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*Topical Review*

# Physiological and Pathological Roles of α3β1 Integrin

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**Abstract.** α3β1 integrin has been considered to be a mysterious adhesion molecule due to the pleiotropy in its ligand-binding specificity. However, recent studies have identified laminin isoforms as high-affinity ligands for this integrin, and demonstrated that α3β1 integrin plays a number of essential roles in development and differentiation, mainly by mediating the establishment and maintenance of epithelial tissues. Furthermore, α3β1 integrin is also implicated in many other biological phenomena, including cell growth and apoptosis, angiogenesis and neural functions. This integrin receptor forms complexes with various other membrane proteins, such as the transmembrane-4 superfamily proteins (tetraspanins), cytoskeletal proteins and signaling molecules. Recently, lines of evidence have been reported showing that complex formation regulates integrin functions in cell adhesion and migration, signal transduction across cell membranes, and cytoskeletal organization. In addition to these roles in physiological processes, α3β1 integrin performs crucial functions in various pathological processes, especially in wound healing, tumor invasion and metastasis, and infection by pathogenic microorganisms.

**Key words:** Integrin — Cell adhesion molecule — Cell migration — Tumor invasion — Laminin — Extracellular matrix — Tetraspanin — Organogenesis

## Introduction

The integrin family of cell adhesion molecules plays important roles in various physiological and pathological processes by mediating cell-extracellular matrix (ECM) and cell-cell interactions. These adhesion receptors are heterodimers of transmembrane glycoproteins (α and β subunits); various combinations of the α and β subunits produce polymorphisms of ligand specificity (reviewed by Albelda & Buck, 1990; Ruoslahti, 1991; Hynes, 1992; Sheppard, 2003). In mammals, 18 α-subunits and 8 β-subunits have been defined, which assemble to form 24 different adhesion receptors. The α3β1 integrin (CD49c/CD29) is a member of the β1 integrin subfamily, and in early studies was referred to as very late activation antigen (VLA)-3 (Takada et al., 1987), extracellular matrix receptor (ECMR)-1 (Wayner et al., 1988), galactoprotein b3 (Tsuji et al., 1990), and chicken integrin complex band 2 (Hynes et al., 1989). This integrin has been suggested to be a functional receptor for a variety of extracellular matrix (ECM) proteins, including fibronectin, collagen, and laminin-1 (a prototype of laminin). However, its physiological function was until recently not well defined, in contrast to other integrins of interest, such as α5β1 integrin as a specific receptor for fibronectin, members of the β2 integrin subfamily (αLβ2, αMβ2 and αXβ2 integrins) in terms of leukocyte function in immunity, and αIIbβ3 integrin in platelet aggregation. However, recent studies have demonstrated that α3β1 integrin serves as a high-affinity receptor for isoforms of laminin, including laminin-5, and that this integrin exerts crucial functions by mediating specific adhesion events in a variety of biologically important processes. Mice deficient in α3 integrin have greatly contributed to functional analyses of this integrin in the development and differentiation of various tissues and organs such as the kidneys, lungs, and skin (Kreidberg et al., 1996; Kreidberg, 2000). Moreover, α3β1 integrin forms complexes with other cell-membrane and cytoskeletal proteins, and these complexes are thought to play essential roles in cell adhesion, motility, signaling, transport, and other cell mem-

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*Abbreviations:* ECM extracellular matrix; MMP, matrix metalloproteinase

brane functions. In addition, this integrin is implicated in tumor invasion and metastasis, and also in infection by pathogenic microorganisms.

## Structure

The α3β1 integrin is a transmembrane glycoprotein consisting of a non-covalently associated heterodimer (α3 and β1 subunits). The integrin α3 subunit is associated exclusively with the β1 subunit, as distinct from some other α subunits, such as α4, α6, and αV subunits, which can be associated with other β subunits as well as with β1. The cDNA for the hamster integrin α3 subunit has been cloned (Tsuji et al., 1990), as has that for the corresponding human (Tsuji, Hakomori & Osawa, 1991; Takada et al., 1991) and mouse (Takeuchi et al., 1995) subunits. The human α3 subunit in its mature form is composed of 1,019 amino-acid residues, including a large extracellular domain, a single transmembrane domain and a short cytoplasmic domain; the overall structure is similar to that of other integrin α subunits (Fig. 1). Electron microscopic observation has revealed that an integrin heterodimer is composed of a globular domain and two rod-like domains (Nermut et al., 1988).

The integrin α3 subunit is characterized by the absence of an I-domain (insertion domain) and the presence of a cleavage site. The I-domain is homologous to the von Willebrand factor (vWF) A domain that is present in several integrin α subunits, such as α2, αL, and αM subunits. This domain plays a pivotal role in the ligand-binding function of these integrins. The cleavage site is also present in the α5, α6, αIIb, and αV subunits in the region proximal to cell membranes, and these α subunit polypeptides are posttranslationally cleaved by a specific protease to generate heavy and light chains that remain associated by a disulfide bond (between Cys-814 and Cys-872, numbered from Phe at the N-terminus of the mature α3 subunit) (Krokhin et al., 2003). Integrin α subunits contain seven repeated sequences, each with a 60–70 amino-acid stretch, in their N-terminal regions. In the α3 subunit, three repeats out of the seven each have a metal-binding sequence of the general structure DX(D/N)X(D/N)GXXD, as is the case with the other integrin α subunits. All integrins require divalent cations ($Mg^{2+}$ and $Mn^{2+}$) for binding to their ligands. The seven repeated sequences were predicted by Springer (1997) to fold into a seven-bladed β-propeller structure, as based on their sequence homology with phospholipase D and the trimeric G-protein β subunit; moreover, the β-propeller structure is thought to be involved in ligand binding by I-domain-lacking integrins.

The αVβ3 integrin, a receptor for vitronectin, fibronectin, thrombospondin, and osteopontin, is structurally the best characterized integrin, as based on X-ray crystallography of its extracellular domains (Xiong et al., 2001). Their study was the first case of a crystallographic analysis of integrin molecules and they revealed that the integrin heterodimer is composed of a globular "head" and two rod-like "tail" domains. This overall shape is in agreement with previous electron microscopic observations. The crystallographic analyses have demonstrated that the major point of contact between the α and β subunits is located in the respective N-terminal globular domains, and supported the prediction made by Springer (1997) that the seven homologous repeats in the α subunit form a β-propeller structure with seven blades. The β-propeller structure in the α subunit interacts with the vWF A domain (βA domain or I-like domain) present in the N-terminal region of the β subunit, and the interface formed by the β-propeller structure and the βA domain creates the ligand-binding pocket (Springer 2002; Xiong et al., 2002; Humphries et al., 2003). Such crystallographic data regarding the αVβ3 integrin have provided great insight into the structure of other integrin molecules.

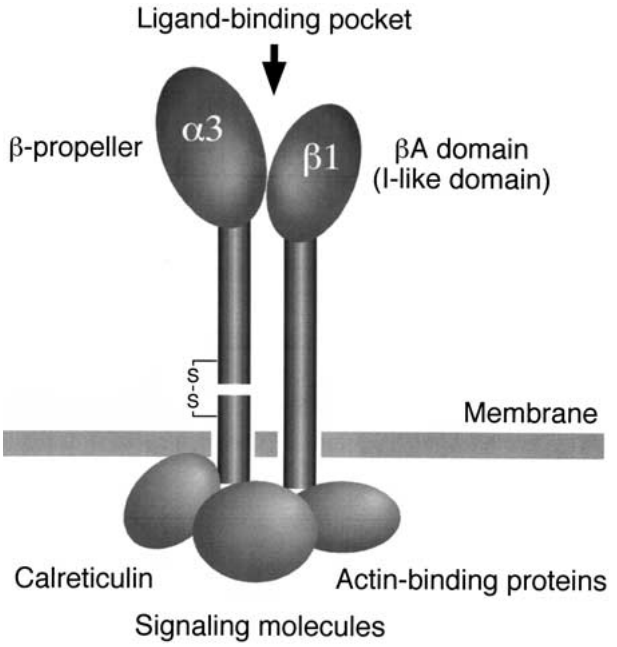


**Fig. 1.** Schematic structure of α3β1 integrin. Both α3 and β1 subunits are transmembrane glycoproteins, which are non-covalently associated and form a heterodimer. The globular domain of the α3 subunit consists of a seven-bladed β-propeller structure with seven repeats of homologous sequences, and it associates with the βA domain of the β1 subunit to constitute a ligand-binding pocket. The α3 subunit is posttranslationally cleaved by a protease to generate heavy and light chains that remain associated by a disulfide bond. The cytoplasmic domains of the α3β1 heterodimer are associated with various proteins, including calreticulin (a multifunctional calcium-binding protein), cytoskeletal proteins, and signaling molecules.

The region in the α3 subunit that is critical for the binding of α3β1 integrin to ECM containing laminin-5 (a high-affinity ligand for α3β1 integrin) was analyzed by the epitope-mapping of several function-blocking anti-α3 integrin antibodies and by site-directed mutagenesis (Zhang et al., 1999). The results demonstrated that the boundary between repeats 1 and 2 (residues 75–80) and the boundary between repeats 2 and 3 including the Thr-162 and Gly-163 residues (numbering in the mature form of the α3 subunit) are crucial for ligand binding. These two regions are separated in the primary structure; however, they are close to each other in the proposed β-propeller model. Another report has demonstrated that a mutation of the Tyr-186 and Trp-188 residues located in repeat 3 of the α3 subunit failed to promote adhesion to laminin-5 (Krukonis et al., 1998). These results suggest that the second and/or third blades of the β-propeller are critical for the recognition of the ECM ligand.

In the cytoplasmic tail of the α3 subunit, an amino-acid sequence motif, GFFKR, is found in the region proximal to the transmembrane domain. This motif is well-conserved among integrin α subunits, and is recognized by a multifunctional calcium-binding protein referred to as calreticulin. The binding of calreticulin to the GFFKR motif regulates the state of activation of integrin receptors (Coppolino et al., 1995a) and it mediates a signal transduction pathway in cells (Ito, Seyama & Kubota, 2001). The Ser-1010 (the 10th amino-acid residue from the C-terminus) in a QPSXXE motif, which is conserved in multiple α subunits (α3A, α6A, and α7A subunits), was found to be phosphorylated (Zhang et al., 2001a). Such phosphorylation may not be directly catalyzed by any of the known protein kinase C (PKC) isoforms, although PKC does play an indirect role in promoting phosphorylation at this site. The phosphorylation appeared to be implicated in the tyrosine phosphorylation of cellular proteins, such as focal adhesion kinase and paxillin, as well as in cell spreading and migration, suggesting that phosphorylation in this context regulates the interaction of α3β1 integrin with the cytoskeleton.

A structural variant of the α3 subunit in the cytoplasmic domain was identified in the murine heart and brain (Tamura et al., 1991). This variant is referred to as the α3B subunit, whereas the authentic α3 subunit is referred to as the α3A subunit. These two splice variants are generated by alternative exon usage (Tsuji et al., 1999). To date, no difference in the ligand-binding specificity of the α3A and α3B subunits has been detected, although their respective tissue distributions differ. The expression of the α3B subunit in adult tissues is more restricted than that of the α3A subunit, which is widely distributed in almost all tissues (de Melker et al., 1997). In heart tissue, for example, the α3A subunit is strongly expressed on vascular smooth muscle cells, whereas the α3B subunit is detected only on endothelial cells of veins. A recent report has suggested that the cytoplasmic domain of the α3 subunit plays a role in regulating the cell surface expression of the heterodimer (DiPersio, Trevithick & Hynes, 2001). The α3B subunit was found to accumulate intracellularly in keratinocytes on collagen matrices, although both α3Aβ1 and α3Bβ1 localized to focal contacts in keratinocytes on laminin-5-rich ECM. This finding suggests that binding to the high-affinity ligand may stabilize the surface expression of the α3Bβ1 heterodimer. Cytoplasmic variants such as those of the α3A and α3B subunits have also been identified for the α6 and α7 subunits.

The amino-acid sequences of the human α3 and β1 subunits contain 14 and 12 potential N-glycosylation sites (Asn-X-Ser, where X is not Pro), respectively, in their extracellular domains (Argraves et al., 1987; Tsuji et al., 1991). In addition to containing N-linked carbohydrate chains, α3β1 integrin purified from HT29 colon carcinoma cells contains O-linked oligosaccharides (Prokopishyn et al., 1999). The N-linked carbohydrates are mainly of the complex-type of oligosaccharides with β1–6 branched side chains and short poly-N-acetyllactosamine units (Galβ1-4GlcNAcβ1-; $n < \text{or} = 3$), and α3β1 integrin expressed in colon cancer is a major carrier of oncodevelopmental carbohydrate epitopes defined by monoclonal antibody. α3β1 integrin is a sialoglycoprotein and the linkages of the sialic acid residues may affect the tumorigenicity and invasiveness of malignant glioma cells (Yamamoto et al., 2001). The replacement of α2-3-linked sialic acid residues abundantly present in N-glycans of the α3β1 integrin with α2-6-linked sialic acids by the transfection of glioma cells with α2-6 sialyltransferase cDNA resulted in a decrease in the extent of in vitro invasion and intracranial tumor formation of these cells. The glycosylation of α3β1 integrin is likely to modulate the function of the α3β1 integrin receptor.

## Cell Adhesion and Motility

In early studies using function-blocking monoclonal antibodies, α3β1 integrin was implicated as an adhesion receptor for fibronectin, collagen, and laminin-1 (classical laminin) (Wayner & Carter, 1987; Takada et al., 1988; Elices, Urry & Hemler, 1991). However, the expression of α3β1 integrin in α3-negative cells by cDNA transfection did not exhibit strong adhesion to these ECM proteins, suggesting that none of them has a high affinity for this integrin (Weitzman et al., 1993). A glycoprotein found in basement membranes in a complex with laminin, an entactin/nidogen, was also proposed as a ligand for α3β1 integrin (Dedhar et al., 1992; Wu, Chung &

McDonald, 1995). Moreover, this integrin was reported to be involved in intercellular adhesion through α3β1-α3β1 homophilic or α2β1-α3β1 heterophilic interactions (Sriramarao, Steffner & Gehlsen, 1993; Symington, Bkada & Carter, 1993). The ligand-binding specificity of α3β1 integrin remains somewhat unclear, as cell adhesion is usually mediated by multivalent interactions with various adhesion molecules.

It has been reported that a high-affinity ligand for α3β1 integrin, designated as epiligrin, is present in most epithelial basement membranes (Carter, Ryan & Gahr, 1991). Two epiligrin-related ECM proteins, kalinin and nicein, have subsequently been identified as ligands for α3β1 integrin (Marinkovich et al., 1993). Both ECM proteins are defective in junctional epidermolysis bullosa (JEB) Herlitz's disease, a group of heritable blistering diseases. Furthermore, another protein that possessed cell scattering activity, designated as ladsin, was isolated from a conditioned medium of a human gastric carcinoma cell line, and subsequently was found to bind to α3β1 integrin (Miyazaki et al., 1993; Kikkawa, Umeda & Miyazaki, 1994). Biochemical and immunological analyses have demonstrated that epiligrin, kalinin, nicein, and ladsin are essentially identical and are members of the laminin family of ECM proteins. They are currently referred to collectively as laminin-5, which consists of three specific subunits, α3, β3, and γ2. All laminins are structurally heterotrimeric, and the combination of the three subunits produces a variety of laminin isoforms (Engvall & Wewer, 1996). The interactions between laminin isoforms and α3β1 integrin have been studied extensively. In addition to laminin-5, laminin-10 (α5β1γ1), laminin-11 (α5β2γ1), and laminin-8 (α4β1γ1) are recognized by α3β1 integrin (Eble et al., 1998; Kikkawa, Sanzen & Sekiguchi, 1998; Fujiwara et al., 2001). However, it has also been reported that anti-α3 integrin antibody did not inhibit cell attachment to laminin-10/11 (Ferletta & Ekblom, 1999). The reason for these apparently controversial results is not clear, but the discrepancy may have been the result of the use of two different methods, and/or by differences in the state of posttranslational processing of the laminin isoforms. Hemler and co-workers quantitatively analyzed the binding of α3β1 integrin to laminin-5 by using recombinant soluble chimeric proteins, in which α3β1 integrin was fused with a Fos/Jun heterodimer (Eble et al., 1998). They found that binding affinity was markedly increased upon the addition of $Mn^{2+}$ or an activating antibody (anti-integrin β1 subunit), and that the $K_d$ value for the binding to laminin-5 was estimated to be 27 nM in the presence of $Mn^{2+}$. Subsequently, Sekiguchi and co-workers performed a similar analysis, but instead used natural α3β1 integrin purified from human placenta and incorporated into liposomes; they demonstrated that the $K_d$ value for binding to laminin-5 was 4 nM (in the presence of $Mn^{2+}$) (Nishiuchi et al., 2003). Moreover, the binding affinity for laminin-10/11 was approximately 3-fold higher than that for laminin-5. They suggested the importance of the interaction between α3β1 integrin and laminin-10/11, because of the wide distribution of laminin-10/11 in the basement membranes of various adult tissues.

Several recent reports have added other proteins to a line-up of ligands for α3β1 integrin. Thrombospondin-1 (TSP-1), a major component of the α-granules of platelets, is also known as an ECM protein that is produced in many cell types upon stimulation with growth factors, and TSP-1 also supports adhesion in certain cell types, including small cell lung carcinoma (SCLC) cells. The adhesion of SCLC cells to TSP-1 was inhibited by antibodies that recognize integrin α3 or β1 subunits (Guo et al., 2000). Furthermore, neurite-like outgrowth and growth inhibition of SCLC cells on a TSP-1 substrate were abolished by treatment with anti-α3 integrin antibody. The high-affinity binding of TSP-1 to SCLC cells was dependent on heparin, suggesting cooperation between sulfated glycosaminoglycans or glycolipids and α3β1 integrin in the process of cell adhesion.

More recently, tissue inhibitor of metalloproteinase (TIMP)-2 was proposed as a ligand for α3β1 integrin. Matrix metalloproteinases (MMPs) are involved in angiogenesis, and TIMP-2, an inhibitor of these enzymes, abrogates endothelial cell proliferation in vitro and angiogenesis in vivo. However, these processes are independent of the inhibition of MMPs, and they require α3β1 integrin-mediated binding of TIMP-2 to endothelial cells (Seo et al., 2003). The action of TIMP-2 was shown to involve both a decrease in total protein tyrosine phosphatase activity associated with β1 integrin subunits as well as a dissociation of the phosphatase SHP-1 from the β1 subunits. Thus, it is likely that α3β1 integrin is the second target for TIMP-2, and the binding of TIMP-2 to α3β1 integrin modulates cell growth by intracellular signaling.

The urokinase-type plasminogen activator receptor (uPAR, CD87), a glycosylphosphatidylinositol (GPI)-anchored surface protein, was proposed as a *cis*-acting ligand for α3β1 integrin (Wei et al., 2001). uPAR is present on the surface of many cell types, and is implicated in cell migration and tissue remodeling during inflammation, wound healing, and cancer metastasis through 1) the proteolytic cascade initiating from the activation of uPA, 2) the modulation of integrin function such as that of αMβ2 integrin (CD11b/CD18, Mac-1), and 3) direct binding to vitronectin, which supports cell adhesion and migration. uPARs physically associate with α3β1 integrin ($K_d$ < 20 nM) via their extracellular domains, and they are also known to be functionally associated, i.e., antibodies against α3 integrin have been

shown to block uPAR-mediated cell adhesion to vitronectin. Moreover, α3β1 integrin mediates uPA/uPAR signaling, leading to the functional modulation of α2β1 and α5β1 integrins, as verified by studies of epithelial cells from α3 integrin-deficient mice. The binding of uPA to uPAR enhanced α2β1-dependent adhesion to collagen and α5β1-dependent adhesion to fibronectin in α3 integrin-positive cells, but not in α3 integrin-deficient cells. The region of the integrin α3 subunit responsible for the binding to uPAR was assigned as being located in a surface loop within the β-propeller (repeat 4), which is close to, although still outside of the laminin-5 binding site (Zhang et al., 2003a). This suggests that the integrin α3 subunit has at least two distinct sites for interactions with distinct ligands within its β-propeller structure. A novel ligand for α3β1 integrin, Reelin, was recently identified in the central nervous system and was found to play a crucial role in the development of the cerebral cortex (Dulabon et al., 2000). This protein will be discussed in more detail in the following section.

Invasin is a protein that is present in the outer membranes of *Yersinia* species bacteria, and it attaches to the surface of the eukaryotic host cell, followed by internalization of the bacterium. Several different integrins, including α3β1 integrin, are recognized by invasin (Isberg & Leong, 1990). Cross-inhibition studies with soluble α3β1 integrin have indicated that invasin displaced laminin-5 from the integrin receptor, suggesting that the binding sites for both proteins are either identical or sterically overlapping (Eble et al., 1998). However, analyses using function-blocking monoclonal antibodies and site-directed mutagenesis have indicated that both binding sites were mutually very close in the N-terminal β-propeller structure, but the precise locations were slightly different (Krukonis et al., 1998; Zhang et al., 1999). Recently, human herpes virus 8 (HHV-8), known as Kaposi's sarcoma-associated herpes virus, was reported to interact with α3β1 integrin on the surface of host cells via envelope glycoprotein B of the virus (Akula et al., 2002). The binding of α3β1 integrin to the virus glycoprotein is dependent on an Arg-Gly-Asp (RGD) sequence, suggesting a distinct mechanism from the recognition of laminin-5. The entry of HHV-8 into the target cells is likely to involve α3β1 integrin, because HHV-8 infectivity was inhibited by antibody against α3β1 integrin and by a soluble form of α3β1 integrin.

Ladsin is one of the previous names for laminin-5; the older term is derived from the known biological activities, i.e., promoting cell adhesion and motility (a large cell-adhesive scatter factor) (Miyazaki et al., 1993; Kikkawa et al., 1994). Laminin-5 was shown to stimulate chemotactic and chemokinetic migration as well as the adhesion of various cell types. In addition, laminin-5 was reported to promote the migration and invasion of malignant glioma and melanoma cells in an α3β1 integrin-dependent manner (Fukushima et al., 1998; Tsuji et al., 2002). Thus, laminin-5 (ladsin) is a unique ECM component, which may be involved in cell migration. The expression of α3β1 integrin on human melanoma cells is correlated with their migratory phenotype, and anti-α3 integrin antibodies are good inhibitors of the migration of these cells on low-affinity ligands (laminin-1, fibronectin, and collagen IV) and of chemoinvasion through a reconstituted basement membrane (Matrigel) (Natali et al., 1993; Melchiori et al., 1995).

In contrast, laminin-5 inhibits human keratinocyte migration driven by collagen matrices. The inhibitory effect of laminin-5 on keratinocyte motility is known to be reversed by anti-α3 integrin antibody (O'Toole et al., 1997). In keratinocytes, the interaction of laminin-5 with α3β1 integrin appears to be associated with immobility. However, another report indicated that keratinocyte migration was accompanied by the deposition of endogenous laminin-5, and this type of migration was blocked by anti-α3 integrin and anti-laminin 5 antibodies (Zhang & Kramer, 1996). These results suggest that migrating keratinocytes use laminin-5 freshly deposited by themselves during locomotion. This apparent inconsistency regarding the effects of laminin-5 on keratinocyte migration might be accounted for by a closer consideration of the processing of laminin-5 following its secretion from the cell. The C-terminal globular domain of the α3 chain of laminin-5 is cleaved by specific proteases such as plasmin to generate the mature form (Goldfinger, Stack & Jones, 1998). Keratinocyte migration on fibronectin or collagen IV requires endogenous laminin-5 deposition, which is predominantly detected in its unprocessed form (Decline & Rousselle, 2001). Interestingly, the migration of keratinocytes requires the interaction of laminin-5 with α2β1 integrin, which normally serves as a receptor for collagen. In addition to the interactions between ECM proteins and integrins, the subsequent cellular signaling cascades are important for the regulation of cell migration. These cascades are likely to include complex pathways involving a variety of signaling molecules (reviewed by Holly, Larson & Parise, 2000 and by Ridley et al., 2003).

## Association with Other Membrane Proteins

It is known that α3β1 integrin forms complexes with other membrane proteins, including receptors and transporters for bioactive molecules, and transmembrane-4 superfamily (TM4SF, tetraspanin) proteins, as well as with cytoskeleton-linked proteins. These complexes are thought to play key roles in cell adhesion, motility, signaling, transport, and other cell membrane functions (Table 1). In some prostatic carcinoma cell lines, the covalent association of the

**Table 1.** Complex formation of α3β1 integrin with other membrane components

| Associated protein | Cell | Reference |
|---|---|---|
| Receptors and transporter | | |
| Transferrin receptor | Prostatic carcinoma | Coppolino et al., 1995b |
| Amino acid transporter | Ehrlich cells | McCormick & Johnstone, 1995 |
| CD87 (uPAR) | Breast carcinoma (MDA-MB-231) | Wei et al., 2001 |
| Tetraspanins (TM4SF) | | |
| CD9 | Vero, A431, MG63 cells | Nakamura et al., 1995 |
| (DRAP27, MRP-1) | Breast carcinoma (MDA-MB-231) | Berditchevski et al., 1996 |
| | S-16 Schwann cells | Hadjiargyrou et al., 1996 |
| | Keratinocytes | Jones, Bishop & Watt, 1996; Okochi et al., 1997 |
| CD63 | Fibrosarcoma (HT1080) | Berditchevski et al., 1995 |
| | Breast carcinoma (MDA-MB-231) | Berditchevski et al., 1996 |
| CD81 (TAPA-1) | Breast carcinoma (MDA-MB-231) | Berditchevski et al., 1996 |
| | Neuron-like cells (NT2N) | Stipp & Hemler, 2000 |
| CD82 | Breast carcinoma (MDA-MB-231) | Berditchevski & Odintsova, 1999 |
| CD151 | Endothelial cells | Yanez-Mo et al., 1998 |
| (PETA-3) | | Sincock et al., 1999 |
| | HeLa, A431 cells | Serru et al., 1999 |
| | Neuron-like cell (NT2N) | Stipp & Hemler, 2000 |
| | Fibrosarcoma (HT1080) | Yauch et al., 2000 |
| NAG-2 | Breast carcinoma (MDA-MB-435) | Tachibana et al., 1997 |
| CO-029 | Colon and pancreatic carcinoma | Claas et al., 1998 |
| Ig superfamily | | |
| CD147 | Fibrosarcoma (HT1080) | Berditchevski et al., 1997b |
| (EMMPRIN, Basigin, OX47, M6) | DMBA-induced papilloma | Owens & Watt, 2001 |
| EWI-2 | Epidermoid carcinoma (A431) | Stipp, Kolesnikora & Hemler, 2003 |
| Other proteins | | |
| Calreticulin | Osteosarcoma (TE-85) | Leung-Hagesteijn et al., 1994 |
| | Rhabdomyosarcoma (RD) | Ito et al., 2001 |
| DRAL/FHL2 | HEK293 (transfected) | Wixler et al., 2000 |

transferrin receptor (CD71) and the integrin α3 subunit has been demonstrated (Coppolino et al., 1995b). The α3 subunit heavy chain (approximately 130 kDa) was linked to a monomer of the transferrin receptor (approximately 95 kDa) via a disulfide bond to form a complex (approximately 225 kDa). The transferrin receptor is normally a homodimer that is involved in the internalization of iron-bound transferrin into cells. It has not yet been clarified how transferrin receptor function and/or that of α3 integrin are modulated by complex formation. During purification of the $Na^+$-dependent A-system amino-acid transporter from the octyl glucoside extract of Ehrlich cell plasma membranes, the integrin α3 subunit was co-purified (McCormick & Johnstone, 1995). The involvement of α3β1 integrin in transporter function was suggested by findings showing that anti-α3β1 integrin antibody partially inhibited transport activity and that the transfection of cells with α3 integrin cDNA increased A-system transport.

Tetraspanins (TM4SF), a family of membrane proteins characterized by the presence of four transmembrane domains, are physically associated with various integrin heterodimers. Two tetraspanins, CD9 and CD63, were reported to form complexes with α3β1 integrin (Berditchevski, Bazzoni & Hemler, 1995; Nakamura et al., 1995). The CD9-α3β1 integrin complex was found in Vero cells, A431 epidermoid carcinoma cells, and MG63 osteosarcoma cells, and was also associated with a membrane-anchored form of heparin-binding epidermal growth factor-like growth factor (proHB-EGF). Since this growth factor stimulates neighboring cell proliferation in a juxtacrine mode, it is possible that this complex of growth factors and cell adhesion molecules is involved in controlling cell proliferation during the cell–cell adhesion process (Nakamura et al., 1995). The CD9 molecule functions in a variety of biologically important phenomena, including cell motility (Miyake et al., 1991) and adhesion, migration, proliferation, and the neurite outgrowth of Schwann cells (Hadjiargyrou & Patterson, 1995; Banerjee, Hadjiargyrou & Patterson, 1997); some of these functions may be carried out by the CD9-α3β1 integrin complex. On the other hand, in fibrosarcoma cells, CD63 specifically forms a complex with a phosphatidyl-

inositol 4-kinase (PI 4-K) (Berditchevski et al., 1997a). Thus, CD63 may link PI 4-K activity to α3β1 integrin and may thus act as a transducer of phosphoinositide signaling, leading to integrin-dependent cell adhesion and motility.

Other members of the tetraspanin family, CD81 (Berditchevski, Zutter & Hemler, 1996), CD82 (Berditchevski & Odintsova, 1999), and CD151 (Yanez-Mo et al., 1998; Serru et al., 1999), were subsequently reported to associate with α3β1 integrin. Among these, the CD151-α3β1 integrin complex has been most extensively studied. Generally, integrin-tetraspanin interactions are observed only under "mild detergent" (such as Brij 97 and CHAPS) conditions, but the CD151-α3β1 integrin complex is rather stable under "stringent detergent" (such as digitonin and Triton X-100) conditions (Serru et al., 1999; Stipp & Hemler, 2000). The CD151-α3β1 integrin complex contributes to a variety of cell adhesion and motility functions. For example, in endothelial cells, this complex is localized at endothelial cell-to-cell junctions. Antibodies against CD151 as well as anti-α3β1 integrin antibody inhibited the migration of endothelial cells and modulated in vitro angiogenesis, suggesting the involvement of this complex in their motility (Yanez-Mo et al., 1998; Sincock et al., 1999). Moreover, antibodies to either CD151 or α3β1 integrin both reduced neutrophil migration induced by a chemotactic peptide (Yauch et al., 1998). PI 4-K was also associated with α3β1 integrin via CD151, as in the case of CD63, and PI 4-K plays a role in neutrophil migration as well. One report has suggested the function of the CD 151-α3β1 integrin complex in neurite formation (Stipp & Hemler, 2000). The neurite outgrowth of NT2N neuron-like cells induced by laminin-5 was inhibited by anti-CD151 and anti-CD81 antibodies. The regions responsible for the interaction between CD151 and the integrin α3 subunit are thought to be the extracellular domains of both molecules; the large extracellular loop of CD151 (one of two extracellular loops of the tetraspanin), and the "stalk" region of the α3 subunit (Yauch et al., 2000). It is likely that the association of tetraspanins modulates integrin function; however, to date, no evidence has been provided demonstrating that this association induces conformational changes in integrin molecules.

Another group of integrin-associated membrane proteins consists of members of the immunoglobulin superfamily. For example, CD147 (EMMPRIN/basigin/OX47/M6) is associated with α3β1 integrin and α6β1 integrin (Berditchevski et al., 1997b). CD147 is a membrane protein with homophilic counter-receptor binding activity and it plays a role in cancer metastasis and angiogenesis by the regulation of MMP production (EMMPRIN is derived from extracellular matrix metalloproteinase inducer). Antibodies to CD147 and an inhibitor of homophilic interactions (recombinant CD147-Fc fusion protein) inhibited both MMP-2 production by breast cancer cells and their invasion using Matrigel-reconstituted basement membranes. However, it remains unclear whether or not the integrins are involved in these processes (Sun & Hemler, 2001). A member of the immunoglobulin superfamily of membrane proteins, referred to as EWI-2, is indirectly associated with α3β1 integrin via tetraspanin CD9 or CD81 (Stipp et al., 2003). The function of EWI-2 is not known; however, the overexpression of EWI-2 in A431 epidermoid carcinoma cells was shown to impair the α3β1 integrin-dependent migration of these cells on a laminin-5-coated surface.

Thus, α3β1 integrin, as well as other integrins, forms a large complex with various membrane proteins including those of the tetraspanin family and the immunoglobulin superfamily of glycoproteins. These complexes have been reported to be localized within specialized membrane microdomains, referred to as glycosphingolipid-enriched microdomains (GEMs, sometimes also called "lipid rafts" (Claas et al., 2001; Kawakami et al., 2002; Hakomori, 2002). Recently, much attention has been focused on these microdomains, because the compartmentalization of various membrane proteins with receptor and/or signaling function facilitates efficient cascades for signal transduction (Simons & Toomre, 2000). The CD9-α3β1 integrin and CD81-α3β1 integrin complexes were, at least in part, found to localize in GEMs (Claas, Stipp & Hemler, 2001). GM3 ganglioside is present in GEMs, and it promoted the CD9-α3β1 integrin complex formation, causing the suppression of laminin-5/α3β1 integrin-dependent cell motility. This finding suggests that GM3 regulates cell motility through the formation of the CD9-α3β1 integrin complex (Kawakami et al., 2002).

As described in the previous section, the cytoplasmic region of integrin α subunits interacts with calreticulin via a GFFKR motif present at the border of the transmembrane and cytoplasmic domain. Various other proteins, including cytoskeletal proteins, actin-binding proteins, and signaling molecules, are associated with the cytoplasmic region of integrin heterodimers; most of the associated proteins interact with β subunits (*reviewed* by Liu, Calderwood & Ginsberg, 2000). These proteins are most likely to regulate integrin function in cell adhesion and motility, and they also regulate the signal transduction for many cellular events. Some enzymes involved in signal transduction (e.g., protein kinase C (PKC) and phosphatidylinositol 4-kinase (PI 4-K)) also interact indirectly with integrin α3 subunits via tetraspanins (Berditchevski et al., 1997a; Zhang et al., 2001b). The α3β1 integrin-tetraspanin complexes may not be directly linked to the cytoskeleton. However, the treatment of the cell with cytochalasin B resulted in the relocation of the complexes into intracellular

vesicles, suggesting that the actin cytoskeleton plays a role in the distribution of tetraspanins into adhesion structures (Berditchevski & Odintsova, 1999).

Recent reports have shown that the α3β1 integrin-CD151 complex is also located at the lateral surface of epithelial monolayer cells and is involved in intercellular adhesion (Chattopadhyay et al., 2003; Shigeta et al., 2003). The association of α3β1 integrin with CD151 in that location promotes E-cadherin-mediated cell-cell adhesion. This promotion of adhesion was regulated in part by the gene expression of PTPμ, a membrane-associated protein tyrosine phosphatase known to be involved in cadherin-mediated adhesion (Chattopadhyay et al., 2003). The upregulation of PTPμ facilitates the further association of the α3β1 integrin-CD151 complex with PKCβII (protein kinase CβII), RACK1 (receptor for activated C kinase-1), β-catenin and E-cadherin to form a multimolecular complex (Fig. 2). This large complex is thought to modulate E-cadherin-mediated cell–cell adhesion of epithelial cells, probably through the dephosphorylation of β-catenin. The crosstalk between the integrin-tetraspanin complex and the cadherin-catenin complex is likely to be crucial for the morphogenesis of epithelial tissues. It has also been reported that the interaction between α3β1 integrin and laminin-5-containing ECM promoted the formation of gap junctional intercellular communication (GJIC) of a monolayer culture of keratinocytes (discussed in the following section).

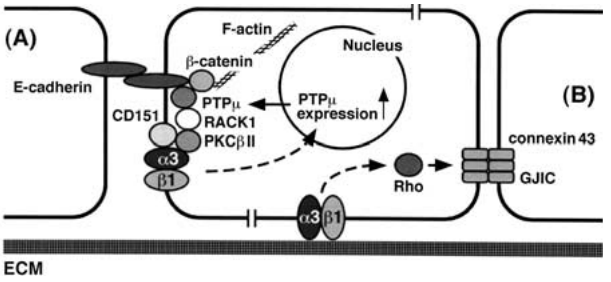


**Fig. 2.** The involvement of α3β1 integrin in cell-cell adhesion. (*A*) The association of α3β1 integrin with CD151 in the lateral membranes of epithelial cells induces the expression of PTPμ. A multimolecular complex composed of α3β1 integrin-CD151-PKCβII-RACK1-PTPμ-β-catenin-E-cadherin is organized and the E-cadherin-containing complex mediates cell-cell adhesion (Chattopadhyay et al., 2003). PKCβII, protein kinase CβII; RACK1, receptor for activated C kinase-1; PTPμ, protein tyrosine phosphatase μ. (*B*) The binding of α3β1 integrin of keratinocytes to ECM containing laminin-5 promotes the assembly of connexin 43 to facilitate gap junctional intercellular communication (GJIC). This promotion is mediated by a Rho-dependent signaling pathway (Lampe et al., 1998).

## Genes

Human genes for the integrin α3 and β1 subunits are located on chromosomes 17 and 10, respectively (Jones, van der Flier & Sonnenberg, 1998). Both the human and mouse genes for the integrin α3 subunit contain 26 exons (Jones et al., 1998; Tsuji et al., 1999). The number of exons of the α3 subunit gene is similar to that of the other integrin α subunits. However, the exon/intron organization differs somewhat among α subunits. In the α subunits of members of the β2 integrin subfamily (αL and αM subunits), for example, each metal-binding sequence is encoded by a single exon, whereas the first metal-binding sequence of the α3 or αIIb subunit is encoded by two separate exons. These differences are of interest from an evolutionary perspective. As described above, a cytoplasmic variant of the α3B subunit is generated by alternative exon usage. Exon 25, which encodes the entire cytoplasmic domain of α3A subunit, is skipped in the mRNA for the α3B subunit, the cytoplasmic domain of which is instead encoded by exon 26 (Tsuji et al., 1999).

A number of studies have demonstrated the aberrant expression of α3β1 integrin in various tumor cells in association with changes in their invasive and metastatic potentials (to be discussed in the last section). The transcriptional regulation of the integrin α3 subunit is therefore of considerable interest. The promoter region of the mouse integrin α3 subunit was recently characterized (Kato et al., 2002). The gene contains a CCAAT box, but lacks a TATA box. Most integrin α subunits lack both TATA and CCAAT boxes, except for the integrin α4 subunit gene that includes both TATA and CCAAT boxes, and also excepting the integrin α6 subunit gene that contains a TATA-like box but lacks a CCAAT box. Luciferase assay following the transfection of gastric carcinoma cells with a series of deletion constructs of the 5′-flanking region revealed that the sequence between positions −260 and −119 bp (relative to the major transcription start site) was required for efficient transcription. The introduction of mutation in one of the Ets-binding sequences greatly decreased promoter activity, suggesting that the transcription of the α3 integrin gene in these cells is regulated by the Ets-family of transcription factors. The sequence surrounding the Ets-binding consensus sequence is well conserved within human and mouse genes. The members of the Ets-family of transcription factors control the expression of numerous genes that are critical for cellular proliferation, differentiation, development, transformation, and apoptosis (Sementchenko & Watson, 2000).

## Development and Differentiation

Mice deficient in α3 integrin die during the neonatal period due to defects in the lungs and the kidneys, suggesting that this integrin plays a crucial role in development and differentiation (Kreidberg et al., 1996). This integrin is located at podocytes and

Bowman's capsule in the kidneys, and in the pulmonary alveoli of the lung; the epithelial organization of these organs may be impaired in α3 integrin-deficient mice. In addition to these tissues, this integrin is detected in most keratinizing and glandular epithelia, perineurium, basal lamina of smooth muscular fibers, vascular media, and myoepithelial cells of the parotid and breast (Bartolazzi et al., 1993a). The α3β1 integrin is expressed on the basolateral surfaces of epithelial cell membranes, and is thought to function in the establishment and maintenance of epithelial tissues of these organs.

In the basal layer of the skin epidermis, keratinocytes abundantly express α3β1 and α6β4 integrins, which serve as receptors for laminin isoforms including laminin-5, a major component of the basement membrane between the epidermis and the dermis. The α6β4 integrins constitute the hemidesmosome, the structural machinery connecting basal keratinocytes with the underlying basement membranes (Borradori & Sonnenberg, 1999). A causal relationship has been suggested between inactivating mutations in the genes coding for laminin-5 subunits, α6β4 integrin or other hemidesmosomal proteins, and epidermolysis bullosa, a blistering disorder of the skin. On the other hand, α3β1 integrin is recruited into focal contacts in cultured cells. In α3β1 integrin-deficient mice, matrix disorganization and concomitant blistering were observed at the dermal-epidermal junction in the skin (DiPersio et al., 1997). Those results suggest the role of α3β1 integrin in the establishment and/or maintenance of basement membrane integrity, while α6β4 integrin is required for stable adhesion of the epidermis to the basement membrane through hemidesmosomes. It was reported that cultured cells with a high level of α3β1 integrin expression deposited in vitro increased amounts of ECM proteins such as entactin and fibronectin into the pericellular matrices, as compared to cells with a low level of expression (Wu et al., 1995). Recently, the roles played by this integrin in the organization of laminin-5 in the ECM produced by keratinocytes were investigated (deHart, Healy & Jones, et al., 2003). Keratinocytes derived from α3β1 integrin-deficient mice were unable to assemble proper laminin-5 matrix organization. In terms of supporting laminin-5-dependent cell adhesion and migration, the characteristics of the matrix produced by the α3 integrin-null keratinocytes were distinct from those produced by the matrix of wild-type cells. These observations suggest that α3β1 integrin is very important for determining the incorporation of laminin-5 into its proper higher-order structure within the ECM of keratinocytes; moreover, the organizational state of laminin-5 is thought to exert an influence on laminin-5 matrix function.

In addition to their adhesion to basement membranes, keratinocytes are known to laterally connect

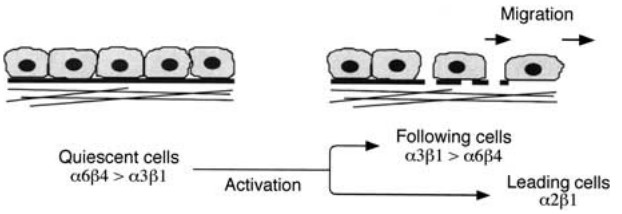


**Fig. 3.** Keratinocyte migration in wound repair. Quiescent epidermal keratinocytes anchored to basement membranes via α6β4 integrin are activated upon injury, and cells at the wound edge are committed to become "leading" and "following" keratinocytes. Activated leading keratinocytes migrate over exposed dermal collagen via α2β1 integrin and they deposit laminin-5 as a provisional basement membrane. By utilizing the deposited laminin-5 as a scaffold, the following keratinocytes migrate mainly via α3β1 integrin. The leading keratinocyte migration via α2β1 integrin is Rho-dependent, whereas the following keratinocyte migration via α3β1 integrin is Rho-independent (Goldfinger et al., 1999; Nguyen et al., 2000).

to adjacent cells through adhesion structures including desmosomes and gap junctions. It has been demonstrated that the adhesion of keratinocytes on laminin-5 via α3β1 integrin promoted the formation of gap junctional intercellular communication (GJIC) (Lampe et al., 1998). Keratinocytes that adhered to laminin-5 were more efficiently able to assemble with gap junctional protein connexin 43 to form functional GJIC than were those keratinocytes adhering to collagen or fibronectin; furthermore, this type of promotion was thought to be mediated by a Rho-dependent signaling pathway (Fig. 2).

During wound healing, the profile of integrin expression is dramatically changed, and keratinocyte migration is stimulated, accompanying the increased expression and deposition of laminin-5 (Larjava et al., 1993). The cooperative roles played by α2β1, α3β1, and α6β4 integrins in keratinocyte migration and wound repair have been proposed (Nguyen et al., 2000). Quiescent epidermal keratinocytes anchored to laminin-5 in basement membranes via α6β4 integrin are activated upon wounding, and cells at the wound edge are transformed into an epidermal outgrowth composed of "leading" and "following" subpopulations of keratinocytes (Fig. 3). Activated leading keratinocytes are characterized by the expression of elevated levels of a precursor form of laminin-5. These cells migrate over exposed dermal collagen by the use of α2β1 integrin and they deposit laminin-5 as a provisional basement membrane. By utilizing the deposited laminin-5 as a scaffold, the following keratinocytes migrate mainly via α3β1 integrin. The precursor form of laminin-5 preferentially interacts with α3β1 integrin to mediate migration (Goldfinger et al., 1998; 1999). Subsequently, the following events take place: 1) GJIC is promoted by α3β1 integrin-mediated adhesion, 2) laminin-5 is processed by proteases to generate its mature form, which is a

high-affinity ligand for α6β4 integrin, and 3) α6β4 integrin assembles to form hemidesmosomes. The migratory process of keratinocytes involves reversible changes in cell adhesion between the stable anchoring mediated by α6β4 integrin and the dynamic transient adhesion system mediated by α2β1 and α3β1 integrins. Change in the intracellular signaling of these cells has also been suggested, i.e., leading keratinocyte migration via α2β1 integrin is dependent on a low-molecular-weight G protein, Rho (involved in actin stress fiber regulation), whereas the following keratinocyte migration via α3β1 integrin is Rho-independent (Nguyen et al., 2001).

The function of α3β1 integrin in keratinocyte apoptosis (Fujisaki & Hattori, 2002), cell proliferation via a MAP kinase pathway (Gonzales et al., 1999), the regulation of stress fiber formation (Hodivala-Dilke et al., 1998), and hair follicle maintenance and morphogenesis (Conti et al., 2003) have also been suggested.

Podocytes of the renal glomerulus are specialized cells that wrap the glomerular capillaries with long, interdigitated foot processes, and these cells are involved in kidney function as an ultrafiltration barrier along with the glomerular basement membrane (GBM). In the kidney of α3β1 integrin-deficient mice, the GBM was extremely fragmented and disorganized, and glomerular podocytes were unable to form mature foot processes (Kreidberg et al., 1996). The α3β1 integrin is thought to be responsible for the attachment of these foot processes to the GBM (Kreidberg, 2000). Similar defects in glomerulogenesis associated with an abnormal GBM were observed in laminin α5 mutant embryos, suggesting that the interaction between α3β1 integrin and laminin 10/11 plays a crucial role in normal glomerulogenesis (Miner & Li, 2000). The kidneys of α3β1 integrin-deficient mice are also known to have defects in the branching morphology of the collecting tubules (Kreidberg et al., 1996). Subclones of Madin-Darby canine kidney (MDCK) cells exhibit branching tubulogenesis in collagen gel in vitro. A decrease in the levels of expression of α3β1 integrin by antisense RNA resulted in the inhibition of branching tubulogenesis (Jiang et al., 2001). Moreover, α3β1 integrin has been implicated in the pathogenesis of kidney failure. In renal ischemic injury, the expression of both α3β1 integrin and laminin-5 was enhanced (Zuk & Matlin, 2002). This receptor–ligand combination may be involved in the pathogenesis of acute renal failure and/or in repair of the injured kidney through the promotion of the regeneration of the damaged epithelium.

Another major defect in α3β1 integrin-deficient mice has been observed in the lung. In mutant mice, branching of the lung bronchi was decreased, and the large bronchi extended to the periphery (Kreidberg et al., 1996). It is known that α3β1 integrin is located at the basal surface of basal cells in the adult airway epithelium and it is thought to play a role in organizing the basement membranes into an ordered structure and in branching morphogenesis (Sheppard, 2003).

The integrin expression profile is dramatically changed during liver organogenesis, but the functional roles played by α3β1 integrin remain unclear. However, in the differentiation of a liver-derived cell line in vitro, an antisense RNA construct directed to α3 mRNA inhibited morphological and proliferative responses of the cell (Lora et al., 1998). In intrahepatic biliary epithelial cells, the enhanced expression of the integrin α3 subunit as well as that of the α2, α6, and β4 subunits was observed in association with laminin deposition during liver organogenesis (Couvelard et al., 1998).

The roles played by the integrin receptors in the differentiation of intestinal epithelial cells were studied by using a Caco-2 colon adenocarcinoma cell line (Zhang et al., 2003b). When cultured in 3-dimensional Matrigel, Caco-2 cells display epithelial-like phenotypes such as cell polarization, cytoskeletal reorganization, adherens junction rearrangement, and decreased proliferation in association with an increased expression of α3 integrin. Some of these phenotypic changes have been blocked, either by antibodies directed to the integrin α2, α3, and β1 subunits, or by transfection with α2 and α3 integrin antisense DNA.

Epithelial cells from various tissues are able to produce important cytokines and chemokines in response to stimulation by proinflammatory cytokines, and these cytokines are known to regulate the immune system. Colonic Caco-2 cells produce interleukin (IL)-6, IL-8 and monocyte chemoattractant protein (MCP)-1 upon stimulation with IL-1 proinflammatory cytokine. The pretreatment of these cells with anti-α3 integrin antibody suppressed the secretion of these cytokines (Lubin, Segal & McGee, 2003). A similar suppressive effect of the antibody was observed in tumor necrosis factor (TNF)-α-stimulated IL-6 secretion from A549 lung adenocarcinoma cells. Antibody binding to α3β1 integrin appears to generate a negative intracellular signal against cytokine production.

Electron microscopic studies of the submandibular glands of mice carrying a targeted mutation in the α3 integrin gene exhibited an aberrant differentiation phenotype with defects in cell polarity and the basement membrane (Menko et al., 2001). Biochemical analysis has demonstrated the altered expression and/or localization of several ECM proteins and adhesion molecules. These changes correlated with alterations in the state of activation of Ras-extracellular signal-regulated kinase (ERK), as well as changes in the expression and/or localization of Rho GTPases (Cdc 42 and Rho A),

which regulate the organization of the actin cytoskeleton.

In addition to epithelial tissue function, endothelial cells and myocytes are regulated by α3β1 integrin. Anti-α3 integrin antibody inhibited angiotensin II-induced angiogenesis (Dominguez-Jimenez et al., 2001). It has also been shown that the α3β1 integrin-CD151 complex normally located at the lateral junctions of endothelial cells was downregulated upon angiotensin II-induced angiogenesis. The role of α3β1 integrin in endothelial cell motility was discussed in the previous section (Yanez-Mo et al., 1998; Sincock et al., 1999). Examination of α3β1 integrin isoforms and their function in cultured neonatal myocytes suggested the possibility that this integrin is involved in the stabilization of myofibril assembly and in the maintenance of sarcomere structure (Kim et al., 1999). One possible function of α3β1 integrin, i.e., aiding in the development of intestinal smooth muscle, was also suggested by analyses of mice deficient in the laminin α5 chain (a component of laminin-10/11) (Bolcato-Bellemin et al., 2003).

## Function in the Nervous System

The involvement of α3β1 integrin in the development of the nervous system was suggested by severe defects in the cerebral cortex of mice deficient in this integrin (Kreidberg et al., 1996; Anton, Kreidberg & Rakic, 1999). Related defects in the cerebral cortex include abnormal laminar organization, abnormal radial glia differentiation, and impaired neuron-glia interactions. Recently, a new ECM protein with serine protease activity, referred to as Reelin, was identified, and this protein controls neuronal migration during the development of laminar structures of the mammalian brain, including the cerebral cortex, hippocampus, and cerebellum (D'Arcangelo et al., 1995; Hong et al, 2000; Quattrocchi et al., 2002). Reelin has been shown to bind to α3β1 integrin, and this interaction may promote proper laminar organization of the cortex (Dulabon et al., 2000).

The outgrowth of retinal neurons is induced by proteolytically processed or antibody-activated laminin-1, and the primary receptors used in the retinal neuron response are α3β1 and α6β1 integrins (Ivins et al., 1998). One means by which the CD151-α3β1 integrin complex is involved in the neurite outgrowth of NT2 N neuron-like cells induced by laminin-5 was described in the previous section (Stipp & Hemler, 2000). Additional roles played by other integrin members in the development of the cerebral cortex have recently been reviewed by Schmid & Anton (2003).

The possible involvement of α3β1 integrin in the establishment of learning and memory has also been suggested by several lines of evidence. Kramar et al. (2002) examined the effects of anti-α3 integrin antibody on rat hippocampal long-term potentiation (LTP), an experimental model of synaptic plasticity, and found that this integrin contributes to the consolidation of LTP. Subcellular fractionation experiments have demonstrated that α3 integrin is localized in synaptic membranes. Chan et al. (2003) found that heterozygous mutant mice with decreased expression of α3 integrin reduce the magnitude of NMDA receptor-dependent hippocampal LTP. Moreover, a deficiency in spatial memory, assessed by a water maze, was shown in mice with reduced expression of α3, α5, and α8 integrins. These results suggest the implication of α3β1 integrin in synaptic function in cooperation with other integrins. The mechanism by which α3β1 integrin and other adhesion molecules influence LTP still remains unclear, and therefore it remains to be elucidated in future studies.

## Expression in Tumor Cells

Galactoprotein b3, a historical term for α3β1 integrin, was given to a cell-surface membrane glycoprotein showing enhanced expression upon the oncogenic transformation of fibroblasts by SV40 or polyoma virus (Carter & Hakomori, 1976; Tsuji et al., 1990), suggesting its relevance to the phenotype of tumor cells. A number of studies have demonstrated the altered expression of α3β1 integrin in various tumors (Table 2). In gastric carcinoma, hepatocellular carcinoma, melanoma, and brain tumors, the expression of α3β1 integrin in these cells is increased when compared to that in normal counterparts, and frequently a positive correlation is observed with the malignant phenotypes, including invasive and metastatic potential. On the other hand, in lung, colon, ovary, breast, and prostate carcinomas, a decrease in the expression of α3β1 integrin is associated with tumorigenic transformation. Statistical analyses of the clinical specimens from lung and colon cancer cases have shown that reduced expression of α3 integrin is a poor prognosis factor in patients with lung adenocarcinomas and colon cancer (Adachi et al., 1998; Hashida et al., 2002).

The influence of the aberrant expression of integrins was assessed by utilizing transgenic mice in which the α2β1 or α3β1 integrin was expressed in the epidermis and the mice were subjected to skin carcinogenesis (Owens & Watt, 2001). Although equal numbers of benign squamous papillomas were observed in the transgenic and the wild-type animals, the frequency of the conversion of papillomas to malignant squamous cell carcinomas was lower in α3β1 transgenic mice than in α2β1 transgenic and wild-type mice. Furthermore, papillomas in the α3β1 integrin-transgenic mice showed less proliferative capacity and more differentiated

**Table 2.** Expression of α3β1 integrin in various tumors

| Organ | Phenomenon | Reference |
|---|---|---|
| Positive correlation between α3β1 integrin expression and tumorigenesis/malignancy: | | |
| Gastric | Association of α3 integrin expression with liver and peritoneal metastases. | Ura et al., 1998 |
| Liver | TGF-β1 stimulates α3 integrin expression in hepatocellular carcinoma concomitant with transformation into an invasive phenotype. Higher expression in metastatic tumors. | Giannelli et al., 2001<br>Giannelli et al., 2002 |
| Melanoma | Increased expression of α3 integrin in association with the degree of dermal invasiveness. | Natali et al., 1993<br>Schumacher & Schaumburg-Lever, 1999 |
| Glioma and Medulloblastoma | Correlation between α3 integrin expression and the grade of malignancy. | Kishima et al., 1999 |
| Astrocytoma | Increased expression of α3 and β1 subunits (some also show increased expression of α5, αV, β3, and β4 subunits). | Paulus et al., 1993 |
| Negative correlation between α3β1 integrin expression and tumorigenesis/malignancy: | | |
| Lung | Decreased α3 integrin expression in small cell lung carcinomas. | Bartolazzi et al., 1995<br>Barr et al., 1998 |
| | Inverse correlation between α3 integrin expression and prognosis. | Adachi et al., 1998 |
| Colon | Inverse correlation between α3 integrin expression and prognosis. | Hashida et al., 2002 |
| Ovary | Reduced α3 integrin expression with loss of basal localization in tumors. | Bartolazzi et al., 1993b |
| Breast | Low expression of β1, α2 and α3 subunits in tumors. | Pignatelli, Hanby & Stamp, 1991 |
| Prostate | Reduced expression of α3 integrin on invasive cells. | Dedhar et al., 1993 |

phenotypes, suggesting the inverse correlation of α3β1 integrin expression in papillomas and their malignancy.

Two integrins, α2β1 and α3β1, are involved in gastric cancer metastasis in different ways. Immunohistochemical studies of clinical specimens have revealed that α2β1 integrin expression was associated with lymph node and liver metastases, and that α3β1 integrin expression was associated with liver and peritoneal metastases (Ura et al., 1998). An experimental model also indicated that the expression of α2β1 and/or α3β1 integrin on gastric carcinoma cells was correlated with their potential for peritoneal metastasis (Nishimura et al., 1996). These data suggest that the integrin molecules, at least in part, determine the organ specificity for metastasis of gastric cancer. In an experimental mouse model of metastasis, CHO cells that expressed α4β1 integrin developed bone metastasis, and the same cells, but with an appropriate level of expression of α5β1 integrin, developed kidney metastasis (Matsuura et al., 1996; Tani et al., 2003). Furthermore, a subclone of a human non-small cell lung cancer cell line with a high potential for brain metastasis was shown to express a higher level of α3 integrin than did a subclone with bone metastatic potential or a parental line, and blocking of integrin α3β1 integrin was found to decrease the incidence of brain metastasis. (Yoshimasu et al., 2004).

The positive and negative correlations between integrin expression on tumor cells and their malignant phenotypes (Table 2) are based primarily on the statistical analysis of data derived from clinical specimens, and therefore such results do not necessarily apply to individual cases. In breast cancer, for example, the expression of α3β1 integrin on metastatic mammary carcinoma cells was specifically increased in comparison to that of non-metastatic cells (Tawil et al., 1996). In contrast, the treatment of metastatic breast carcinoma cells (MDA-MB-435) with Maspin, a product of a tumor suppressor gene, resulted in the inhibition of their motility and invasion concomitant with the increased expression of both α3 and α5 integrin (Seftor et al., 1998).

The expression of α3β1 integrin is likely to exert an influence on the malignant behavior of tumor cells by regulating their motility. Migration and invasion of glioma and melanoma cells on either a high-affinity ligand (laminin-5) or low-affinity ligands (fibronectin, collagen, and laminin-1) inhibited the antibodies against integrin α3 and β1 subunits (Melchiori et al., 1995; Fukushima et al., 1998; Tsuji et al., 2002). In colon carcinoma cells (Lovo), the expression of α3β1 integrin as well as that of CD63 tetraspanin was upregulated in low metastatic cells; in this case, migration and invasion were enhanced by anti-CD63 antibody in a phosphoinositide 3-kinase (PI 3-K)-dependent manner (Sordat et al., 2002). However, in

other colon carcinoma cells (LIM1215), the cell migration induced by epidermal growth factor (EGF) was inhibited by either an EGF receptor kinase inhibitor or a MAP kinase kinase inhibitor, but not by a PI3-K inhibitor. Inhibition experiments using antibodies have indicated that basal cell migration on laminin-10 is mediated by α3β1, but not by α2β1 or α6β4 integrins (Pouliot, Nice & Burgess, 2001).

It should be noted that the migration and invasion of tumor cells involves the production of ECM-degrading enzymes, including matrix metalloproteinase (MMP), by these cells. Recently, Morini et al. (2000) reported that the treatment of invasive mammary carcinoma (MDA-MB-231) cells with an anti-α3 integrin antibody reduced the production of MMP-9 (type IV collagenase), in addition to reducing migration and invasion by these cells. Moreover, immobilized laminin-5 induced A375 melanoma cells to secrete matrix metalloproteinase-9 (Tsuji et al., 2002). These results suggest that the interaction of α3β1 integrin with its ligand transduces an "outside-in signal" into a cell, leading to the production of MMP. In contrast, in human glioma (SNB19 and U251) and rhabdomyosarcoma (RD) cells, anti-α3 integrin antibodies demonstrated enhanced invasion into Matrigel, in association with increased levels of MMP-2 secretion from these cells (Chintala et al., 1996; Kubota et al., 1997). In these cells, anti-α3 integrin antibodies acted as agonists of MMP-2 secretion. The antibody-induced MMP-2 secretion from RD cells was mediated through a calreticulin-dependent pathway (Ito et al., 2001). In addition, the ligation of the α3β1 integrin-tetraspanin complex in MDA-MB-231 cells embedded in Matrigel, as observed using monoclonal antibodies, stimulated the production of MMP-2 and increased the invasive potential of these cells in a Matrigel penetration assay (Sugiura & Berditchevski, 1999). The enhanced MMP-2 production was inhibited by an inhibitor of PI 3-K, but not by an inhibitor of protein tyrosine kinases.

Currently, there is only a limited understanding of the regulatory mechanism of integrin α3 subunit expression; however, α3 integrin was found to be downregulated in osteosarcoma and neuroblastoma cells due to the overexpression of N-myc oncogene (Judware & Culp, 1997a, b); a similar downregulation was observed in small cell lung carcinoma cells as a result of the overexpression of c-myc oncogene (Barr et al., 1998). These results are in agreement with the observed correlation between the reduced expression of α3 integrin in these cells and the malignancy associated with the amplification of these oncogenes. On the other hand, transforming growth factor (TGF)-β1 upregulated α3 integrin expression in non-invasive hepatocellular carcinoma (HCC) cells, causing transformation into a motile and invasive phenotype (Giannelli et al., 2001; 2002). Invasive HCC cells secrete a high level of TGF-β1, and patients with metastasis tend to show increased concentrations of TGF-β1 in the serum; these patients also show stronger expression of TGF-β1 and α3 integrin in the HCC tissue itself. By contrast, bone morphogenetic protein (BMP)-2, a TGF-β-related growth factor, was shown to specifically downregulate α3 integrin expression in osteogenic sarcoma-derived cell lines (Nissinen, Pirila & Heino, 1997). These contrasting effects of TGF-β and BMP-2 on α3 integrin expression are likely to be regulated via pathways involving Smad proteins.

Numerous reports have demonstrated the presence of changes in integrin expression profiles in cancer cells, and such reports have addressed the relevance of such changes to malignant phenotypes. (for reviews, *see*: Varner & Cheresh, 1996; Ruoslahti, 1999; Holly et al., 2000; Orr et al., 2000; Hood & Cheresh, 2002).

## Concluding Remarks

During the last decade, the detailed structural and functional characterization of α3β1 integrin has been carried out. As illustrated in this article, α3β1 integrin plays crucial roles in the organization of epithelial and endothelial tissues by exerting functions related to cell adhesion and migration, which in turn are prerequisites for the development and differentiation of various organs. Several lines of evidence have shown that α3β1 integrin function is regulated by various types of associated molecules, including other membrane proteins, intracellular signaling molecules, and cytoskeletal proteins. The regulatory mechanisms of α3β1 integrin function, as well as the cooperation of α3β1 integrin with other integrins and other families of adhesion molecules, remain to be elucidated. In addition, as α3β1 integrin serves as a receptor for "outside-in signals", it will be important in future studies to account for how such signals are transduced into cells, and how such processes can lead to the modification of cellular function. Finally, the aberrant expression of α3β1 integrin in various tumor cells and the relevance of such atypical expression to malignant phenotypes remain important issues in the diagnosis and treatment of cancer.

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## References

Adachi, M., Taki, T., Huang, C., Higashiyama, M., Doi, O., Tsuji, T., Miyake, M. 1998. Reduced integrin α3 expression as a

factor of poor prognosis of patients with adenocarcinoma of the lung. *J Clin Oncol* **16:**1060–1067

Akula, S.M., Pramod, N.P., Wang, F.Z., Chandran, B. 2002. Integrin α3β1 (CD 49c/29) is a cellular receptor for Kaposi's sarcoma-associated herpesvirus (KSHV/HHV-8) entry into the target cells. *Cell* **108:**407–419

Albelda, S.M., Buck, C.A. 1990. Integrins and other cell adhesion molecules. *FASEB J.* **4:**2868–2880

Anton, E.S., Kreidberg, J.A., Rakic, P. 1999. Distinct functions of α3 and αV integrin receptors in neuronal migration and laminar organization of the cerebral cortex. *Neuron* **22:**277–289

Argraves, W.S., Suzuki, S., Arai, H., Thompson, K., Pierschbacher, M.D., Ruoslahti, E. 1987. Amino acid sequence of the human fibronectin receptor. *J. Cell Biol.* **105:**1183–1190

Banerjee, S.A., Hadjiargyrou, M., Patterson, P.H. 1997. An antibody to the tetraspan membrane protein CD9 promotes neurite formation in a partially α3β1 integrin-dependent manner. *J. Neurosci.* **17:**2756–2765

Barr, L.F., Campbell, S.E., Bochner, B.S., Dang, C.V. 1998. Association of the decreased expression of α3β1 integrin with the altered cell: environmental interactions and enhanced soft agar cloning ability of c-myc-overexpressing small cell lung cancer cells. *Cancer Res.* **58:**5537–5545

Bartolazzi, A., Cerboni, C., Flamini, G., Bigotti, A., Lauriola, L., Natali, P.G. 1995. Expression of α3β1 integrin receptor and its ligands in human lung tumors. *Int. J. Cancer* **64:**248–252

Bartolazzi, A., Cerboni, C., Full, C., Valentini, C., Natali, P.G., Venture, I., Bigotti, A. 1993a. Vla-3 distribution in normal and neoplastic non-lymphoid human tissues. *Pathol. Res. Pract.* **189:**387–393

Bartolazzi, A., Kaczmarek, J., Nicolo, G., Risso, A.M., Tarone, G., Rossino, P., Defilippi, P., Castellani, P. 1993b. Localization of the α3β1 integrin in some common epithelial tumors of the ovary and in normal equivalents. *Anticancer Res.* **13:**1–11

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

Berditchevski, F, Chang, S., Bodorova, J., Hemler, M.E. 1997b. Generation of monoclonal antibodies to integrin-associated proteins. Evidence that α3β1 complexes with EMMPRIN/basign/OX47/M6. *J. Biol. Chem.* **272:**29174–29180

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

Berditchevski, F., Zutter, M.M., 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

Bolcato–Bellemin, A.L., Lefebvre, O., Arnold, C., Sorokin, L., Miner, J.H., Kedinger, M., Simon-Assmann, P. 2003. Laminin α5 chain is required for intestinal smooth muscle development. *Dev. Biol.* **260:**376–390

Borradori, L., Sonnenberg, A. 1999. Structure and function of hemidesmosomes: more than simple adhesion complexes. *J. Invest. Dermatol.* **112:**411–418

Carter, W.G., Hakomori, S. 1976. Isolation and partial characterization of "galactoprotein a" (LETS) and "galactoprotein b" from hamster embryo fibroblasts. *Biochem. Biophys. Res. Commun.* **76:**299–308

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

Chan, C.S., Weeber, E.J., Kurup, S., Sweatt, J.D., Davis, R.L. 2003. Integrin requirement for hippocampal synaptic plasticity and spatial memory. *J. Neurosci.* **23:**7107–7116

Chattopadhyay, N., Wang, Z., Ashman, L.K., Brady-Kalnay, S.M., Kreidberg, J.A. 2003. α3β1 integrin-CD151, a component of the cadherin–catenin complex, regulates PTPμ expression and cell-cell adhesion. *J. Cell Biol.* **163:**1351–1362

Chintala, S.K., Sawaya, R., Gokaslan, Z.L., Rao, J.S. 1996. Modulation of matrix metalloprotease-2 and invasion in human glioma cells by α3β1 integrin. *Cancer Lett.* **103:**201–208

Claas, C., Seiter, S., Claas, A., Savelyeva, L., Schwab, M., Zoller, M. 1998. Association between the rat homologue of CO-029, a metastasis-associated tetraspanin molecule and consumption coagulopathy. *J. Cell Biol.* **141:**267–280

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

Conti, F.J., Rudling, R.J., Robson, A., Hodivala-Dilke, K.M. 2003. α3β1-integrin regulates hair follicle but not interfollicular morphogenesis in adult epidermis. *J Cell Sci.* **116:**2737–2747

Coppolino, M., Leung-Hagesteijn, C., Dedhar, S., Wilkins, J. 1995a. Inducible interaction of integrin α3β1 with calreticulin. Dependence on the activation state of the integrin. *J. Biol. Chem.* **270:**23132–23138

Coppolino, M., Migliorini, M., Argraves, W.S., Dedhar, S. 1995b. Identification of a novel form of the α3 integrin subunit: covalent association with transferrin receptor. *Biochem. J.* **306:**129–134

Couvelard, A., Bringuier, A.F, Dauge, M.C., Nejjari, M., Darai, E., Benifla, J.L., Feldmann, G., Henin, D., Scoazec, J.Y. 1998. Expression of integrins during liver organogenesis in humans. *Hepatology* **27:**839–847

D'Arcangelo, G., Miao, G.G., Chen, S.C., Scares, H.D., Morgan, J.I, Curran, T. 1995. A protein related to extracellular matrix proteins deleted in the mouse mutant reeler. *Nature* **374:**719–723

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

Decline, F, Rousselle, P. 2001. Keratinocyte migration requires α2β1 integrin-mediated interaction with the laminin 5 γ2 chain. *J. Cell Sci.* **114:**811–823

Dedhar, S., Jewell, K., Rojiani, M., Gray, V. 1992. The receptor for the basement membrane glycoprotein entactin is the integrin α3/β1. *J. Biol. Chem.* **267:**18908–18914

Dedhar, S., Saulnier, R., Nagle, R., Overall, C.M. 1993. Specific alterations in the expression of α3β1 and α6β4 integrins in highly invasive and metastatic variants of human prostate carcinoma cells selected by in vitro invasion through reconstituted basement membrane. *Clin. Exp. Metastasis.* **11:**391–400

deHart, G.W., Healy, K.E., Jones, J.C. 2003. The role of α3β1 integrin in determining the supramolecular organization of laminin-5 in the extracellular matrix of keratinocytes. Exp. *Cell Res.* **283:**67–79

DiPersio, C.M., Hodivala-Dilke, K.M., Jaenisch, R., Kreidberg, J.A., Hynes, R.O. 1997. α3β1 Integrin is required for normal development of the epidermal basement membrane. *J. Cell Biol.* **137:**729–742

DiPersio, C.M., Trevithick, J.E., Hynes, R.O. 2001. Functional comparison of the α3A and α3B cytoplasmic domain variants of the chicken α3 integrin subunit. *Exp. Cell Res.* **268:**45–60

Dominguez-Jimenez, C., Yanez-Mo, M., Carreira, A., Tejedor, R., Gonzalez-Amaro, R., Alvarez, V., Sanchez-Madrid, F. 2001. Involvement of α3 integrin/tetraspanin complexes in the angiogenic response induced by angiotensin II. *FASEB J.* **15:**1457–1459

Dulabon, L., Olson, E.C., Taglienti, M.G., Eisenhuth, S., McGrath, B., Walsh, C.A., Kreidberg, J.A., Anton, E.S. 2000. Reelin binds α3β1 integrin and inhibits neuronal migration. *Neuron* **27:**33–44

Eble, J.A., Wucherpfennig, K.W., Gauthier, L., Dersch, P., Krukonis, E., Isberg, R.R., Hemler, M.E. 1998. Recombinant soluble human α3β1 integrin: purification, processing, regulation, and specific binding to laminin-5 and invasin in a mutually exclusive manner. *Biochemistry* **37:**10945–10955

Elices, M.J., Urry, L.A., Hemler, M.E. 1991. Receptor functions for the integrin VLA-3: fibronectin, collagen, and laminin binding are differentially influenced by Arg-Gly-Asp peptide and by divalent cations. *J. Cell Biol.* **112:**169–181

Engvall, E., Wewer, U.M. 1996. Domains of laminin. *J. Cell. Biochem.* **61:**493–501

Ferletta, M., Ekblom, P. 1999. Identification of laminin-10/11 as a strong cell adhesive complex for a normal and a malignant human epithelial cell line. *J. Cell Sci.* **112:**1–10

Fujisaki, H., Hattori, S. 2002. Keratinocyte apoptosis on type I collagen gel caused by lack of laminin 5/10/11 deposition and Akt signaling. *Exp. Cell Res.* **280:**255–269

Fujiwara, H., Kikkawa, Y., Sanzen, N., Sekiguchi, K. 2001. Purification and characterization of human laminin-8. Laminin-8 stimulates cell adhesion and migration through α3β1 and α6β1 integrins. *J. Biol. Chem.* **276:**17550–17558

Fukushima, Y., Ohnishi, T., Arita, N., Hayakawa, T., Sekiguchi, K. 1998. Integrin α3β1-mediated interaction with laminin-5 stimulates adhesion, migration and invasion of malignant glioma cells. *Int. J. Cancer* **76:**63–72

Giannelli, G., Bergamini, C., Fransvea, E., Marinosci, F., Quaranta, V., Antonaci, S. 2001. Human hepatocellular carcinoma (HCC) cells require both α3β1 integrin and matrix metalloproteinases activity for migration and invasion. *Lab. Invest.* **81:**613–627

Giannelli, G., Fransvea, E., Marinosci, F., Bergamini, C., Colucci, S., Schiraldi, O., Antonaci, S. 2002. Transforming growth factor-β1 triggers hepatocellular carcinoma invasiveness via α3β1 integrin. *Am. J. Pathol.* **161:**183–193

Goldfinger, L.E., Stack, M.S., Jones, J.C. 1998. Processing of laminin-5 and its functional consequences: role of plasmin and tissue-type plasminogen activator. *J. Cell Biol.* **141:**255–265

Goldfinger, L.E., Hopkinson, S.B., deHart, G.W., Collawn, S., Couchman, J.R., Jones, J.C. 1999. The α3 laminin subunit, α6β4 and α3β1 integrin coordinately regulate wound healing in cultured epithelial cells and in the skin. *J. Cell Sci.* **112:**2615–2629

Gonzales, M., Haan, K., Baker, S.E., Fitchmun, M., Todorov, I., Weitzman, S., Jones, J.C. 1999. A cell signal pathway involving laminin-5, α3β1 integrin, and mitogen-activated protein kinase can regulate epithelial cell proliferation. *Mol. Biol. Cell* **10:**259–270

Guo, N., Templeton, N.S., Al-Barazi, H., Cashel, J.A., Sipes, J.M., Krutzsch, H.C., Roberts, D.D. 2000. Thrombospondin–1 promotes α3β1 integrin-mediated adhesion and neurite-like outgrowth and inhibits proliferation of small cell lung carcinoma cells. *Cancer Res.* **60:**457–466

Hadjiargyrou, M., Patterson, P.H. 1995. An anti-CD9 monoclonal antibody promotes adhesion and induces proliferation of Schwann cells in vitro. *J. Neurosci.* **15:**574–583

Hadjiargyrou, M., Kaprielian, Z., Kato, N., Patterson, P.H. 1996. Association of the tetraspan protein CD9 with integrins on the surface of S-16 Schwann cells. *J. Neurochem.* **67:**2505–2513

Hakomori, S. 2002. The glycosynapse. *Proc. Natl. Acad. Sci. USA* **99:**225–232

Hashida, H., Takabayashi, A., Tokuhara, T., Taki, T., Kondo, K., Kohno, N., Yamaoka, Y., Miyake, M. 2002. Integrin α3 expression as a prognostic factor in colon cancer: association with MRP-1/CD9 and KAI1/CD82. *Int. J. Cancer* **97:**518–525

Hodivala-Dilke, K.M., DiPersio, C.M., Kreidberg, J.A., Hynes, R.O. 1998. Novel roles for α3β1 integrin as a regulator of cytoskeletal assembly and as a trans-dominant inhibitor of integrin receptor function in mouse keratinocytes. *J. Cell Biol.* **142:**1357–1369

Holly, S.P., Larson, M.K., Parise, L.V. 2000. Multiple roles of integrins in cell motility. *Exp. Cell Res.* **261:**69–74

Hong, S.E., Shugart, Y.Y, Huang, D.T., Shahwan, S.A., Grant, P.E, Hourihane, J.O., Martin, N.D., Walsh, C.A. 2000. Autosomal recessive lissencephaly with cerebellar hypoplasia is associated with human RELN mutations. *Nat. Genet.* **26:**93–96

Hood, J.D., Cheresh, D.A. 2002. Role of integrins in cell invasion and migration. *Nat. Rev. Cancer* **2:**91–100

Humphries, M.J., McEwan, P.A., Barton, S.J., Buckley, P.A., Bella, J., Paul Mould, A. 2003. Integrin structure: heady advances in ligand binding, but activation still makes the knees wobble. *Trends Biochem. Sci.* **28:**313–320

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

Hynes, R.O., Marcantonio, E.E., Stepp, M.A., Urry, L.A., Yee, G.H. 1989. Integrin heterodimer and receptor complexity in avian and mammalian cells. *J. Cell Biol.* **109:**409–420

Isberg, R.R., Leong, J.M. 1990. Multiple β1 chain integrins are receptors for invasin, a protein that promotes bacterial penetration into mammalian cells. *Cell* **60:**861–871

Ito, H., Seyama, Y., Kubota, S. 2001. Calreticulin is directly involved in anti-α3 integrin antibody-mediated secretion and activation of matrix metalloprotease-2. *Biochem. Biophys. Res. Commun.* **283:**297–302

Ivins, J.K., Colognato, H., Kreidberg, J.A., Yurchenco, P.D., Lander, A.D. 1998. Neuronal receptors mediating responses to antibody activated laminin-1. *J. Neurosci.* **18:**9703–9715

Jiang, S.T., Chiu, S.J., Chen, H.C., Chuang, W.J., Tang, M.J. 2001. Role of $\alpha_3\beta_1$ integrin in tubulogenesis of Madin-Darby canine kidney cells. *Kidney Int.* **59:**1770–1778

Jones, P.H., Bishop, L.A., Watt, F.M. 1996. Functional significance of CD9 association with β1 integrins in human epidermal keratinocytes. *Cell Adhes. Commun.* **4:**297–305

Jones, S.D., van der Flier, A., Sonnenberg, A. 1998. Genomic organization of the human α3 integrin subunit gene. *Biochem. Biophys. Res. Commun.* **248:**896–898

Judware, R., Culp, L.A. 1997a. N-myc over-expression downregulates α3β1 integrin expression in human Saos-2 osteosarcoma cells. *Clin. Exp. Metastasis* **15:**228–238

Judware, R., Culp, L.A. 1997b. Concomitant down-regulation of expression of integrin subunits by N-myc in human neuroblastoma cells: differential regulation of subunits by N-myc in human neuroblastoma cells: differential regulation of α2, α3 and β1. *Oncogene* **14:**1341–1350

Kato, T., Katabami, K., Takatsuki, H., Han, S.A., Takeuchi, K., Irimura, T., Tsuji, T. 2002. Characterization of the promoter for the mouse α3 integrin gene. *Eur. J. Biochem.* **269:**4524–4532

Kawakami, Y., Kawakami, K., Steelant, W.F., Ono, M., Baek, R.C., Handa, K., Withers, D.A., Hakomori, S. 2002. Tetraspanin CD9 is a "proteolipid," and its interaction with α3 integrin in microdomain is promoted by GM3 ganglioside, leading to inhibition of laminin-5-dependent cell motility. *J. Biol. Chem.* **277:**34349–34358

Kikkawa, Y., Sanzen, N., Sekiguchi, K. 1998. Isolation and characterization of laminin-10/11 secreted by human lung carcinoma cells. Laminin-10/11 mediates cell adhesion through integrin α3β1. *J. Biol. Chem.* **273:**15854–15859

Kikkawa, Y., Umeda, M., Miyazaki, K. 1994. Marked stimulation of cell adhesion and motility by ladsin, a laminin-like scatter factor. *J. Biochem. (Tokyo)* **116:**862–869

Kim, Y.Y., Lim, C.S., Song, Y.H., Ahnn, J., Park, D., Song, W.K. 1999. Cellular localization of $\alpha_3\beta_1$ integrin isoforms in association with myofibrillogenesis during cardiac myocyte development in culture. *Cell. Adhes. Commun.* **7:**85–97

Kishima, H., Shimizu, K., Tamura, K., Miyao, Y., Mabuchi, E., Tominaga, E., Matsuzaki, J., Hayakawa, T. 1999. Monoclonal antibody ONS-M21 recognizes integrin α3 in gliomas and medulloblastomas. *Br. J. Cancer* **79:**333–339

Kramar, E.A., Bernard, J A., Gall, C.M., Lynch, G. 2002. α3 integrin receptors contribute to the consolidation of long-term potentiation. *Neuroscience* **110:**29–39

Kreidberg, J.A. 2000. Functions of α3β1 integrin. *Curr. Opin. Cell Biol.* **12:**548–553

Kreidberg, J.A., Donovan, M.J., Goldstein, S.L., Rennke, H., Shepherd, K., Jones, R.C., Jaenisch, R. 1996. α3β1 integrin has a crucial role in kidney and lung organogenesis. *Development* **122:**3537–3547

Krokhin, O.V., Cheng, K., Sousa, S.L., Ens, W., Standing, K.G., Wilkins, J.A. 2003. Mass spectrometric based mapping of the disulfide bonding patterns of integrin a chains. *Biochemistry* **42:**12950–12959

Krukonis, E.S., Dersch, P., Eble, J.A., Isberg, R.R. 1998. Differential effects of integrin α chain mutations on invasin and natural ligand interaction. *J. Biol. Chem.* **273:**31837–43183

Kubota, S., Ito, H., Ishibashi, Y., Seyama, Y. 1997. Anti-α3 integrin antibody induces the activated form of matrix metalloprotease-2 (MMP-2) with concomitant stimulation of invasion through matrigel by human rhabdomyosarcoma cells. *Int. J. Cancer* **70:**106–111

Lampe, P.D., Nguyen, B.P., Gil, S., Usui, M., Olerud, J., Takada, Y., Carter, W.G. 1998. Cellular interaction of integrin α3β1 with laminin 5 promotes gap junctional communication. *J. Cell Biol.* **143:**1735–1747

Larjava, H., Salo, T., Haapasalmi, K., Kramer, R.H., Heino, J. 1993. Expression of integrins and basement membrane components by wound keratinocytes. *J. Clin. Invest.* **92:**1425–1435

Leung-Hagesteijn, C.Y., Milankov, K., Michalak, M., Wilkins, J., Dedhar, S. 1994. Cell attachment to extracellular matrix substrates is inhibited upon downregulation of expression of calreticulin, an intracellular integrin α-subunit-binding protein. *J. Cell Sci.* **107:**589–600

Liu, S., Calderwood, D.A., Ginsberg, M.H. 2000. Integrin cytoplasmic domain-binding proteins. *J. Cell Sci.* **113:**3563–3571

Lora, J.M., Rowader, K.E., Scares, L., Giancotti, R., Zaret, K.S. 1998. α3β1-integrin as a critical mediator of the hepatic differentiation response to the extracellular matrix. *Hepatology* **28:**1095–1104

Lubin, F.D., Segal, M., McGee, D.W. 2003. Regulation of epithelial cell cytokine responses by the α3β1 integrin. *Immunology* **108:**204–210

Marinkovich, M.P., Verrando, P., Keene, D.R., Meneguzzi, G., Lunstrum, G.P., Ortonne, J.P., Burgeson, R.E. 1993. Basement membrane proteins kalinin and Basement membrane proteins kalinin and nicein are structurally and immunologically identical. *Lab Invest.* **69:**295–299

Matsuura, N., Puzon-McLaughlin, W., Irie, A., Morikawa, Y., Kakudo, K., Takada, Y. 1996. Induction of experimental bone metastasis in mice by transfection of integrin α4β1 into tumor cells. *Am. J. Pathol.* **148:**55–61

McCormick, J.I., Johnstone, R.M. 1995. Identification of the integrin α3β1 as a component of a partially purified A-system amino acid transporter from Ehrlich cell plasma membranes. *Biochem. J.* **311:**743–751

Melchiori, A., Mortarini, R., Carlone, S., Marchisio, P.C., Anichini, A., Noonan, D.M., Albini, A. 1995. The α3β1 integrin is involved in melanoma cell migration and invasion. *Exp. Cell Res.* **219:**233–242

Menko, A.S., Kreidberg, J.A., Ryan, T.T., Van Bockstaele, E., Kukuruzinska, M.A. 2001. Loss of α3β1 integrin function results in an altered differentiation program in the mouse submandibular gland. *Dev. Dyn.* **220:**337–349

Miner, J.H., Li, C. 2000. Defective glomerulogenesis in the absence of laminin α5 demonstrates a developmental role for the kidney glomerular basement membrane. *Dev. Biol.* **217:**278–289

Miyake, M., Koyama, M., Seno, M., Ikeyama, S. 1991. Identification of the motility-related protein (MRP–1), recognized by monoclonal antibody M31-15, which inhibits cell motility. *J. Exp. Med.* **174:**1347–1354

Miyazaki, K., Kikkawa, Y., Nakamura, A., Yasumitsu, H., Umeda, M. 1993. A large cell-adhesive scatter factor secreted by human gastric carcinoma cells. *Proc. Natl. Acad. Sci. USA.* **90:**11767–11771

Morini, M., Mottolese, M., Ferrari, N., Ghiorzo, F., Buglioni, S., Mortarini, R., Noonan, D.M., Natali, P.G., Albini, A. 2000. The α3β1 integrin is associated with mammary carcinoma cell metastasis, invasion, and gelatinase B (MMP-9) activity. *Int. J. Cancer* **87:**336–342

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

Natali, P.G., Nicotra, M.R., Bartolazzi, A., Cavaliere, R., Bigotti, A. 1993. Integrin expression in cutaneous malignant melanoma: association of the α3/β1 heterodimer with tumor progression. *Int. J. Cancer* **54:**68–72

Nermut, M.V., Green, N.M., Eason, P., Yamada, S.S., Yamada, K.M. 1988. Electron microscopy and structural model of human fibronectin receptor. *EMBO J.* **7:**4093–4099

Nguyen, B.P., Ren, X.D., Schwartz, M.A., Carter, W.G. 2001. Ligation of integrin α3β1 by laminin 5 at the wound edge activates Rho-dependent adhesion of leading keratinocytes on collagen. *J. Biol. Chem.* **276:**43860–43870

Nguyen, B.P., Ryan, M.C., Gil, S.G., Carter, W.G. 2000. Deposition of laminin 5 in epidermal wounds regulates integrin signaling and adhesion. *Curr. Opin. Cell Biol.* **12:**554–562

Nishimura, S., Chung, Y.S., Yashiro, M., Inoue, T., Sowa, M. 1996. Role of α2β1- and α3β1-integrin in the peritoneal implantation of scirrhous gastric carcinoma. *Br. J. Cancer* **74:**1406–1412

Nishiuchi, R., Murayama, O., Fujiwara, H., Gu, J., Kawakami, T., Aimoto, S., Wada, Y., Sekiguchi, K. 2003. Characterization of the ligand-binding specificities of integrin α3β1 and α6β1 using a panel of purified laminin isoforms containing distinct α chains. *J. Biochem. (Tokyo).* **134:**497–504

Nissinen, L., Pirila, L., Heino, J. 1997. Bone morphogenetic protein-2 is a regulator of cell adhesion. *Exp. Cell Res.* **230:**377–385

Okochi, H., Kato., M., Nashiro, K., Yoshie, O., Miyazono, K., Furue, M. 1997. Expression of tetra-spans transmembrane family (CD9, CD37, CD53, CD63, CD81 and CD82) in normal and neoplastic human keratinocytes: an association of CD9 with α3β1 integrin. *Br. J. Dermatol.* **137:**856–863

Orr, F.W., Wang, H.H., Lafrenie, R.M., Scherbarth, S., Nance, D.M. 2000. Interactions between cancer cells and the endothelium in metastasis. *J. Pathol.* **190:**310–329

O'Toole, E.A., Marinkovich, M.P., Hoeffler, W.K., Furthmayr, H., Woodley, D.T. 1997. Laminin-5 inhibits human keratinocyte migration. *Exp. Cell Res.* **233:**330–339

Owens, D.M., Watt, F.M. 2001. Influence of β1 integrins on epidermal squamous cell carcinoma formation in a transgenic

mouse model: α3β1, but not α2β1, suppresses malignant conversion. *Cancer Res.* **61:**5248–5254

Paulus, W., Baur, I., Schuppan, D., Roggendorf, W. 1993. Characterization of integrin receptors in normal and neoplastic human brain. *Am. J. Pathol.* **143:**154–163

Pignatelli, M., Hanby, A.M., Stamp, G.W. 1991. Low expression of β1, α2 and α3 subunits of VLA integrins in malignant mammary tumours. *J. Pathol.* **165:**25–32

Pouliot, N., Nice, E.C., Burgess, A.W. 2001. Laminin-10 mediates basal and EGF-stimulated motility of human colon carcinoma cells via $\alpha_3\beta_1$ and $\alpha_6\beta_4$ integrins. *Exp. Cell Res.* **266:**1–10

Prokopishyn, N.L., Puzon-McLaughlin, W., Takada, Y., Laferte, S. 1999. Integrin α3β1 expressed by human colon cancer cells is a major earner of expressed by human colon cancer cells is a major carrier of oncodevelopmental carbohydrate epitopes. *J. Cell. Biochem.* **72:**189–209

Quattrocchi, C.C., Wannenes, F., Persico, A.M., Ciafre, S.A., D'Arcangelo, G., Farace, M.G., Keller, F. 2002. Reelin is a serine protease of the extracellular matrix. *J. Biol. Chem.* **277:**303–309

Ridley, A.J., Schwartz, M.A., Burridge, K., Firtel, R.A., Ginsberg, M.H., Borisy, G., Parsons, J.T., Horwitz, A.R. 2003. Cell migration: integrating signals from front to back. *Science* **302:**1704–1709

Ruoslahti, E. 1991. Integrins. *J. Clin. Invest.* **87:**1–5

Ruoslahti, E. 1999. Fibronectin and its integrin receptors in cancer. *Adv. Cancer Res.* **76:**1–20

Schmid, R.S., Anton, E.S. 2003. Role of integrins in the development of the cerebral cortex. *Cereb. Cortex.* **13:**219–224

Schumacher, D., Schaumburg-Lever, G. 1999. Ultrastructural localization of alpha-3 integrin subunit in malignant melanoma and adjacent epidermis. *J. Cutan. Pathol.* **26:**321–326

Seftor, R.E., Seftor, E.A., Sheng, S., Pemberton, P.A., Sager, R., Hendrix, M.J. 1998. maspin suppresses the invasive phenotype of human breast carcinoma. *Cancer Res.* **58:**5681–5685

Sementchenko, V.I., Watson, D.K. 2000. Ets target genes: past, present and future. *Oncogene* **19:**6533–6548

Seo, D.W., Li, H., Guedez, L., Wingfield, P.T., Diaz, T., Salloum, R., Wei, B.Y., Stetler-Stevenson, W.G. 2003. TIMP-2 mediated inhibition of angiogenesis: an MMP-independent mechanism. *Cell* **114:**171–180

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

Sheppard, D. 2003. Functions of pulmonary epithelial integrins: from development to disease. *Physiol. Rev.* **83:**673–686

Shigeta, M., Sanzen, N., Ozawa, M., Gu, J., Hasegawa, H., Sekiguchi, K. 2003. CD151 regulates epithelial cell-cell adhesion through PKC- and Cdc42-dependent actin cytoskeletal reorganization. *J. Cell Biol.* **163:**165–176

Simons, K., Toomre, D. 2000. Lipid rafts and signal transduction. *Nat. Rev. Mol. Cell Biol.* **1:**31–39

Sincock, P.M., Fitter, S., Parton, R.G., Berndt, M.C., Gamble, J.R., 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

Sordat, I., Decraene, C., Silvestre, T., Petermann, O., Auffray, C., Pietu, G., Sordat, B. 2002. Complementary DNA arrays identify CD63 tetraspanin and α3 integrin chain as differentially expressed in low and high metastatic human colon carcinoma cells. *Lab. Invest.* **82:**1715–1724

Springer, T.A. 1997. Folding of the N-terminal, ligand-binding region of integrin α-subunits into a β-propeller domain. *Proc. Natl. Acad. Sci. USA* **94:**65–72

Springer, T.A. 2002. Predicted and experimental structures of integrins and β-propellers. *Curr. Opin. Struct. Biol.* **12:**802–813

Sriramarao, P., Steffner, P., Gehlsen, K.R. 1993. Biochemical evidence for a homophilic interaction of the α3β1 integrin. *J. Biol. Chem.* **268:**22036–22041

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

Stipp, C.S., Kolesnikova, T.V., Hemler, M.E. 2003. EWI-2 regulates α3β1 integrin-dependent cell functions on laminin-5. *J. Cell Biol.* **163:**1167–1177

Sugiura, T., 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

Sun, J., Hemler, M.E. 2001. Regulation of MMP-1 and MMP-2 production through CD147/extracellular matrix metalloproteinase inducer interactions. *Cancer Res.* **61:**2276–2281

Symington, B.E., Takada, Y., Carter, W.G. 1993. Interaction of integrins α3β1 and α2β1: potential role in keratinocyte intercellular adhesion. *J. Cell Biol.* **120:**523–535

Tachibana, I., Bodorova, J., Berditchevski, F, Zutter, M.M., Hemler, M.E. 1997. NAG-2, a novel transmembrane-4 superfamily (TM4SF) protein that complexes with integrins and other TM4SF proteins. *J. Biol. Chem.* **272:**29181–29189

Takada, Y., Strominger, J.L., Hemler, M.E. 1987. The very late antigen family of heterodimers is part of a superfamily of molecules involved in adhesion and embryogenesis. *Proc. Natl. Acad. Sci. USA* **84:**3239–3243

Takada, Y, Wayner, E.A., Carter, W.G., Hemler, M.E. 1988. Extracellular matrix receptors, ECMRII and ECMRI, for collagen and fibronectin correspond to VLA-2 and VLA-3 in the VLA family of heterodimers. *J. Cell. Biochem.* **37:**385–393

Takada, Y., Murphy, E., Pil, P., Chen, C., Ginsberg, M.H., Hemler, M.E. 1991. Molecular cloning and expression of the cDNA for α3 subunit of human α3β1 (VLA-3), an integrin receptor for fibronectin, laminin, and collagen. *J. Cell Biol.* **115:**257–266

Takeuchi, K., Hirano, K., Tsuji, T., Osawa, T., Irimura, T. 1995. cDNA cloning of mouse VLA-3 α subunit. *J. Cell. Biochem.* **57:**371–377

Tamura, R.N., Cooper, H.M., Collo, G., Quaranta, V. 1991. Cell type-specific integrin variants with alternative α chain cytoplasmic domains. *Proc. Natl. Acad. Sci. USA* **88:**10183–10187

Tani, N., Higashiyama, S., Kawaguchi, N., Madarame, J., Ota, I., Ito, Y., Ohoka, Y., Shiosaka, S., Takada, Y., Matsuura, N. 2003. Expression level of integrin α5 on tumour cells affects the rate of metastasis to the kidney. *Br. J. Cancer* **88:**327–333

Tawil, N.J., Gowri, V., Djoneidi, M., Nip, J., Carbonetto, S., Brodt, P. 1996. Integrin α3β1 can promote adhesion and spreading of metastatic breast carcinoma cells on the lymph node stroma. *Int. J. Cancer* **66:**703–710

Tsuji, T., Hakomori, S., Osawa, T. 1991. Identification of human galactoprotein b3, an oncogenic transformation-induced membrane glycoprotein, as VLA-3 α subunit: the primary structure of human integrin α3. *J. Biochem. (Tokyo)* **109:**659–665

Tsuji, T., Han, S.A., Takeuchi, K., Takahashi, N., Hakomori, S., Irimura, T. 1999. Characterization of mouse integrin α3 subunit gene. *J. Biochem. (Tokyo)* **125:**1183–1188

Tsuji, T., Kawada, Y., Kai-Murozono, M., Komatsu, S., Han, S.A., Takeuchi, K., Mizushima, H., Miyazaki, K., Irimura, T.

2002. Regulation of melanoma cell migration and invasion by laminin-5 and α3β1 integrin (VLA-3). *Clin. Exp. Metastasis* **19:**127–134

Tsuji, T., Yamamoto, F., Miura, Y., Takio, K., Titani, K., Pawar, S., Osawa, T., Hakomori, S. 1990. Characterization through cDNA cloning of galactoprotein b3 (Gap b3), a cell surface membrane glycoprotein showing enhanced expression on oncogenic transformation. Identification of Gap b3 as a member of the integrin superfamily. *J. Biol. Chem.* **265:**7016–7021

Ura, H., Denno, R., Hirata, K., Yamaguchi, K., Yasoshima, T. 1998. Separate functions of α2β1 and α3β1 integrins in the metastatic process of human gastric carcinoma. *Surg. Today* **28:**1001–1006

Varner, J.A., Cheresh, D.A. 1996. Integrins and cancer. *Curr. Opin. Cell Biol.* **8:**724–730

Wayner, E.A., Carter, W.G. 1987. Identification of multiple cell adhesion receptors for collagen and fibronectin in human fibrosarcoma cells possessing unique α and common β subunits. *J. Cell Biol.* **105:**1873–1884

Wayner, E.A., Carter, W.G., Piotrowicz, R.S., Kunicki, T.J. 1988. The function of multiple extracellular matrix receptors in mediating cell adhesion to extracellular matrix: preparation of monoclonal antibodies to the fibronectin receptor that specifically inhibit cell adhesion to fibronectin and react with platelet glycoproteins Ic-IIa. *J. Cell Biol.* **107:**1881–1891

Wei, Y., Eble, J.A., Wang, Z., Kreidberg, J.A., Chapman, H.A. 2001. Urokinase receptors promote β1 integrin function through interactions with integrin α3β1. *Mol. Biol. Cell* **12:**2975–2986

Weitzman, J.B., Pasqualini, R., Takada, Y., Hemler, M.E. 1993. The function and distinctive regulation of the integrin VLA-3 in cell adhesion, spreading, and homotypic cell aggregation. *J. Biol. Chem.* **268:**8651–8657

Wixler, V., Geerts, D., Laplantine, E., Westhoff, D., Smyth, N., Aumailley, M., Sonnenberg, A., Paulsson, M. 2000. The LIM-only protein DRAL/FHL2 binds to the cytoplasmic domain of several α and β integrin chains and is recruited to adhesion complexes. *J. Biol. Chem.* **275:**33669–33678

Wu, C., Chung, A.E., McDonald, J.A. 1995. A novel role for α3β1 integring in extracellular matrix assembly. *J. Cell Sci.* **108:**2511–2523

Xiong, J.P., Stehle, T., Diefenbach, B., Zhang, R., Dunker, R., Scott, D.L., Joachimiak, A., Goodman, S.L., Arnaout, M.A. 2001. Crystal structure of the extracellular segment of integrin αVβ3. *Science* **294:**339–345

Xiong, J.P., Stehle, T., Zhang, R., Joachimiak, A., Frech, M., Goodman, S.L., Arnaout, M.A. 2002. Crystal structure of the extracellular segment of integrin αVβ3 in complex with an Arg-Gly-Asp ligand. *Science* **296:**151–155

Yamamoto, H., Oviedo, A., Sweeley, C., Saito, T., Moskal, J.R. 2001. α2, 6-sialylation of cell-surface N-glycans inhibits glioma formation in vivo. *Cancer Res.* **61:**6822–6829

Yanez-Mo, M., Alfranca, A., Cabanas, C., Marazuela, M., Tejedor, R., Ursa, M.A., Ashman, L.K., de Landazuri, M.O., 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

Yauch, R.L., Berditchevski, F., Harler, M.B., Reichner, J., 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., Hemler, M.E. 2000. Direct extracellular contact between integrin $\alpha_3\beta_1$ and TM4SF protein CD151. *J Biol. Chem.* **275:**9230–9238

Yoshimasu, T., Sakurai, T., Oura, S., Hirai, I., Tanino, H., Kokawa, Y., Naito, Y., Okamura, Y., Ota, I., Tani, N., Matsuura, N. 2004. Increased expression of integrin α3β1 in highly brain metastatic subclone of a human non-small cell lung cancer cell line. *Cancer Sci.* **95:**142–148

Zhang, K., Kramer, R.H. 1996. Laminin 5 deposition promotes keratinocyte motility. *Exp. Cell Res.* **227:**309–322

Zhang, X.P., Puzon-McLaughlin, W., Irie, A., Kovach, N., Prokopishyn, N.L., Laferte, S., Takeuchi, K., Tsuji, T., Takada, Y. 1999. α3β1 adhesion to laminin-5 and invasin: critical and differential role of integrin residues clustered at the boundary between α3 N-terminal repeats 2 and 3. *Biochemistry* **38:**14424–14431

Zhang, X.A., Bontrager, A.L., Stipp, C.S., Kraeft, S.K., Bazzoni, G., Chen, L.B., Hemler, M.E. 2001a. Phosphorylation of a conserved integrin α3 QPSXXE motif regulates signaling, motility, and cytoskeletal engagement. *Mol. Biol. Cell* **12:**351–365

Zhang, X.A., Bontrager, A.L., Hemler, M.E. 2001b. Transmembrane-4 superfamily proteins associate with activated protein kinase C (PKC) and link PKC to specific $\beta_1$ integrins. *J. Biol. Chem.* **276:**25005–25013

Zhang, F., Tom, C.C., Kugler, M.C., Ching, T.T., Kreidberg, J.A., Wei, Y., Chapman, H.A. 2003a. Distinct ligand binding sites in integrin α3β1 regulate matrix adhesion and cell-cell contact. *J. Cell Biol.* **163:**177–188

Zhang, X., Cromwell, J.W., Kunjummen, B.D., Yee, D., Garcia-Aguilar, J. 2003b. The α2 and α3 integrins are required for morphologic differentiation of an intestinal epithelial cell line. *Surgery* **133:**429–437

Zuk, A., Matlin, K.S. 2002. Induction of a laminin isoform and $\alpha_3\beta_1$-integrin in renal ischemic injury and repair in vivo. *Am. J. Physiol.* **283:**F971–984