Functional Consequences of Integrin Gene Mutations in Mice

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Abstract—Integrins are cell-surface receptors responsible for cell attachment to extracellular matrices and to other cells. The application of mouse genetics has significantly increased our understanding of integrin function in vivo. In this review, we summarize the phenotypes of mice carrying mutant integrin genes and compare them with phenotypes of mice lacking the integrin ligands. (Circ Res. 2001;89:211-223.)

Key Words: integrin ■ knockout ■ extracellular matrix

Cell-cell and cell-matrix interactions play essential roles in development, tissue function, and repair. During morphogenesis and tissue homeostasis, specific signals emerge from the environment, cross the cell membrane, and control many aspects of cell behavior, including migration, proliferation, survival, and differentiation. Integrins are cell adhesion molecules that can transmit such signals. They are heterodimeric transmembrane glycoproteins, composed of noncovalently-associated α and β subunits. To date, 24 integrin receptors assembled from various combinations of 18 α and 8 β chains have been described (Figure 1). The extracellular domain of integrins binds the cognate ligand, whereas the cytoplasmic domain is linked to the actin cytoskeleton. Integrins can bind extracellular matrix (ECM) proteins, such as collagens, fibronectins, and laminins, and cellular receptors, such as vascular cell adhesion molecule-1 (VCAM-1) and the intercellular cell adhesion molecule (ICAM) family.1 Certain integrins share the same extracellular ligand, but on the other hand, several ligands are recognized by the same integrin. Ligand binding is followed by recruitment of several signaling proteins to the integrin and initiation of a signaling cascade that modulates cell behavior and gene transcription.2,3

During the last two decades, most of the information about integrin function has been derived from in vitro cell culture systems. Gene targeting technology recently made it possible to generate mice that lack specific integrins in a constitutive or cell type–specific manner. Analyses of these mice demonstrate how integrin-mediated adhesion and signal transduction affect development and maintenance of tissues and provide additional insight into integrin function in various diseases. In this review, we summarize the phenotypes of mice carrying mutant integrin genes and compare them with the relevant genetic models bearing mutations in some integrin ligands (Table).

Early Mouse Development
Ablation of integrin genes leads to various phenotypes during mouse development, ranging from apparently normal mice to early lethality (Figure 2). For example, disruption of the ubiquitously expressed β1 integrin gene leads to the loss of at least 12 different integrin receptors and results in peri-
implantation lethality characterized by an inner cell mass (ICM) failure.\textsuperscript{4,5} Fertilization of $\beta_1$-null oocytes and the entire preimplantation development is normal. This is most likely because of the presence of maternal $\beta_1$ integrin mRNA and protein.\textsuperscript{6} A possible explanation of the ICM failure could be the loss of $\beta_1$-mediated survival signals. At peri-implantation stage, the ICM cells facing the blastocyst cavity differentiate into primitive endodermal cells and lay down a basal membrane (BM). This leads to polarization of adjacent ICM cells and their differentiation into ectodermal cells. The inability of the ectodermal cells to interact with BM components through integrins could lead to their loss by apoptosis and arrest of additional development.\textsuperscript{7} Another possibility could be that the lack of $\beta_1$ integrins leads to abnormal BM assembly. $\beta_1$ integrin is crucial for normal expression and correct assembly of BM components into a supramolecular structure.\textsuperscript{8-12} The complete absence of a BM is observed in mice carrying a null mutation in the laminin $\gamma_1$ chain gene.\textsuperscript{13} Laminins are heterotrimeric glycoproteins composed of $\alpha$, $\beta$, and $\gamma$ subunits. Laminin $\gamma_1$-null mice fail to develop a BM between primitive endoderm and ICM, and, like the $\beta_1$-null mice, they die at the peri-implantation stage.\textsuperscript{13} A recent study demonstrated the role of the BM in regulating apoptosis and cavity formation using embryoid bodies derived from laminin $\gamma_1$-null embryonic stem (ES) cells.\textsuperscript{14} In laminin $\gamma_1$-null embryoid bodies, the ectoderm never polarizes and the inner cells never apoptose to form cavities. An identical phenotype is observed in $\beta_1$-null embryoid bodies (R. Fässler, unpublished data, 1995).

Deletion of the integrin $\alpha_5$ gene leads to an embryonic lethality around embryonic day 9.5 (E9.5) and E10 with variable degrees of embryonic and extra-embryonic defects that partly depend on the genetic background.\textsuperscript{15,16} $\alpha_5\beta_1$ plays a role in neural crest survival and maintenance of mesoderm derivatives after the gastrulation stage but is dispensable for initial commitment of mesodermal cells.\textsuperscript{17} Knockout of the $\alpha_5\beta_1$ ligand fibronectin (FN) results in a more severe phenotype, suggesting that other FN-binding integrins compensate in the $\alpha_5$-null mouse. Fibronectin-null embryos implant and initiate gastrulation normally. Shortly thereafter, they show defects in neural tube closure, in the cardiovascular system, and in somite formation and die at E8.5.\textsuperscript{18}

$\alpha_5$-null embryos fail to fuse the allantois with the chorion during placentation, show defects in the heart and vasculature, and display abnormalities in the cranial and facial structure.\textsuperscript{19} $\alpha_5$-null embryos die at two different stages during development. The first wave is around E9.5 to E11.5 and is caused by the failure of allantois-chorion fusion, and the second is between E11.5 and E14 and is caused by massive heart hemorrhage. $\alpha_5\beta_1$ Integrin can bind fibronectin and VCAM-1. The mutant phenotype is mainly attributable to a defect in VCAM-1 binding, because mice lacking VCAM-1 also fail to fuse the allantois with the chorion and have cardiac defect similar to $\alpha_5$-null mice.\textsuperscript{20,21}

$\alpha_6$ Integrins can associate with $\beta_1$, $\beta_4$, $\beta_5$, $\beta_6$, and $\beta_8$ subunits. They bind to both fibronectin and vitronectin, except for $\alpha_5\beta_1$ integrin, which binds to fibronectin, vitronectin, and tenascin-C, and $\alpha_6\beta_4$, which binds only to fibronectin.\textsuperscript{1} Several reports have shown that $\alpha_5\beta_1$ and $\alpha_6\beta_4$ integrins are crucial for tumor angiogenesis.\textsuperscript{22} Therefore, it came by surprise that $\alpha_5$-null mice, which lack both of these receptors, have no defects in vasculogenesis and angiogenesis. Approximately 80% of the $\alpha_5$-null mice die between E10 and E12 because of a placenta defect characterized by a poorly developed labyrinthine zone and reduced interdigitation of fetal and maternal vessels. The remaining 20% die at birth from massive hemorrhages and cleft palates.\textsuperscript{23} Because vitronectin, tenascin-C, and osteopontin-null mice have no obvious phenotype during the early mouse development,\textsuperscript{24} the $\alpha_5$-null phenotype is most likely attributable to a lack of fibronectin binding.

A recent study investigated $\alpha_5^{-/-}$/$\alpha_6^{-/-}$ double knockouts, which lack several major fibronectin receptors. The mutants die between E7.5 and E8 and display a severe gastrulation defect, with a lack of anterior mesoderm.\textsuperscript{16} It is noteworthy that this phenotype is even more severe than that observed in the fibronectin-null mutants, suggesting the presence of additional ligands for these two receptors at this developmental stage.

**Hematopoiesis**

Hematopoietic stem cells (HSCs) and endothelial cells are derived from a common precursor cell called hemangioblast. HSCs are generated outside the embryo proper, in the yolk sac (YS), and within the embryo in the para-aortic splanchnopleura/aorta-gonad mesonephros region. At around E8.5, when circulation starts in mouse, the HSCs are present in the fetal blood, and at E10 they start to colonize the fetal liver. Later, hematopoietic progenitors seed the thymus, spleen, other lymphoid organs, and bone marrow. In the adult mouse, HSCs reside in the bone marrow, but differentiated leukocytes circulate through the body and extravasate into infected tissues. Because of the early embryonic lethality of integrin $\beta_1$-null mice, chimeric mice have been used to analyze $\beta_1$ function in the hematopoietic system. These mice revealed that $\beta_1$ integrin is not required for the generation of progenitor cells in the para-aortic splanchnopleura/aorta-
gonad mesonephros region and the YS but is essential for them to colonize the fetal liver. Differentiation of \( \beta_1 \)-null precursor cells in the presence of cytokines in vitro and in reaggregated fetal organ cultures of liver and thymus is normal, indicating that \( \beta_1 \) integrins are crucial for the migration of HSCs into, but not for their development within, hematopoietic organs. Also, the homing of adult HSCs to the bone marrow is critically dependent on \( \beta_1 \) integrin expression. Accumulation of transferred \( \beta_1 \)-null precursor cells in the blood in vivo and reduced adhesion of other cells to endothelioma cells in vitro suggests that \( \beta_1 \) integrin is essential for the adhesion of HSC to the vessel wall. Whether it plays an additional role at later processes (transmigration through the endothelium or extravascular migration) is not clear. Mouse HSCs express \( \alpha_4 \beta_1 \), \( \alpha_5 \beta_1 \), and \( \alpha_6 \beta_1 \) integrin. Because individual knockouts of these \( \alpha \) subunits do not interfere with HSC homing, either a combination of these receptors or another integrin of \( \beta_1 \) family mediates HSC migration to the bone marrow.

Deletion of the \( \beta_7 \) integrin subunit, which can associate with \( \alpha_4 \) and \( \alpha_6 \), results in defective migration of T cells to Peyer’s patches and a reduced number of lamina propria and intraepithelial lymphocytes. Migration of lymphocytes to the Peyer’s patches is mediated by \( \alpha_4 \beta_7 \) binding to the endothelial MadCAM-1 receptors on the high endothelial venules in the gut. This was confirmed with mice lacking MadCAM-1 because of ablation of the transcription factor NKX2.3 gene, which controls MadCAM-1 expression. These mice have very small Peyer’s patches only visible after microscopic inspection. Targeted disruption of the \( \alpha_E \) gene leads to a reduction of T cells in lamina propria and gut epithelium but not in Peyer’s patches. Mice lacking both \( \beta_7 \) integrin and L-selectin suffer from a nearly complete impairment of lymphocyte migration to mesenterial lymph nodes. Because of the early embryonic lethality of \( \alpha_4 \)-null mice, the function of \( \alpha_4 \) integrin in the hematopoietic system was studied in \( \alpha_4 \)-null chimeric mice. The \( \alpha_4 \) subunit can associate with \( \beta_1 \) and \( \beta_7 \) integrin and is important for the differentiation

### Ablation of Integrin Genes in Mice and Their Resulting Phenotypes In Vivo

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<thead>
<tr>
<th>Gene</th>
<th>Phenotype</th>
<th>References</th>
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<tbody>
<tr>
<td>( \alpha_1 )</td>
<td>V, F</td>
<td>Increased collagen synthesis, reduced tumor vascularization</td>
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<tr>
<td>( \alpha_2 )</td>
<td>...</td>
<td>Not reported</td>
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<tr>
<td>( \alpha_3 )</td>
<td>L, birth</td>
<td>Defects in kidneys, lungs, and cerebral cortex; skin blistering</td>
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<tr>
<td>( \alpha_4 )</td>
<td>L, E11–E14</td>
<td>Defects in choioallantois fusion and cardiac development</td>
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<tr>
<td>( \alpha_5 )</td>
<td>L, E10</td>
<td>Defects in extraembryonic and embryonic vascular development</td>
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<tr>
<td>( \alpha_6 )</td>
<td>L, birth</td>
<td>Defects in cerebral cortex and retina; skin blistering</td>
</tr>
<tr>
<td>( \alpha_7 )</td>
<td>V, F</td>
<td>Muscular dystrophy</td>
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<tr>
<td>( \alpha_8 )</td>
<td>L+/V/F</td>
<td>Small or absent kidneys; inner ear defects</td>
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<td>( \alpha_9 )</td>
<td>L, perinatal</td>
<td>Bilateral chylothorax</td>
</tr>
<tr>
<td>( \alpha_{10} )</td>
<td>V, F</td>
<td>No apparent phenotype</td>
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<tr>
<td>( \alpha_{11} )</td>
<td>...</td>
<td>Not reported</td>
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<tr>
<td>( \alpha_{12} )</td>
<td>L, E12–birth</td>
<td>Defects in placenta and in CNS and GI blood vessels; cleft palate</td>
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<td>( \alpha_{13} )</td>
<td>...</td>
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<tr>
<td>( \alpha_{14} )</td>
<td>V, F</td>
<td>Inflammatory skin lesions</td>
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<td>( \alpha_{15} )</td>
<td>V, F</td>
<td>Defective platelet aggregation</td>
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<tr>
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<td>L, E5.5</td>
<td>Inner cell mass deterioration</td>
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<td>( \beta_2 )</td>
<td>V, F</td>
<td>Impaired leukocyte recruitment; skin infections</td>
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<td>V, F</td>
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<tr>
<td>( \beta_6 )</td>
<td>V, F</td>
<td>Skin and lung inflammation and impaired lung fibrosis</td>
</tr>
<tr>
<td>( \beta_7 )</td>
<td>V, F</td>
<td>Abnormal Peyer’s patches; decreased No. of intraepithelial lymphocytes</td>
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<tr>
<td>( \beta_{12} )</td>
<td>L, E12–birth</td>
<td>Defects in placenta and in CNS and GI blood vessels; cleft palate</td>
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V indicates viable; F, fertile; L, lethal; L+/V/F, mutations that disrupt development but also allow survival in a fraction of mice; CNS, central nervous system; GI, gastrointestinal; and PMN, polymorphonuclear neutrophil.
of erythroid, myeloid, and B cell progenitors during embryogenesis. Because in vitro differentiation in colony assays is normal, the defects observed in vivo are quite likely attributable to impaired interaction of the α5-null progenitors with the microenvironment in the hematopoietic organs. Furthermore, no α5-null erythrocytes, almost no B lymphoid cells, and only a few myeloid cells are present in adult chimeric mice, indicating an important role of α5 integrins in the postnatal hematopoietic development.

The underlying mechanism proposed for this severe phenotype is an important role for the α5 integrin for transmigration of progenitors through the stroma. In addition, proliferation of α5-null precursor cells might be decreased, because α5-deficient preB cells that did transmigrate in vitro showed a poor mitotic activity. Similar to β2-deficient mice, the contribution of α5-null T cells to Peyer’s patches is impaired. α5-Null chimeras that are older than 1 month after birth lack α5-deficient thymocytes in the thymus, although T cell precursors are present in the bone marrow. The few peripheral α5-null T cells present show a reduced migration into the inflamed peritoneum. Because mice with a disrupted VCAM-1 gene have normal hematopoiesis, the α5-null phenotype is most likely attributable to impaired interactions of α5β1 with fibronectin. Mice with a conditional inactivation of the VCAM-1 gene show reduced number of mature B cells in the BM attributable to a defective migration of lymphocyte to the BM. In addition, T cell–dependent immune response is impaired.

Integrins play a central role in leukocyte adhesion and migration. Leukocytes express several integrins in a low-affinity state, ensuring that they do not make inappropriate interactions with ligands normally present in blood or on endothelial cells. To leave the blood, leukocytes attach loosely to activated endothelium via selectins and begin to roll. Chemokines released by the endothelium or the surrounding tissue shift the leukocyte integrin into a high-affinity state that allows binding to endothelial cell adhesion molecules such as ICAMs and VCAM-1, resulting in firm adhesion of the blood cells to the vessel wall. Finally, the leukocytes cross the endothelial cell layer and underlying BM and adhere and migrate within the extravascular tissue.

β2 integrin is specifically expressed on leukocytes and associates with α4 (lymphocyte function–associated antigen-1 [LFA-1]), α5 (Mac-1), α6, and α9 subunits. Important counter-receptors of β2 integrins are members of the ICAM family. However, Mac-1, α5β2, and α6β2 also bind to other proteins: Mac-1 to inactivated complement factor (iC3b), fibrinogen, factor X, α5β2 to iC3b and type I collagen, and α6β1 to VCAM-1. In addition, Mac-1 and α5β2 can interact specifically with various polysaccharides, such as β-glucan, mannose, N-acetyl-D-glucosamine, and glucose, which mediate attachment to bacteria.

Humans with a mutation in the β2 gene develop leukocyte adhesion deficiency type I. These patients have a massive leukocytosis, recurrent bacterial infections, impaired wound healing, and severe gingivitis. Almost no neutrophils extravasate into infected tissues. Mice lacking β2 integrin display a very similar phenotype. The degree of neutrophil extravasation, however, is variable and depends on the model of inflammation. Some neutrophil extravasation processes like the croton oil-induced migration into skin are completely abolished, whereas others, like the sterile peritonitis, still occur. These migrations are perhaps mediated by β1 integrins. In chronic inflammations, α6β1 integrin seems to play an important role. Another candidate is α9β6, which is highly expressed on human neutrophils and involved in transendothelial migration in vitro. β5 integrin also plays an important role in T cell proliferation and contributes to the accumulation of dendritic cells in the lung.

LFA-1 and, to a lesser degree, Mac-1 contribute to neutrophil adhesion to endothelium. In a tumor necrosis factor (TNF)-induced air pouch inflammation model, neutrophil migration was equally reduced in β2-deficient and LFA-1-deficient mice, whereas mice lacking Mac-1 showed a marked increase of neutrophil extravasation. In the absence of LFA-1, immune responses against systemic viral infections are normal, but alloantigen triggered T cell proliferation and cytotoxicity are severely impaired, leading to defective host-versus-graft reaction and abrogated tumor rejection. LFA-1 is also involved in the homing of lymphocytes to peripheral lymph nodes and, to a lesser degree, to mesenteric lymph nodes and Peyer’s patches. Mac-1–null neutrophils have an impaired phagocytosis and degranulation, leading to...
a delayed neutrophil apoptosis.\textsuperscript{54,55} Furthermore, mast cells were reduced in some but not all locations of Mac-1–
deficient mice.\textsuperscript{56} In an acute glomerulonephritis model, Mac-
1–null neutrophils extravasated normally but did not stay in the
tissue, apparently because of an absent cytoskeletal
rearrangement mediated by Mac-1.\textsuperscript{57} Unexpectedly, mice
lacking Mac-1 or the Mac-1 ligand ICAM-1 are obese, suggesting a role of Mac-1 in the regulation of fat metabo-

Vasculogenesis and Angiogenesis

Studies of mice carrying integrin-null mutations support the
concept that endothelial integrins are involved in the forma-
tion and maintenance of the vascular network. Endothelial
cells express at least 11 different integrins, including \(\alpha_\text{IIb}\beta_1\),
\(\alpha_\text{IIb}\beta_2\), \(\alpha_\text{IIb}\beta_3\), \(\alpha_\text{Iib}\beta_3\), \(\alpha_\text{IIb}\beta_1\), \(\alpha_\text{IIb}\beta_1\), \(\alpha_\text{IIb}\beta_2\), \(\alpha_\text{IIb}\beta_3\), \(\alpha_\text{IIb}\beta_1\), \(\alpha_\text{IIb}\beta_1\), and \(\alpha_\text{IIb}\beta_3\).

The most severe vascular defect is present in mice lacking the
\(\alpha_\text{v}\) subunit. They have impaired development of the
extraembryonic (YS) and embryonic (heart and aorta) vascu-
lature.\textsuperscript{15} Blood islands form, and hematopoiesis occurs in the
YS. Subsequently, the mesoderm and endoderm layers are
separated, and the primitive blood cells lie in sacs between the
two germ layers and leak into the exocoelomic space. This
splitting of the germ layers could be explained by defective
cell-matrix interaction or mesoderm defects. All \(\alpha_\text{v}\)-null
embryos form heart and blood vessels that contain a few
primitive blood cells. The vessels, however, are distended and
leak. A very similar but more severe vascular phenotype is
present in mice lacking fibronectin,\textsuperscript{18,70} the major ligand of
\(\alpha_\text{IIb}\beta_3\). The fibronectin mutants display variable cardiovascular
defects depending on the genetic background.\textsuperscript{70} On the
C57BL/6 background, the defects are less severe, whereas on the
129/Sv background, no hearts have properly formed
vessels. In C57BL/6 mutants that form hearts, the myocardial
and endocardial cells are disorganized, resulting in a bulbous
heart tube. The aorta contains endothelial cells that lack
contact with the surrounding mesenchyme, whereas the yolk
sac is completely devoid of vessels. On a 129/Sv background,
endothelial cells differentiate but fail to assemble into vessels.
These findings clearly suggest that \(\alpha_\text{IIb}\beta_3\) is not required for
initial vasculogenesis but is necessary for the proper forma-
tion and maintenance of blood vessels. Fibronectin, on the
other hand, seems necessary for vasculogenesis, suggesting
that in the absence of \(\alpha_\text{v}\), other fibronectin receptors can
compensate. Similar to the \(\alpha_\text{v}\)– and fibronectin-null mice, mice
lacking vascular endothelial growth factor (VEGF)-R1 (flt-1) have normal hematopoietic progenitors but show
impaired blood vessel formation.\textsuperscript{71} Mice lacking the tie-2
receptor\textsuperscript{72} or its ligand angiopoietin-1\textsuperscript{73} show defects in the
interactions of endothelial cells with the surrounding matrix
and mesenchyme. These abnormalities are reminiscent of
those observed in \(\alpha_\text{v}\)- and fibronectin-deficient mice, suggest-
ing that these signaling systems cooperate in blood vessel
assembly.

Null mutations of other \(\alpha\) subunits associated with \(\beta_3\) show
milder or no obvious vascular defects. Mice lacking integrin
\(\alpha_\text{v}\) die during embryonic development because of defects in
heart and placenta.\textsuperscript{19} In addition, these mutants lack coronary
vessels.\textsuperscript{19} Because epicardial coronary vessels connect with
those in the myocardium, blood from the myocardial vessels
leaks, resulting in cardiac hemorrhage in the \(\alpha_\text{v}\)-deficient
mice. It is not clear, however, if this is a direct consequence
of the loss of \(\alpha_\text{v}\), or an indirect secondary defect attributable to
a loss of the epicardium. A vascular abnormality is also
present in \(\alpha_\text{v}\)-null mice. Their glomerular capillaries show
reduced branching and a distended lumen.\textsuperscript{74} Glomerular
endothelial cells express \( \alpha_\beta_1 \), and, therefore, the defect could result from an abnormal formation of capillary structure of the glomerulus. It is also possible that the altered capillaries are caused by the failure of podocytes to provide adequate scaffolding, within which the newly formed capillaries would form. Mice lacking the \( \alpha_1 \) subunit show normal development of the blood vessel system but have a reduced tumor angiogenesis. This reduction in tumor capillary numbers is caused by overexpression of metalloproteinase 7 (MMP-7) and MMP-9 in the mutants leading to elevation of angiotatin that has been shown to inhibit endothelial cell proliferation in tumors but not during embryogenesis. Isolated \( \alpha_\beta_1 \)-null endothelial cells from adult lung, however, show a reduced proliferation on both \( \alpha_\beta_1 \)-dependent and -independent substrata, indicating that this response is not restricted to tumor endothelial cells. The formation of a normal embryonic vasculature suggests that there are phenotypic differences between embryonic versus adult and tumor endothelial cells. These data demonstrate that \( \alpha \)-integrins regulate MMP expression and that a tightly controlled MMP expression is crucial for angiogenesis. Although MMP synthesis is essential for new blood vessel formation by degrading the ECM and thus facilitating remodelling and invasion, an increased MMP expression can also lead to the release of factors that inhibits cell growth, such as angiotatin.

Endothelial cells express both \( \alpha_\beta_1 \) and \( \alpha_\beta_3 \) integrins. Mice lacking \( \alpha_\beta_1 \) or \( \beta_1 \) integrin show no obvious vascular defects. Other members of the integrin family or \( \alpha \)-dystroglycan may compensate for the loss of \( \alpha_\beta_1 \) function. Mice lacking \( \alpha \)-dystroglycan die before the first vessels form, and, therefore, only tissue-specific gene inactivation of the \( \alpha \)-dystroglycan gene will reveal whether it has a function during vessel formation. Targeted null mutation of the \( \beta_1 \) integrin gene leads to embryonic lethality before the vascular system begins to form. With the use of \( \beta_1 \)-null ES cells, however, it is possible to demonstrate an important role for the \( \beta_1 \) subfamily during blood vessel formation. Chimeric mice derived from \( \beta_1 \)-null ES cells lack \( \beta_1 \)-null endothelial cells in liver, indicating that endothelial cells either fail to form or fail to participate in blood vessel formation. In vitro differentiation of \( \beta_1 \)-null ES cells into embryoid bodies show that endothelial cells differentiate but initiation of vasculoigenesis is significantly delayed. In addition, the responsiveness to VEGF is impaired, suggesting that VEGF action is upstream of \( \beta_1 \) integrin function. Finally, in \( \beta_1 \)-null teratomas, the \( \beta_1 \) mutant ES cells fail to participate in tumor vessel formation, which is in sharp contrast to wild-type ES cells.

Endothelial cells express at least 4 members of the \( \alpha \)-integrin subfamily, \( \alpha_\beta_1 \), \( \alpha_\beta_2 \), \( \alpha_\beta_3 \), and \( \alpha_\beta_6 \). Numerous studies using blocking antibodies suggested that this family of integrins is of crucial importance for angiogenesis, as shown in chorioallantoic membrane assays and tumor models. and neovascularization studies of the mouse retina. The generation and analyses of \( \alpha_\beta_1 \)-null mice, however, revealed that 20% of the mutants are born alive with no defects in vasculoigenesis and angiogenesis. Basic endothelial processes of proliferation, migration, tube formation, branching, and basement membrane assembly occur in the mutants. The intracerebral and intestinal vasculature, however, becomes distended and eventually ruptures, resulting in severe hemorrhage. Interestingly, \( \beta_1 \)-null mutant mice share many similarities to those of the \( \alpha_\beta_1 \) mutants, with early defects in placental development and later deficits in formation and stabilization of the vascularization of the central nervous system. Mice lacking \( \beta_1 \) or \( \beta_6 \) two of the \( \alpha_\beta_1 \)-associated \( \beta \) subunits, are viable and show no obvious defects in vascular development. In addition, null mutants of many \( \alpha \) ligands, including vitronectin, tenascin-\( \alpha \)-C, osteopon- tin, 89 fibrinogen, perlecan, and von Willebrand factor show no defects in vessel formation.

**Skin**

The skin is composed of an epidermal and a dermal layer, separated by a basement membrane. The epidermis is made of keratinocytes at different stages of differentiation. The proliferating basal keratinocytes adhere to the basement membrane via specialized adhesion junctions called hemidesmosomes, containing \( \alpha_\beta_4 \) integrin, and via other ECM receptors, including \( \alpha_\beta_1 \) integrin and \( \alpha \)-dystroglycan. Both \( \alpha_\beta_1 \) and \( \alpha_\beta_3 \) integrins bind laminin 5 (\( \alpha_\beta_2 \gamma_1 \)) of the BM. During differentiation, the keratinocytes lose their integrin expression, detach from the basement membrane, move to the suprabasal layers, and terminally differentiate to form the stratum corneum. Mutations in the \( \alpha_\beta_1 \) or the \( \beta_1 \) gene result in a reduction of hemidesmosomes, causing the epidermolysis bullosa in humans, a severe blistering disease leading in most cases to early postnatal lethality. Deletion of the \( \alpha_\beta_1 \) or the \( \beta_1 \) gene in mice leads to a complete absence of hemidesmosomes, a severe detachment of the epidermis and death shortly after birth. Interestingly, the basal lamina is not affected by the loss of \( \alpha_\beta_3 \) integrin.

\( \alpha_\beta_4 \)-Null mice die shortly after birth because of defects in kidney and lung organogenesis. These mice also display mild blisters caused by disruption of the basement membrane organization. Keratinocyte-restricted disruption of the \( \beta_1 \) integrin gene during early development causes a similar but more severe phenotype with death shortly after birth, suggesting that \( \alpha_\beta_4 \) or \( \alpha_\beta_6 \), which both are expressed in skin, play an additional role in basement membrane formation and skin homeostasis. Skin-specific ablation of the \( \beta_1 \) integrin gene at around birth demonstrated a role of \( \beta_1 \) integrin in the processing of the BM components and in the growth and maintenance of hair follicles. \( \beta_1 \)-deficient basal keratinocytes have an aberrant morphology and a reduced proliferation rate, but are still able to terminally differentiate. Interestingly, in the absence of both \( \alpha_\beta_4 \) or \( \beta_1 \) integrin, basal keratinocytes can initially attach to the basal lamina and proliferate, indicating additional adhesion mechanisms. A candidate receptor for maintaining proliferation could be \( \alpha \)-dystroglycan.

The critical importance of laminin-5 as a ligand for \( \alpha_\beta_4 \) integrin is demonstrated by patients with mutations in the laminin subchains \( \alpha_\beta_1 \), \( \beta_1 \), or \( \gamma_1 \), which develop lethal junctional epidermolysis bullosa. Similarly, mice lacking a functional laminin \( \alpha_\beta_4 \) gene have severe blistering and abnormal hemidesmosomes but an ultrastructurally normal basal
Adhesion experiments with laminin-5-deficient basement membrane membrane suggested that αβ1 also interacts with other basement membrane components, in contrast to αβ1 integrin.

The integrin αβ3, which interacts with fibronectin, vitronectin, and tenascin-C, is only expressed on epithelial cells during local injury or inflammation. Mice deficient for β3 integrin show normal embryonic development and wound healing.99 Surprisingly, however, mutant mice exhibit juvenile baldness attributable to macrophage infiltration of the dermis of the affected areas. Furthermore, β3-null mice demonstrate an increased airway responsiveness, a hallmark of asthma, and an infiltration of activated B and T cells into the lung. This phenotype was explained by the finding that αβ3 can bind to a latent form of the transforming growth factor-β1 (TGF-β1), inducing a conformational change in the ligand, which allows its interaction with TGF-β receptors. Therefore, loss of αβ3 reduces the amount of active TGF-β1 in epithelia, which at least partially mimics a local TGF-β1-null phenotype.100 Expression of αβ3 in injury and inflammation, therefore, leads to increased local activation of TGF-β1, which, among other effects, modulates inflammatory cell function.

Skeletal Muscle

The β1 subunit is expressed throughout skeletal muscle development. Initially, muscle precursor cells and fetal muscle express the cytoplasmic β1A splice variant. After birth, the β1A splice variant becomes substituted by the β1B variant.101 During early muscle development, β1A is expressed with α1, α5, α6, α7, αv, and α. With the exception of the α1 integrin, the other α subunits are downregulated around birth. In vitro studies using Drosophila cells revealed an important role for integrins in generating the sarcomeric cytoarchitecture. Normal Drosophila embryo cellsfuse and develop sarcomeres in which integrins and actins concentrate at Z-bands. In contrast, integrin-deficient fly cells fail to form sarcomeres.102 In mice, antibody perturbation experiments suggested an important role of β1 integrin for migration of muscle precursor cells from the dermomyotome to the limbs, fusion of myoblasts into primary and secondary myotubes, and assembly of the contractile proteins/apparatus.103–105 Interaction between αβ1 on the primary myotubes and VCAM-1 on the secondary myoblasts was suggested to be essential for the formation of the secondary myotubes.105 Such a function for α1 integrin, however, was neither confirmed in αβ1-null chimeric mice nor in αβ1-null embryoid bodies.14 In addition, no muscle phenotype was found in the few VCAM-1 null mice that survive the embryonic defects.20,21

αβ1 Integrin is expressed in adhesion plaque-like structures along the myotube during development106 but is downregulated in adult muscle. αβ3-Null mice die during early development before muscle is formed. αβ3-Null chimeras with a high contribution of αβ3-deficient cells to skeletal muscles develop typical alterations resembling muscular dystrophy, including giant muscle fibers, vacuoles, and centrally located nuclei.28 The cause of the muscle degeneration is reduced adhesion and survival of αβ3-null myoblasts.28

The most abundant integrin in skeletal muscle is α5β1, which is expressed during all stages of muscle development107,108 and interacts with laminin 2 (α5β1γ1) and laminin 4 (α5β1γ2) in skeletal muscle.109–111 α5 exists in two extra-cellular (X1 and X2) and three cytoplasmic (A, B, and C) splice variants, which display a tissue-specific and developmentally regulated expression pattern.112–115 Skeletal muscles express α5X3, α5BC, α5C, and α5X2, whereas α5A, α5X1, and α5X3 are found in the heart. After birth, α5X1 and α5X3 concentrate at neuromuscular and myotendinous junctions, whereas α5C is the predominant extrajunctional splice variant.107,113 Mice with a targeted deletion of the α5 integrin develop a progressive muscular dystrophy after birth. The major defect is severe disruption of the myotendinous junctions.116 Mutations in the α5 gene have also been identified in patients suffering from congenital myopathies characterized by delayed motor milestones.117 The genetic findings from mouse and human suggest no role for α5β1 in myogenesis but a maintenance function on the mature muscle by firmly linking the muscle fibers to the extracellular matrix. Muscle expresses laminin 4 that binds the α5β1 integrin. Naturally occurring mutations in the laminin α5 gene are found in humans and mouse (dydy mouse strain) and lead to a congenital muscular dystrophy118 resembling the phenotype produced by loss of α5 integrin expression.

These data demonstrate an important function of αβ1A and αβ1B integrins in the maintenance of muscles and show that they cannot compensate each other. However, in β1-null chimeric mice, β1-deficient myoblasts migrate, form myotubes, and develop a normal sarcomeric cytoarchitecture in β1-null chimeric mice.119 The formation of myotubes in β1-null embryoid bodies is delayed but otherwise normal.119,120 The absence of any obvious muscular dystrophy in the β1-null chimeric mice could be explained by both a low contribution of β1-null muscle cells and the presence of wild-type cells, which can functionally compensate. Replacement of the muscle-specific splice variant β1B by β1A in mouse strain) and lead to a muscular dystrophy121 No muscle phenotype has been reported from knockouts of α1, α5, α6, αv, αβ integrins, respectively.

Heart

Cardiomyocytes express several integrin subunits during development: β1A, β1D, α1, α3, α6, αv, αβ, and α. Like in the skeletal muscle, after birth, adult cardiac muscle expressed the β1D variant. Expression of α1, α5, and αβ is downregulated at birth. On the basis of in vitro analysis, integrin-mediated attachment to the ECM has been suggested to be important for controlling growth and differentiation of cardiomyocyte.122 Furthermore, integrins were proposed to function as mechanoreceptors that transform mechanical stimuli into biochemical signals that affect cellular function.122 Several genetic mouse models demonstrate an important function of β1 integrin in cardiac muscle in vivo. Mice expressing β1A instead of β1D in heart develop, without exposure to mechanical stress, mild cardiac defects characterized by an increase in atrial natriuretic peptide (ANP) synthesis. ANP is a vasorelaxant diminishing volume overload and hypertension. Increased levels of ANP have been correlated to, among other
things, reduced ventricular function and induction of ventricular hypertrophy. The increased expression of ANP suggests that the β1,α1 subunit, at least in the heart, is not fully compensating for β1,α0.121 Even more severe defects occur when both β1,α and β1,β are absent or functionally inactivated. The areas with β1-null cardiac muscle cells in the heart of β1-null chimeras become smaller with time and show signs of degeneration. Ultrastructural analysis revealed alterations in the sarcomeric architecture. In addition, transgenic mice expressing a dominant-negative form of β1 integrin, in which the extracellular and transmembrane domain of CD4 is fused to cytoplasmic domain of β1 integrin, show hypertrophic changes in the heart. Mice that express high levels of the transgene die around birth and display a replacement fibrosis.123 Overexpression of a constitutively active form of α5 integrin in the heart results in electrocardiographic abnormalities, cardiomyopathy, and death within one month after birth.124

Skeleton
The vertebrate skeleton develops via two distinct ways: by replacing cartilaginous precursors (endochondral ossification) or by direct development within a mesenchymal tissue (intramembranous ossification). The major cell types of the skeleton, the chondrocytes, the bone depositing osteoblasts, and the bone resorbing osteoclasts, express various integrin receptors at their surfaces. Despite numerous in vitro studies, their function in vivo during skeletogenesis is not well understood. Mice deficient in β1 integrin produce dysfunctional osteoclasts, leading to osteosclerosis characterized by increased bone mass.125 The mutant osteoclasts fail to form the proper ruffled membrane important for their resorptive function, leading to hypocalcemia in vivo and decreased resorption of whale dentin in vitro. Furthermore, β1-deficient osteoclasts do not spread in culture and lack the characteristic actin rings, suggesting that α,β1-mediated signals are important for the organization of cytoskeleton in these cells.

The most severe skeletal abnormalities have been identified in α5/α6 double-knockout mice.126 They display exencephaly attributable to the failure of neural tube closure, kinked tail, and limb anomalies characterized by shortening, abnormal shape, lack of digit separation, and fusion of phalanges between digits 2 and 3. Detailed analyses revealed discontinuity of the surface ectoderm of the distal limb and defective formation of a specialized limb bud epithelial structure, called apical ectodermal ridge (AER). AER is located at the tip of the bud and is involved in the control of limb outgrowth and differentiation. Both α1 and α6 integrin chains are expressed in this region, and a lack of both integrins results in structural disorganization and reduced proliferation of the ridge cells. Because single α5 or α6 mutants do not show such defects, the observations suggest that the presence of both integrin chains is crucial for the organization and proper function of AER and reveal the synergistic actions of these integrins in limb morphogenesis. Similar abnormalities of the distal limbs are present in the laminin α5 knockout mice.127 Altogether, these observations indicate that α5 and α6 integrins are the major cell-surface receptors for laminins containing α5 chains in the distal limb ectoderm.

Transgenic mice expressing a dominant-negative β1 integrin subunit under the control of the osteoblast-specific osteocalcin promoter show impaired membranous bone formation.128 The phenotype is characterized by a decreased rate of cortical bone formation and reduced bone mass of cortical and flat bones in young animals, indicating the importance of β1 integrin in osteoblast function. The defect of flat bones was normalized in older males but not in females, suggesting sex-specific differences in adult animals.

Kidney
So far, two integrins, αβ3 and αβ3, have been shown to be crucial for kidney development. In αβ3-/- mutant mice, the glomerular basement membrane of the kidney is disorganized and glomerular podocytes are unable to form mature foot processes, suggesting that αβ3 integrin might be important for basal membrane organization. In addition, these mutant mice have a lung defect with reduced branching of the conducting airway74 and show an alteration of the basal membrane organization of the submandibular gland.129 Inactivation of the αβ integrin leads to a failure in kidney development, with different levels of severity ranging from a reduction of kidney size to a complete loss of kidneys.130 The kidney defect is characterized by abnormal growth of the ureteric bud and abnormal branching and formation of the ureteric epithelium. Fibronectin, tenascin-C, and vitronectin bind to αβ. However, they seem not to be involved in the phenotype, because they are not detectably expressed during this stage of kidney development. Osteopontin can also bind αβ and has been proposed to mediate this interaction. Osteopontin-null mice, however, are normal and develop no apparent phenotype in the kidney.131,132 Most integrin αβ-null mice die soon after birth, but a few survive until adulthood. These mice are deaf and have balance defects attributable to a structural inner-ear defect, indicating a role of αβ integrin and its ligand fibronectin in hair-cell differentiation and function.133

Brain
Evidence coming from fly and worm suggest a critical role for the integrin family during brain development and maintaining brain function.134 This was also confirmed in mice. αβ-Null mice display a defect in neuron migration and a disorganized layering of the cerebral cortex, suggesting that this integrin is involved in radial neuronal cell migration.135 A very similar phenotype is observed in reeler mice, which lack the extracellular matrix protein reelin. In these mutant mice, migration of Cajal-Retzius cells is impaired, leading to an abnormal lamination of the cerebral cortex. A recent study showed that αβ integrin can bind reelin.136 This interaction may provide a stop signal and arrest neuronal migration.

αβ-Null mice have abnormalities in the laminar organization of the developing cerebral cortex and retina, ectopic neuroblastic outgrowth on the brain surface and in the eye, and an abnormal laminin deposition.137 In the αβ/αβ double-knockout mice, the cortical disorganization appears earlier and is more severe, likely because of an additive effect.
of the two mutations. Ablation of the laminin $\alpha_2$ gene leads also to a brain phenotype similar to the $\alpha_1^{-/-} \alpha_6^{-/-}$ double knockouts. In addition, the $\alpha_1^{-/-} \alpha_6^{-/-}$ mutants have several other abnormalities, including exencephaly, syndactyly, and kidney defects.126,127,138

Axotomy leads to an increased expression of $\alpha_6\beta_1$ integrin on axons and growth cones during peripheral nerve regeneration. An axonal reconnection has been reported in $\alpha_6$-null mutants, suggesting that this integrin plays a role in this process.139

**Signaling**

Integrin-mediated adhesion results in the activation of various signaling cascades inside the cell, including activation of tyrosine kinases like focal adhesion kinase (FAK) and Src, serine/threonine kinases like mitogen activated protein kinase (MAPK), and small G proteins.3,140 Direct evidence for signaling defects in integrin-deficient mice is scarce. In $\alpha_6$-null mice, a constitutive activation of c-Raf-1 kinase was observed in muscle, suggesting that $\alpha_6\beta_1$ integrin may be involved in the negative control of this pathway.141 However, this finding could also be caused indirectly by the muscle degeneration observed in these mice. Analysis of the inner ear development in $\alpha_6$-null mice revealed a defect in stereocilia formation correlating with a loss of FAK at the cell membrane.133

Cell cultures studies revealed that integrin-mediated signals are involved in cell proliferation, cell survival, prevention of apoptosis (also called anoikis), and cell differentiation.142 Presently, it is not clear to what degree the integrin signaling observed in vitro also has a role in vivo. The observations collected so far suggest an involvement of integrins in regulating cell proliferation and programmed cell death in vivo.

Several integrin-null mice show reduced proliferation of certain cell populations. Mice lacking $\alpha_6$ and $\alpha_6$ integrin have a reduction of cell proliferation in the AER,126 and mice lacking $\beta_1$ integrin in keratinocytes lose proliferating hair matrix cells and display a reduced proliferation of basal keratinocytes.10 Deletion of the $\alpha_c$ gene results in decreased proliferation of dermal fibroblasts.143 In vitro culture of these fibroblasts demonstrated a failure to recruit and activate the adaptor protein Shc, which triggers the MAPK activation cascade leading to cell proliferation. In a dominant-negative approach, the cytoplasmic and transmembrane domain of $\beta_1$ integrin was fused to the extracellular domain of CD4, which interferes with endogenous $\beta_1$ integrin function in vitro. Expression of such a chimeric protein in the mammary gland of transgenic mice resulted in decreased proliferation, increased apoptosis, and impaired differentiation of the mammary epithelial cells.144 Additional biochemical analysis of these mice revealed no change in FAK activation in the mammary gland but a significant decrease in the phosphorylation of Shc, Grb2, Akt, c-Jun N-terminal kinase, and extracellular signal–regulated kinase.145 Lack of the $\beta_1$ integrin cytoplasmic domain has been shown to reduce proliferation in the skin and intestine,146 which agrees with in vitro data showing that $\beta_1$ integrin is able to bind and activate Shc.

Cell survival defects have been reported in some integrin-null mice. For instance, $\alpha_5$ integrin was shown to be important for the survival of neural crest cells in vivo,17 and $\alpha_5^{-/-} \alpha_6^{-/-}$ mice have syndactyly resulting from an impaired cell death of interdigital cells.126 On the other hand, loss of $\beta_1$ integrin–mediated adhesion of basal keratinocytes does not result in cell death, in contrast to expectations from cell culture experiments.10

Concerning differentiation processes, so far gene ablation approaches revealed only a minor role for integrins. Absence of $\beta_1$ integrin does not interfere with differentiation of neurons and neural crest–derived cells,4 fetal hematopoietic cells,26 or keratinocytes.10 Double knockouts for $\alpha_5$ and $\alpha_6$ integrin show normal keratinocyte differentiation, despite the absence of the major integrin receptors $\alpha_6\beta_1$ and $\alpha_6\beta_1$.126 In vitro myoblast differentiation and fusion occurs independently of $\beta_1$ integrin but is delayed.119 A similar delay was found in blood vessel formation in $\beta_1$-null embryoid bodies.80 Such a delay could be attributable to the absence of $\beta_1$ integrin or the experimental design that does not reflect the in vivo conditions, eg, growth factors that are absent in fetal calf serum but not in vivo or absence of complex matrices in vitro.

On the other hand, teratomas using $\beta_1$-null cells never give rise to an endothelial cell population, suggesting that $\beta_1$ integrin could be involved during their differentiation or survival.80

**Future**

Analysis of integrin function by gene-targeted mice often confirmed but sometimes also contradicted previous results obtained by antibody or peptide inhibition studies. For instance, blocking $\alpha_6\beta_1$ function by inhibitory antibodies suggested an essential role of $\alpha_6\beta_1$ integrin in angiogenesis. However, both $\alpha_5$ and $\beta_1$ knockout mice show blood vessel formation. Similarly, a few $\alpha_5$ blocking antibodies prevent collagen-induced platelet aggregation, whereas deletion of $\beta_1$ integrin on platelets revealed only a minor and clearly dispensable role for $\alpha_6\beta_1$ during this process. One obvious disadvantage of antibodies is that they do not completely block protein function, whereas a targeted gene disruption leads to a complete loss of the protein. In addition, antibodies might have unexpected side effects by steric hindrance of other ligand-receptor interactions or only partial inhibition of the integrin signaling. However, constitutive gene knockout also has disadvantages. The mutated mouse might react to the loss of a protein by upregulating the activity or expression of other molecules, thus compensating the defect. Because totipotent embryonic cells, at least theoretically, have a high capacity for compensation, this risk is real in constitutive knockouts, and compensation has already been demonstrated on several occasions. A way to reduce the chance of compensation is to induce the knockout in more differentiated cells, which are less able to change their expression pattern. This is possible using the cre/loxP system generating mice with tissue restricted or inducible gene deletions.10,26,69,96 The generation of mice carrying floxed integrin genes to induce cell type–specific deletions, but also the introduction of subtle mutations into the extracellular and intracellular domain of integrin subunits and the interbreeding of integrin-null mice.
to create double and triple mutant animals, will additionally advance our understanding of how integrins function in vivo. In addition, the mutant mice will allow the establishment of cell lines that can be used to study in vivo observations at a molecular level.

More information about the role of integrins in signaling in vivo will come from the analysis of mutant tissues with antibodies specific for activated forms of signaling molecules, from knockin mice carrying subtle mutations of the integrin molecules interfering specifically with certain in vitro signaling functions, from knockouts of integrin-associated proteins, and from the rescue of integrin-null mice with transgenes encoding activated forms of signal transduction molecules suspected to act downstream of the respective integrins.

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