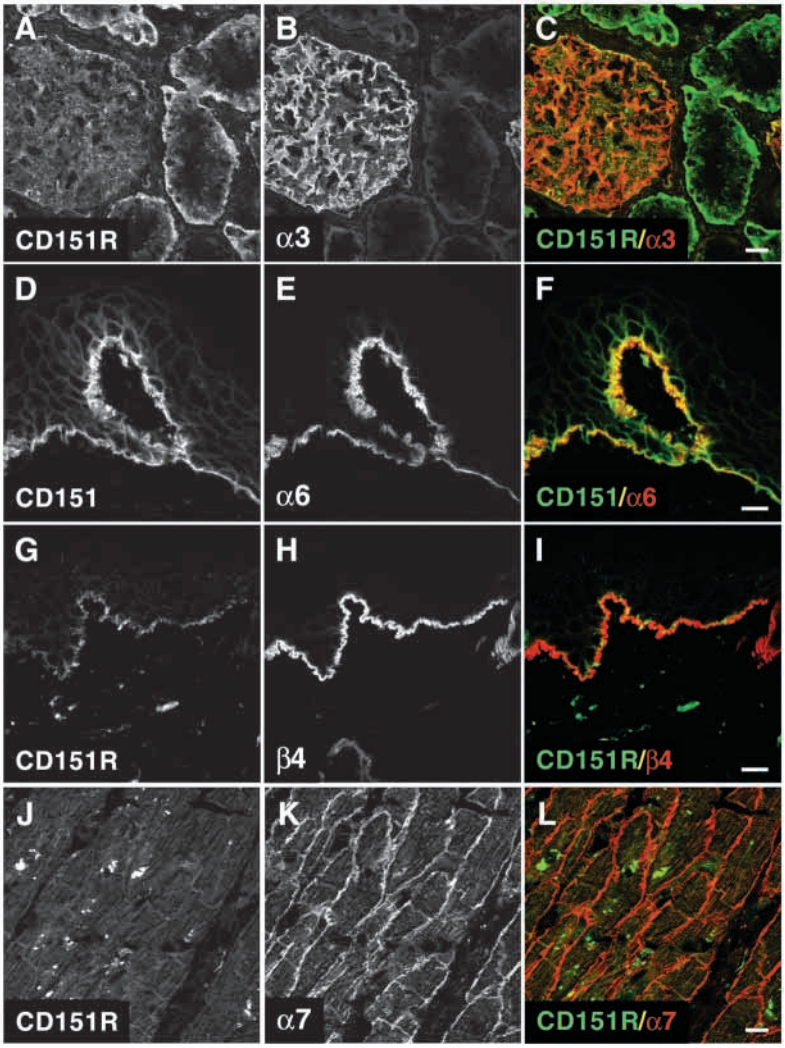

Fig. 8. Reactivity of CD151 with antibody TS151R is cell type specific. Cryostat sections of human kidney, skin and heart muscle were processed for double immunofluorescence confocal microscopy using TS151R (A,G,J) and P48 (D) against CD151, GoH3 against α6 (E), 439-9B against β4 (H) and polyclonal antibodies against α3 (B) or α7 (K). The composite images (C,F,I, L) were generated by superimposition of the green and red signals, with areas of overlap appearing as yellow. TS151R against CD151 reacts with CD151 in tubular epithelial cells but hardly reacts in glomerular cells (A-C), keratinocytes (G-I) and cardiomyocytes (J-L). P48 against CD151 is used in the skin for comparison and shows a prominent staining especially at the dermo-epidermal junction (D-F). Bar, 10 μm.

recognized by this antibody, is masked, and it is not dependent on the type of laminin-binding integrin that the K562 cells were transfected with. These results are consistent with the results of the above biochemical study showing that the TS151R epitope is not expressed on K562 cells expressing different laminin-binding integrins. However, also no CD151 was detected with TS151R on untransfected K562 cells. We therefore cannot exclude non-reactivity of this antibody in fluorescence studies, because of low affinity when the number of available epitopes is restricted. Furthermore, the results indicate that the surface levels of CD151 on cells are strongly influenced by the expression of the laminin-binding integrins.

Lack of complex formation between CD151 and the Lutheran molecule or dystroglycan

The colocalization of CD151 with the different kinds of laminin receptors, DG, LU and integrins, may suggest a role for CD151 in linking these three kinds of laminin receptors to each other. To investigate whether CD151, DG and LU are not only colocalized but also complexed, cultured human podocytes and proximal tubular epithelial cells (PTEC) were used for immunoprecipitation studies (Fig. 11). FACS analysis showed that both cell lines express α3β1, CD151, DG

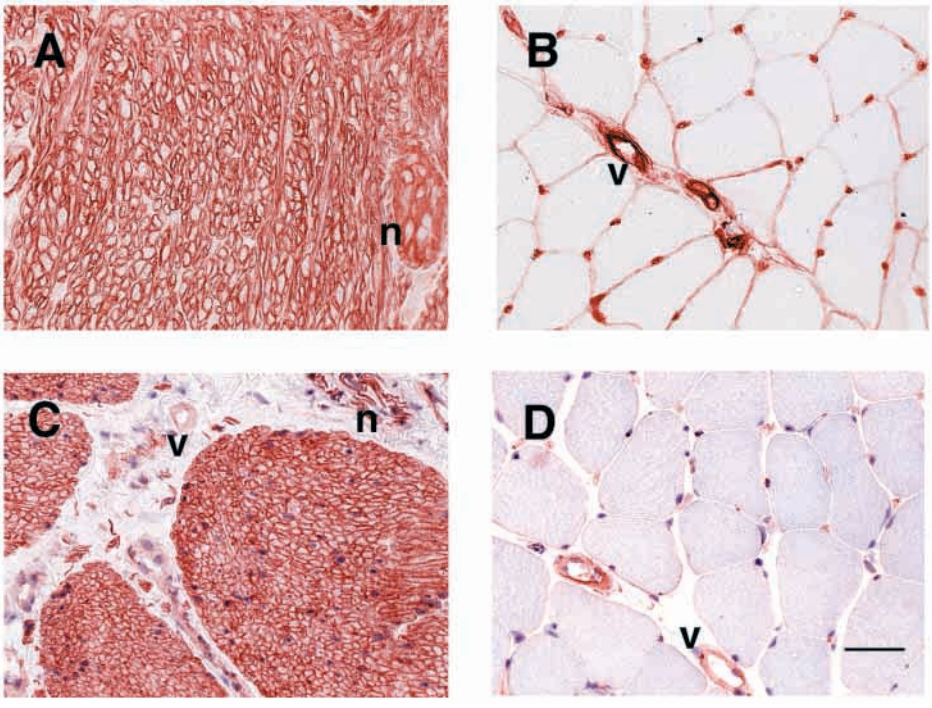

Fig. 9. Immunohistochemical staining of smooth and striated muscle tissue with P48 and TS151R against CD151. Cryostat sections of human smooth (A,C) and striated muscle (B,D) were stained by immunoperoxidase reaction with P48 (A,C) or TS151R (B,D). Both types of muscle cells were strongly stained by P48. Intense staining of CD151 by TS151R was only seen in smooth muscle cells, no staining is found in skeletal muscle. n, nerve; v, vessel. Bar, 10 μm.

and LU, although LU is only weakly expressed by podocytes. These expression profiles correspond with those of podocytes and PTECs in vivo. Lysates from cells prepared with 1% CHAPS were incubated with antibodies against α3, CD151, LU or β-DG. Subsequently the presence of α3 (light chain, 30 kDa) or β-DG (43 kDa) in the precipitates was assessed by immunoblotting using specific antibodies. Protein bands corresponding to α3 could be detected on the immunoblots of the immunoprecipitates containing α3β1 or CD151 from both cell lysates (left panels). LU or DG could not be coprecipitated with α3β1 with any of the cell lysates. P48 against CD151 and antibodies against α3 or LU did not coprecipitate DG (right panels) either. This demonstrates that in the tested cell lines, CD151 bound to the laminin-binding integrins does not associate with the other laminin receptors LU or DG and therefore cannot serve as a link between them.

Discussion

In a former study we have shown that CD151 is associated with α3β1 and α6β4 in the basal keratinocyte layer of the skin and in hemidesmosomes (Sterk et al., 2000). Now we have investigated the association of CD151 with various laminin receptors in kidney, skin and muscle tissues selected for the laminin-binding integrins they express.

As indicated by this study, there are different distribution patterns of CD151. In the first pattern most of the CD151 is colocalized with the laminin-binding integrins present and thus is mainly expressed at the site of cell-matrix interactions where there is a basement membrane

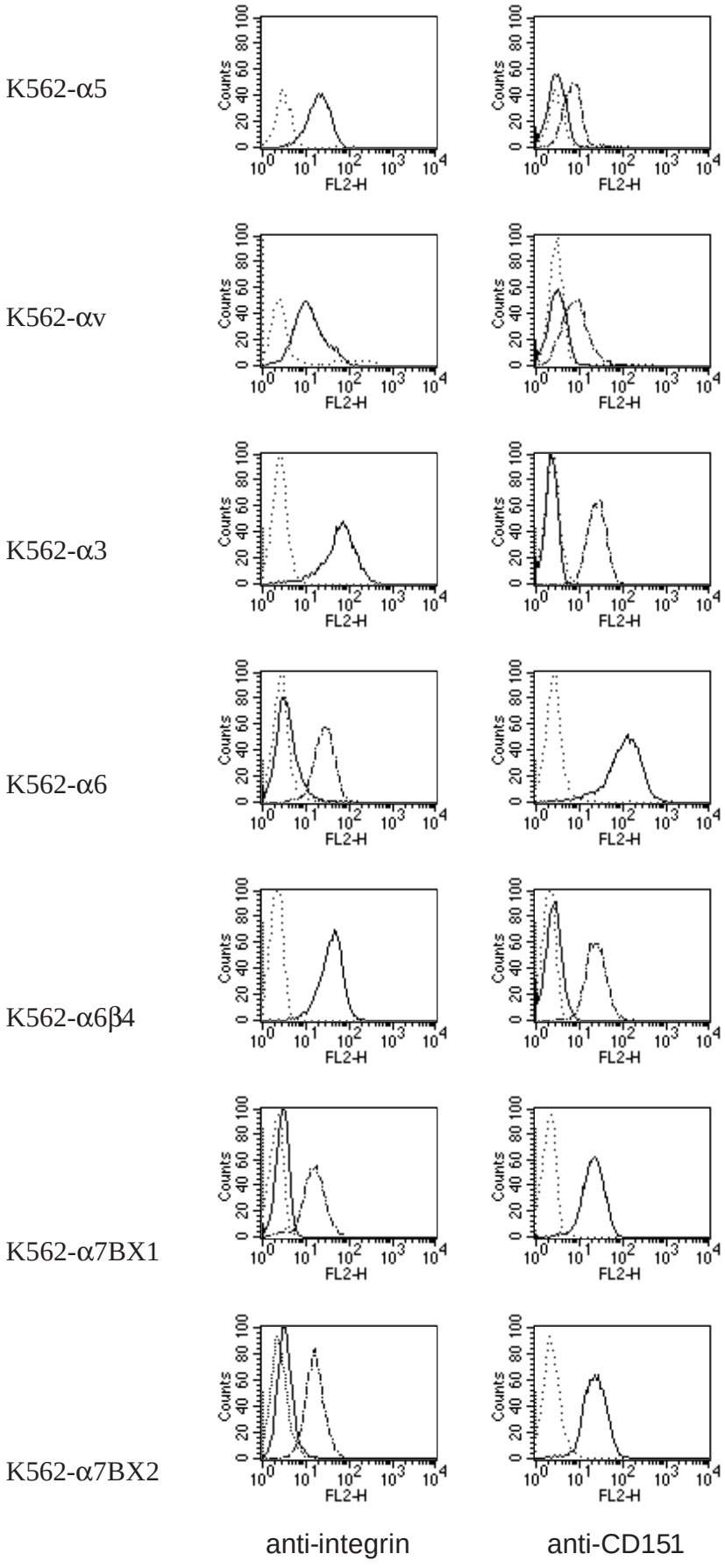

Fig. 10. Expression of laminin-binding integrins on transfected K562 cells increases CD151 cell surface expression. Flow cytometric analysis of wild-type K562 cells endogenously expressing α5β1 and transfected K562 also expressing αvβ1, α3β1, α6β1, α6β4, α7X1β1 or α7X2β1. Solid lines in the left panels indicate staining with anti-integrin antibodies: anti-α5 mAb Sam-1 (K562 cells), anti-αv mAb 13C2 (K562-αv), anti-α3 mAb J143 (K562-α3), anti-α6 mAb GoH3 (K562-α6), anti-β4 mAb 439-9B (K562-α6β4) and anti-α7 mAb CA25 (K562-α7BX1 and K562-α7BX2). Staining of cells with anti-CD151 antibodies is shown in the right panels. Solid lines represent staining of cells with mAb TS151R and broken lines with mAb P48. A negative control (dotted line) with secondary goat anti-mouse or rat IgG alone is shown in each panel. R represents the ratio of the mean fluorescence intensity of cells stained with mAb P48 and with secondary antibody alone. A three- to sixfold increase of CD151 expression is observed in transfected cells that ectopically express laminin-binding integrins. The TS151R epitope is masked in all cells expressing one of the laminin-binding integrins.

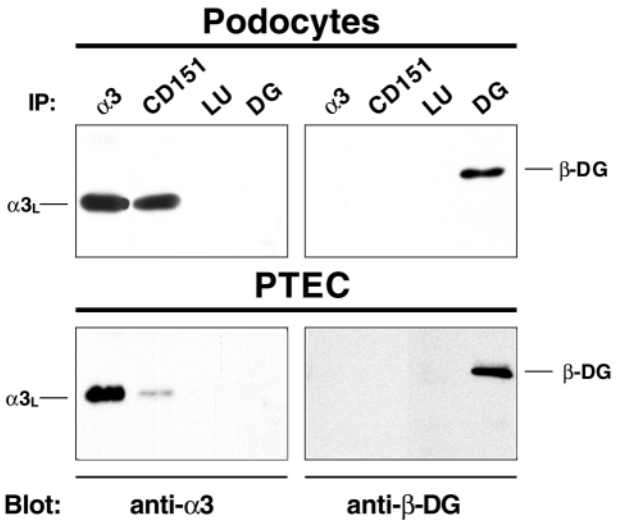

Fig. 11. No complexes are formed between dystroglycan or the Lutheran blood group molecule and CD151-α3β1 complexes in human podocytes and proximal tubular epithelial cells in culture. Human podocytes (upper panels) and proximal tubular epithelial cells (PTEC) (lower panels) expressing the laminin receptors α3β1, dystroglycan (DG) and Lutheran blood group antigens (LU) were lysed in 1% CHAPS and precipitated with polyclonal antibodies against α3 or DG (AP83) and mAbs against CD151 (P48) or LU (LU4F2). The immunoprecipitates were analyzed by immunoblotting for the presence of α3 and DG using specific antibodies. Note that DG is not coprecipitated with α3β1 or CD151 and that α3β1 is not seen in the precipitates obtained by antibodies against LU or DG. $\alpha3_L$, α3 light chain; β-DG, β-dystroglycan.

containing at least one laminin isoform (Miner et al., 1997; Aumailley and Rouselle, 1999). Thus, in glomerular cells, CD151 and α3β1 are colocalized at the GBM side of cells. In renal tubular epithelial cells, CD151 and α6β1 are concentrated at the tubular basement membrane, and in skin CD151 and α3β1 and α6β4 are present at the dermo-epidermal junction. Finally in myocytes, CD151 together with α7β1 is expressed at the sarcolemma. The second pattern is seen in cells such as endothelial and most hematopoietic cells in which the amount of laminin-binding integrins is limited. As shown by others, these cells contain, in addition to a considerable pool of unbound CD151, CD151 that is weakly associated with α5β1 and other surface molecules (Hasegawa et al., 1998; Fitter et al., 1999; Sincock et al., 1999). The third distribution pattern is seen in fibroblasts (stromal cells) and K562 cells, which contain CD151 but no laminin-binding integrins.

In addition to the previously reported association between CD151 and the laminin-binding integrins α3β1, α6β1 and α6β4, we demonstrate here that CD151 also interacts with α7β1. Like CD151-α3β1 complexes, CD151-α7β1 complexes remained intact after Nonidet P-40 lysis of transfected K562 cells, which classifies their interaction as level 1. Also using transfected K562 cells, it was shown that CD151-α6β1 and CD151-α6β4 (Sterk et al., 2000) interact at level 2, the interaction not being resistant to Nonidet P-40 treatment. A correlation between the presence of the laminin-binding integrins and CD151 expression was established by FACS analysis, which showed an increase of CD151 expressed at the cell surface of K562 cells after transfection of either of the laminin-binding integrins.

CD151 does not link the different laminin receptors with each other

Two non-integrin receptors for laminin are DG and LU. DG was originally isolated from skeletal muscle as a component of the large oligomeric DGC, which amongst other proteins contains sarcospan and dystrophin (Durbeej and Campbell, 1999). The third type of laminin receptor is the molecule expressing the LU antigens, which is able to bind to the laminin α5 chain present in the subendothelial matrix (Parsons et al., 2001).

Our in vivo results show a considerable overlap in the distribution of these different types of laminin receptors. Hence, a tight regulation and precise coordination of the signaling mechanisms by the different receptors seems essential. An example of an interaction between the different laminin receptors is shown in glomerulogenesis, during which a switch occurs in expression of the laminin-binding integrins from α6β1 to α3β1 simultaneously with a switch in the deposition of laminin isoforms, that is, from laminin-1 to laminin-11 (Sterk et al., 1998). Others have shown that the symptoms induced by the absence of dystrophin, which disturb the function of the laminin receptor DG, can be partly compensated by increasing the expression of α7β1 (Burkin et al., 2001). Moreover, myocytes of patients with Duchenne's disease lacking dystrophin and of dystrophin-deficient mice express an increased amount of α7β1 (Hodges et al., 1997). This indicates that when one laminin receptor is lacking, another laminin receptor can (partially) compensate for it. A protein present in the DGC that is homologous to tetraspanins is sarcospan (Crosbie et al., 1999). Although sarcospan is a component of the DGC and has a function in maintaining the integrity of muscle tissue, sarcospan-deficient mice have only mild symptoms of muscular atrophy (Lebakken et al., 2000). Moreover, epithelial cells expressing DG do not contain sarcospan (Durbeej and Campbell, 1999). These data suggest that in sarcospan-deficient mice and epithelia expressing DG, other tetraspanins may compensate for the absence of sarcospan. We demonstrate that, except in the skin, in all tissues tested, DG, the laminin-binding integrins and CD151 are co-distributed. Furthermore, this study shows that CD151 is the only known tetraspanin in the glomerulus that is present at the side of the basement membrane, together with DG. Therefore and analogous to the sarcolemma, the sarcospan function in the glomerulus could be taken over by CD151, thereby connecting the integrin family with the DGC. However, despite their co-distribution, coimmunoprecipitation studies could not demonstrate a physical association between α3β1-CD151 complexes and DG or LU in two different cell lines obtained from the kidney, that is, human podocytes and proximal tubular epithelial cells.

Neither the variants of the extracellular or cytoplasmic domains of integrin α subunits nor the cleavage of α6 affect binding of CD151 to integrins

The binding between tetraspanins and integrins is established via the extracellular domains of the tetraspanin and the integrin α subunit (Yauch et al., 2000). Theoretically, splicing of the mRNA for the extracellular or cytoplasmic domains of integrin

subunits or maturation-induced cleavage of the α chain could influence the association of tetraspanins with integrins and thereby alter integrin signaling. However, biochemically no such differences could be demonstrated for the cytoplasmic A and B variants of the integrin α3 and α6 subunits or for the extracellular variants X1 and X2 of α7. Furthermore, mutational changes in the extracellular domain of α6 preventing cleavage seemed to have no influence on the formation of a complex with CD151. This implies that switches of splice variants as described during development (Collo et al., 1993; Thorsteindóttir et al., 1995; Sterk et al., 1998) or cleavage of the α subunit during maturation have no effect on CD151-integrin binding.

All laminin-binding integrins may bind to a single site on CD151

The reactivity of CD151 with TS151R and P48 in immunohistochemistry indicates that masking of the TS151R epitope detected in the biochemical experiments and FACS analysis also occurs in vivo. Glomerular cells expressing α3β1, basal keratinocytes expressing α3β1and α6β4 and cardiac and skeletal muscle cells expressing α7β1 either did not or only weakly react with TS151R, although these cell types were strongly stained by mAb P48. A likely interpretation of this finding is that in these tissues most if not all of the CD151 is bound to a laminin-binding integrin, leading to masking of the TS151R epitope. However, renal tubular and smooth muscle cells, which express α6β1 and α7β1, respectively, were stained equally well by the two antibodies. The reason for this observation is not clear, but there are several explanations. First, the strength of the interaction between CD151 and integrins might be regulated from within the cell and/or by the microenvironment, by which the tightness of the binding may be influenced and thus the exposure of the TS151R epitope. Second, CD151 may not directly interact with one of the laminin-binding integrins but indirectly by forming a tetraspanin web with other molecules. In fact, different binding sites have recently been described on CD151 for tetraspanins and integrins (Berditchevski et al., 2001). Third, CD151, in addition to being complexed with a laminin-binding integrin, could also be bound to other cell surface molecules, as has been described for different tetraspanins and MHCII, DRAP27 and HB-EGF (Angelisova et al., 1994; Nakamura et al., 1995). This binding should, in that case, involve another part of the binding site than that which interacts with integrins. Fourth, in kidney sections from HIV patients, we found that the amount of CD151 reactive with TS151R is greatly increased in the glomeruli and comparable with that reactive with P48 (L.M.T.S., unpublished). This indicates that under certain pathophysiological conditions the TS151R epitope on CD151, which is normally complexed with α3β1, becomes exposed. Finally, a difference in affinities of the two antibodies for CD151, as suggested by our FACS data, may have influenced their reactivity.

In summary, we demonstrate extensive co-distribution of various kinds of laminin receptors in different tissues. We confirm previous findings that CD151 binds to the laminin-binding integrins α3β1 and α6β1 and show for the first time that it also interacts with α7β1. CD151 does not bind to the laminin receptors DG or LU. Moreover, we show that variants of the cytoplasmic or extracellular domains bind equally well to CD151 and that cleavage of the integrin α subunits does not alter their binding. Studies with the mAb TS151R, which has previously been shown to recognize an epitope at the binding site of CD151 with α3β1, indicate that the same site is involved in the binding to α7β1 and possibly also to α6β1 and α6β4. The finding that in immunohistochemical studies few cells and tissues, irrespective of the laminin-binding integrins they express, reacted with TS151R, suggests that the strength of interaction of CD151 is subject to regulation either from within the cell and/or by the cell's environment.

We thank A. Pfauth and E. Noteboom for their assistance with the FACS analysis of cells. We also thank L. Oomen for advice on the use of the CLSM and E. Danen and P. Engelfriet for critical reading of the manuscript. We are grateful to many of our colleagues for providing us with antibody reagents and would especially like to thank D. Mielenz and K. von dem Mark for the full-length cDNAs for the α7BX1 and α7BX2 splice variants. This work was supported by a grant from the Dutch Kidney Foundation (C 96.1581).

References

Angelisova, P., Hilgert, I. and Horejší, V. (1994). Association of four antigens of the tetraspans family (CD37, CD53, TAPA-1, and R2/C33) with MHC class II glycoproteins. *Immunogenetics* **39**, 249-256.

Ashman L. K., Fitter, S., Sincock, P. M., Nguyen, L. Y. and Cambareri, A. C. (1997). CD151 (PETA-3) workshop summary report. In *Leukocyte typing VI. White cell differentiation antigens* (ed. T. Kishimoto, H. Kikutani, A. E. G. Kr. von dem Borne, S. M. Goyert, D. Y. Mason, M. Miyasaka, L. Moretta, K. Okumura, S. Shaw, T. A. Springer, K. Sugamura and H. Zola), pp. 681-683. New York: Garland Publishing Inc.

Aumailley, M. and Rouselle, P. (1999). Laminins of the dermo-epidermal junction. *Matrix Biol.* **18**, 19-28.

Berditchevski, F. and Odintsova, E. (1999). Characterization of integrin-tetraspanin adhesion complexes: role of tetraspanins in integrin signaling. *J. Cell Biol.* **146**, 477-492.

Berditchevski, F., Bazzoni, G. and Hemler, M. E. (1995). Specific association of CD63 with the VLA-3 and VLA-6 integrins. *J. Biol. Chem.* **270**, 17784-17790.

Berditchevski, F., Zutter, M. M. and Hemler, M. E. (1996). Characterization of novel complexes on the cell surface between integrins and proteins with 4 transmembrane domains (TM4 proteins). *Mol. Biol. Cell* **7**, 193-207.

Berditchevski, F., Tolias, K. F., Wong, K., Carpenter, C. L. and Hemler M. E. (1997). A novel link between integrins, transmembrane-4 superfamily proteins (CD63 and CD81), and phosphatidylinositol 4-kinase. *J. Biol. Chem.* **272**, 2595-2598.

Berditchevski, F., Gilbert, E., Griffths, M. R., Fitter, S., Ashman, L. and Jenner, S. J. (2001). Analysis of the CD151-α3β1 integrin and CD151-tetraspanin interactions by mutagenesis. *J. Biol. Chem.* **276**, 41165-41174**.**

Burkin, D. J., Wallace, G. Q., Nicol, K. J., Kaufman, D. J. and Kaufman, S. J. (2001). Enhanced expression of the α7β1 integrin reduces muscular dystrophy and restores viability in dystrophic mice. *J. Cell Biol.* **152**, 1207-1218.

Claas, C., Stipp, C. S. and Hemler, M. E. (2001). Evaluation of prototype transmembrane 4 superfamily protein complexes and their relation to lipid rafts. *J. Biol. Chem.* **276**, 7974-7984.

Collo, G., Starr, L. and Quaranta, V. (1993). A new isoform of the laminin receptor integrin α7β1 is developmentally regulated in skeletal muscle. *J. Biol. Chem.* **268**, 19019-19024.

Colognato, H. and Yurchenco, P. D. (2000). Form and function: the laminin family of heterotrimers. *Dev. Dyn.* **218**, 213-234.

Crosbie, R. H., Lebakken, C. S., Holt, K. H., Venzke, D. P., Straub, V., Lee, J. C., Grady, R. M., Chamberlain, J. S., Sanes, J. R. and Campbell, K. P. (1999). Membrane targeting and stabilization of sarcospan is mediated by the sarcoglycan subcomplex. *J. Cell Biol.* **145**, 153-165.

de Melker, A. A., Sterk, L. M., Delwel, G. O., Fles, D. L., Daams, H., Weening, J. J. and Sonnenberg, A. (1997). The A and B variants of the α3 integrin subunit: tissue distribution and functional characterization. *Lab. Invest.* **76**, 547-563.

de Melker, A. A. and Sonnenberg, A. (1999). Integrins: alternative splicing

as a mechanism to regulate ligand binding and integrin signaling events. *BioEssays* **21**, 499-509.

Defilippi, P., van Hinsbergh, A., Bertolotto, A., Rossino, P., Silengo, L. and Tarone, G. (1991). Differential distribution and modulation of expression of α1β1 on human endothelial cells. *J. Cell Biol.* **114**, 855-863.

Delwel, G. O., Hogervorst, F., Kuikman, I., Paulsson, M., Timpl, R. and Sonnenberg, A. (1993). Expression and function of the cytoplasmic variants of the integrin α6 subunit in transfected K562 cells. Activation-dependent adhesion and interaction with isoforms of laminin. *J. Biol. Chem.* **268**, 25865-25875.

Delwel, G. O., Hogervorst, F. and Sonnenberg, A. (1996). Cleavage of the α6A subunit is essential for activation of the α6β1 integrin by phorbol 12-myristate 13-acetate. *J. Biol. Chem.* **271**, 7293-7296.

DiPersio, C. M., Shah, S. and Hynes, R. O. (1995). α3Aβ1 integrin localizes to focal contacts in response to diverse extracellular matrix proteins. *J. Cell Sci*. **108**, 2321-2336.

Durbeej, M. and Campbell, K. P. (1999). Biochemical characterization of the epithelial dystroglycan complex. *J. Biol. Chem.* **274**, 26609-26616.

El Nemer, W., Gane, P., Colin, Y., Bony, V., Rahuel, C., Galacteros, F., Cartron, J. P. and Le Van, K. C. (1998). The Lutheran blood group glycoproteins, the erythroid receptors for laminin, are adhesion molecules. *J. Biol. Chem*. **273**, 16686-16693.

El Nemer, W., Colin, Y., Bauvy, C., Codogno, P., Fraser, R. H., Cartron, J. P. and Le Van Kim, C. L. (1999). Isoforms of the Lutheran/basal cell adhesion molecule glycoprotein are differentially delivered in polarized epithelial cells. Mapping of the basolateral sorting signal to a cytoplasmic di-leucine motif. *J. Biol. Chem*. **274**, 31903-31908.

Falcioni, R., Sacchi, A., Resau, J. and Kennel, S. J. (1988). Monoclonal antibody to human carcinoma-associated protein complex: quantitation in normal and tumor tissue. *Cancer Res.* **48**, 816-821.

Fitter, S., Sincock, P. M., Jolliffe, C. N. and Ashman, L. K. (1999). Transmembrane 4 superfamily protein CD151 (PETA-3) associates with β1 and αIIbβ3 integrins in haemopoietic cell lines and modulates cell-cell adhesion. *Biochem. J.* **338**, 61-70.

Hasegawa, H., Utsunomiya, Y., Kishimoto, K., Yanagisawa, K. and Fujita, S. (1996). SFA-1, a novel cellular gene induced by human T-cell leukemia virus type 1, is a member of the transmembrane 4 superfamily. *J. Virol.* **70**, 3258-3263.

Hasegawa, H., Nomura, T., Kishimoto, K., Yanagisawa, K. and Fujita, S. (1998). SFA-1/PETA-3 (CD151), a member of the transmembrane 4 superfamily, associates preferentially with α5β1 integrin and regulates adhesion of human T cell leukemia virus type 1-infected T cells to fibronectin. *J. Immunol.* **161**, 3087-3095.

Hemler, M. E., Sánchez-Madrid, F., Flotte, T. J., Krensky, A. M., Burakoff, S. J., Bhan, A. K., Springer, T. A. and Strominger, J. L. (1984). Glycoproteins of 210,000 and 130,000 m.w. on activated T cells: cell distribution and antigenic relation to components on resting cells and T cell lines. *J. Immunol.* **132**, 3011-3018.

Hodges, B. L., Hayashi, Y. K., Nonaka, I., Wang, W., Arahata, K. and Kaufman, S. J. (1997). Altered expression of the α7β1 integrin in human and murine muscular dystrophies. *J. Cell Sci.* **110**, 2873-2881.

Hogervorst, F., Kuikman, I., Noteboom, E. and Sonnenberg, A. (1993). The role of phosphorylation in activation of the α6Aβ1 laminin receptor. *J. Biol. Chem*. **268**, 18427-18430.

Hogervorst, F., Admiraal, L. G., Niessen, C., Kuikman, I., Janssen, H., Daams, H. and Sonnenberg, A. (1993). Biochemical characterization and tissue distribution of the A and B variants of the integrin α6 subunit. *J. Cell Biol.* **121**, 179-191.

Hořejší, V. and Vlček, C. (1991). Novel structurally distinct family of leucocyte surface glycoproteins including CD9, CD37, CD53 and CD63. *FEBS Lett.* **288**, 1-4.

Hynes, R. O. (1992). Integrins: versatility, modulation, and signaling in cell adhesion. *Cell* **69**, 11-25.

Ibraghimov-Beskrovnaya, O., Ervasti, J. M., Leveille, C. J., Slaughter, C. A., Sernett, S. W. and Campbell, K. P. (1992). Primary structure of dystrophin-associated glycoproteins linking dystrophin to the extracellular matrix. *Nature* **355**, 696-702.

Imai, T. and Yoshie, O. (1993). C33 antigen and M38 antigen recognized by monolconal antibodies inhibitory to syncitium formation in human T cell leukemia virus 1 are both members of the transmembrane 4 superfamily and associate with each other and with CD4 or CD8 in T cells. *J. Immunol*. **151**, 6470-6481.

Kantor, R. R. S., Mattes, M. J., Lloyd, K. O., Old, L. J. and Albino, A. P. (1987). Biochemical analyses of two cell surface glycoprotein complexes, very common antigen 1 and very common antigen 2. *J. Biol. Chem*. **262**, 15158-15165.

Keizer, G. D., te Velde, A. A., Schwarting, R., Figdor, C. G. and De Vries, J. E. (1987). Role of p150,95 in adhesion, migration, chemotaxis and phagocytosis of human monocytes. *Eur. J. Immunol*. **17**, 1317-1322

Kinsella, T. M. and Nolan, G. P. (1996). Episomal vectors rapidly and stably produce high-titer recombinant retrovirus. *Hum. Gene Ther.* **7**, 1405-1413.

Krishnamurti, U., Chen, Y., Michael, A., Kim, Y., Fan, W. W., Wieslander, J., Brunmark, C., Rondeau, E., Sraer, J. D., Delarue, F. and Tsilibary, E. C. (1996). Integrin-mediated interactions between primary/T-SV40 immortalized human glomerular epithelial cells and type IV collagen. *Lab. Invest.* **74**, 650-657.

Lebakken, C. S., Venzke, D. P., Hrstka, R. F., Consolino, C. M., Faulkner, J. A., Williamson, R. A. and Campbell, K. P. (2000). Sarcospan-deficient mice maintain normal muscle function. *Mol. Cell. Biol*. **20**, 1669-1677.

Maecker, H. T., Todd, S. C. and Levy, S. (1997). The tetraspanin superfamily: molecular facilitators. *FASEB J.* **11**, 428-442.

Mannion, B. A., Berditchevski, F., Kraeft S. K., Chen, L. B. and Hemler, M. E. (1996). Transmembrane-4 superfamily proteins CD81 (TAPA-1), CD82, CD63, and CD53 specifically associated with integrin α4β1 (CD49d/CD29). *J. Immunol.* **157**, 2039-2047.

Miner, J. H., Patton, B. L., Lentz, S. I., Gilbert, D. J., Snider, W. D., Jenkins, N. A., Copeland, N. G. and Sanes, J. R. (1997). The laminin alpha chains: expression, developmental transitions, and chromosomal locations of α1-5, identification of heterotrimeric laminins 8-11, and cloning of a novel α3 isoform. *J. Cell Biol.* **137**, 685-701.

Nakamura, K., Iwamoto, R. and Mekada, E. (1995). Membrane-anchored Heparin-binding EGF-like growth factor (HB-EGF) and Diphteria toxin receptor-associated protein (DRAP27)/CD9 form a complex with integrin α3β1 at cell-cell contact sites. *J. Cell Biol.* **129**, 1691-1705.

Niessen, C. M., Hogervorst, F., Jaspars, L. H., de Melker, A. A., Delwel, G. O., Hulsman, E. H., Kuikman, I. and Sonnenberg, A. (1994). The α6β4 integrin is a receptor for both laminin and kalinin. *Exp. Cell Res*. **211**, 360-367.

Parsons, S. F., Lee, G., Spring, F. A., Willig, T. N., Peters, L. L., Gimm, J. A., Tanner, M. J., Mohandas, N., Anstee, D. J. and Chasis, J. A. (2001). Lutheran blood group glycoprotein and its newly characterized mouse homologue specifically bind α5 chain-containing human laminin with high affinity. *Blood* **97**, 312-320.

Penas, P. F., Garcia-Diez, A., Sanchez-Madrid, F. and Yanez-Mo, M. (2000). Tetraspanins are localized at motility-related structures and involved in normal human keratinocyte wound healing migration. *J. Invest. Dermatol.* **114**, 1126-1135.

Schober, S., Mielenz, D., Echtermeyer, F., Hapke, S., Poschl, E., von der Mark, H., Moch, H. and von der Mark, K. (2000). The role of extracellular and cytoplasmic splice domains of α7- integrin in cell adhesion and migration on laminins. *Exp. Cell Res.* **255**, 303-313.

Schwartz, M. A., Schaller, M. D. and Ginsberg, M. H. (1995). Integrins: emerging paradigms of signal transduction. *Annu. Rev. Cell Dev. Biol*. **11**, 549-599.

Serru, V., Le Naour, F., Billard, M., Azorsa, D. O., Lanza, F., Boucheix, C. and Rubinstein, E. (1999). Selective tetraspan-integrin complexes (CD81/α4β1, CD151/α3β1, CD151/α6β1) under conditions disrupting tetraspan interactions. *Biochem. J.* **340**, 103-111.

Sincock, P. M., Mayrhofer, G. and Ashman, L. K. (1997). Localization of the transmembrane 4 superfamily (TM4SF) member PETA-3 (CD151) in normal human tissues: comparison with CD9, CD63, and α5β1 integrin. *J. Histochem. Cytochem.* **45**, 515-525.

Sincock, P. M., Fitter, S., Parton, R. G., Berndt, M. C., Gamble, J. R. and Ashman, L. K. (1999). PETA-3/CD151, a member of the transmembrane 4 superfamily, is localised to the plasma membrane and endocytic system of endothelial cells, associates with multiple integrins and modulates cell function. *J. Cell Sci*. **112**, 833-844.

Sonnenberg, A., Janssen, H., Hogervorst, F., Calafat, J. and Hilgers, J. (1987). A complex of platelet glycoproteins Ic and IIa identified by a rat monoclonal antibody. *J. Biol. Chem*. **262**, 10376-10383.

Sterk, L. M., de Melker, A. A., Kramer, D., Kuikman, I., Chand, A., Claessen, N., Weening, J. J. and Sonnenberg, A. (1998). Glomerular extracellular matrix components and integrins. *Cell Adhes. Commun.* **5**, 177-192.

Sterk, L. M., Geuijen, C. A., Oomen, L. C., Calafat, J., Janssen, H. and Sonnenberg, A. (2000). The tetraspan molecule CD151, a novel constituent

of hemidesmosomes, associates with the integrin α6β4 and may regulate the spatial organization of hemidesmosomes. *J. Cell Biol.* **149**, 969-982.

Stipp, C. S. and Hemler, M. E. (2000). Transmembrane-4-superfamily proteins CD151 and CD81 associate with α3β1 integrin, and selectively contribute to alpha 3 beta 1- dependent neurite outgrowth. *J. Cell Sci.* **113**, 1871-1882.

Sugiura, T. and Berditchevski, F. (1999). Function of α3β1-tetraspanin protein complexes in tumor cell invasion. Evidence for the role of the complexes in production of matrix metalloproteinase 2 (MMP-2). *J. Cell Biol.* **146**, 1375-1389.

Testa, J. E., Brooks, P. C., Lin, J. M. and Quigley, J. P. (1999). Eukaryotic expression cloning with an antimetastatic monoclonal antibody identifies a tetraspanin (PETA-3/CD151) as an effector of human tumor cell migration and metastasis. *Cancer Res*. **59**, 3812-3820.

Thorsteinsdóttir, S., Roelen, B. A., Freund, E., Gaspar, A. C., Sonnenberg, A. and Mummery, C. L. (1995). Expression patterns of laminin receptor splice variants α6Aβ1 and α6Bβ1 suggest different roles in mouse development. *Dev. Dyn.* **204**, 240-258.

Udani, M., Zen, Q., Cottman, M., Leonard, N., Jefferson, S., Daymont, C., Truskey, G. and Telen, M. J. (1998). Basal cell adhesion molecule/lutheran protein. The receptor critical for sickle cell adhesion to laminin. *J. Clin. Invest*. **101**, 2550-2558.

van Leeuwen, F. N., Kain, H. E., Kammen, R. A., Michiels, F., Kranenburg, O. W. and Collard, J. G. (1997). The guanine nucleotide exchange factor Tiam1 affects neuronal morphology; opposing roles for the small GTPases Rac and Rho. *J. Cell Biol.* **139**, 797-807.

van der Flier, A. and Sonnenberg, A. (2001). Function and interactions of integrins. *Cell Tissue Res*. **305**, 285-298.

Wright, M. D. and Tomlinson, M. G. (1994). The ins and outs of the transmembrane 4 superfamily. *Immunol. Today* **15**, 588-594.

Yanez-Mo, M., Alfranca, A., Cabanas, C., Marazuela, M., Tejedor, R., Ursa, M. A., Ashman, L. K., de Landazuri, M. O. and Sanchez-Madrid, F. (1998). Regulation of endothelial cell motility by complexes of tetraspan molecules CD81/TAPA-1 and CD151/PETA-3 with α3β1 integrin localized at endothelial lateral junctions. *J. Cell Biol.* **141**, 791-804.

Yanez-Mo, M., Tejedor, R., Rousselle, P. and Madrid, F. (2001). Tetraspanins in intercellular adhesion of polarized epithelial cells: spatial and functional relationship to integrins and cadherins. *J. Cell Sci.* **114**, 577-587.

Yauch, R. L., Berditchevski, F., Harler, M. B., Reichner, J. and Hemler, M. E. (1998). Highly stoichiometric, stable, and specific association of integrin α3β1 with CD151 provides a major link to phosphatidylinositol 4-kinase, and may regulate cell migration. *Mol. Biol. Cell* **9**, 2751-2765.

Yauch, R. L., Kazarov, A. R., Desai, B., Lee, R. T. and Hemler, M. E. (2000). Direct extracellular contact between integrin α3β1 and TM4SF protein CD151. *J. Biol. Chem*. **275**, 9230-9238.

Yauch, R. L. and Hemler, M. E. (2000). Specific interactions among transmembrane 4 superfamily (TM4SF) proteins and phosphoinositide 4-kinase. *Biochem. J.* **351**, 629-637.

Ziober, B. L., Vu, M. P., Waleh, N., Crawford, J., Lin, C. S. and Kramer, R. H. (1993). Alternative extracellular and cytoplasmic domains of the integrin α7 subunit are differentially expressed during development. *J. Biol. Chem*. **268**, 26773-26783.

Zhang, X. A., Bontrager, A. L. and Hemler, M. E. (2001). Transmembrane-4 superfamily proteins associate with activated protein kinase C (PKC) and link PKC to specific β1 integrins. *J. Biol. Chem.* **276**, 25005-25013.