Integrin and Growth Factor Receptor Crosstalk

Brian P. Eliceiri

Abstract—Crosstalk between integrins and growth factor receptors are an important signaling mechanism to provide specificity during normal development and pathological processes in vascular biology. Evidence from several model systems demonstrates the physiological importance of the coordination of signals from growth factors and the extracellular matrix to support cell proliferation, migration, and invasion in vivo. Several examples of crosstalk between these two important classes of receptors indicate that integrin ligation is required for growth factor–induced biological processes. Furthermore, integrins can directly associate with growth factor receptors, thereby regulating the capacity of integrin/growth factor receptor complexes to propagate downstream signaling. Recent data suggest that antagonists of αv integrins can provide a therapeutic benefit in human cancer patients, whereas knockout mice lacking specific integrins can provide an interesting insight into the role of integrins during development. This review will focus on the biological importance of integrin and growth factor receptor crosstalk that occurs during cell growth, migration, and invasion as well as in endothelial cells during angiogenesis. (Circ Res. 2001;89:1104-1110.)

Key Words: integrins | growth factor receptors | cell migration/invasion | angiogenesis

Recent work from a number of laboratories has demonstrated that cell adhesion receptors and growth factor receptors are important molecular determinants in providing specificity for signaling during development and/or during pathological processes. Although integrins and growth factor receptors can independently propagate intracellular signals, the synergy of signals provided by the extracellular matrix (ECM) and growth factors appears to regulate complex processes, including blood vessel development during embryogenesis as well as tumor growth/metastasis and angiogenesis in the adult. Analysis of the crosstalk between the biochemical pathways mediated by integrins and growth factor receptors may ultimately lead to a better understanding of the cell biological processes underlying normal development and the progression of pathological conditions. Several excellent reviews on integrin-mediated1–4 and growth factor receptor–mediated signal transduction5–8 have been recently published; therefore, this review will focus on the evidence for crosstalk between integrins and growth factor receptors in cell biology. These in vitro studies help provide an important insight into the role of integrins in more complex biological questions in vascular biology. This will be followed by an analysis of the recent progress on growth factor–induced angiogenesis as a paradigm for the study of the crosstalk between growth factor receptors and integrins in intact tissues. Recent work provides clinical data on the therapeutic benefit of αv integrin antagonists, whereas studies of knockout mice, lacking various integrins, provide insight into the role of integrins during the embryonic development of the mouse vasculature. Model systems demonstrating the physiological relevance of growth factor receptors and integrins will be discussed at the level of biochemical signaling in the context of complex physiological processes such as cell migration, blood vessel development, and angiogenesis.
Integrins and the ECM

Cell adhesion to the ECM is mediated by integrins, a family of heterodimeric transmembrane proteins comprising at least 16 α and 8 β subunits in mammals. Different combinations of single α and β subunits dimerize to form at least 22 different receptors with distinct and often overlapping specificity for ECM proteins. The biological significance of the range of ECM-integrin specificities during cell adhesion has remained an important question. Although integrins support specific cell-ECM interactions for cell adhesion and migration, the identification of the underlying mechanisms by which specific subsets of integrins mediate distinct cellular processes such as during development, wound healing, cell invasion, or angiogenesis remains a challenge.1–3

In addition to the function of integrins in mediating cell adhesion, integrins have been widely recognized as important molecules in the transduction of positional cues from the ECM to the intracellular signaling machinery. For example, integrin ligation is known to induce a wide range of intracellular signaling events, including the activation of Ras; MAP kinase; focal adhesion kinase (FAK); Src; Rac, Rho, and cdc42 GTPases; phosphatidylinositol-3-kinase (PI3-kinase); Abl; and integrin-linked kinase.1,8–10 Furthermore, direct phosphorylation of integrin cytoplasmic tails can mediate platelet aggregation.11 Adapter proteins including CAS/Crk and She are important for coordinating intracellular signals during integrin-mediated cell migration.12,13 In addition, integrin ligation increases intracellular pH and Ca2+ levels, inositol lipid synthesis, cyclin synthesis, and the expression of immediate-early genes.4 Interestingly, many of the signaling pathways and effectors, which are activated by integrin ligation, are also activated after growth factor stimulation. This suggests that integrin- and growth factor–mediated cellular responses may synergize and may function to coordinate biochemical responses in multiple cell types.

Integrin and Growth Factor Receptor Crosstalk in Cultured Cells

Although integrins are responsible for mediating cell adhesion to the ECM, a role for growth factors in these integrin-dependent processes has been emerging gradually. Growth factor–induced cell proliferation, adhesion, and migration in cultured cell models often depend on specific integrins. For example, optimal cell stimulation with epidermal growth factor (EGF), platelet-derived growth factor (PDGF), insulin, or vascular endothelial growth factor (VEGF)14–17 depends on integrin-mediated cell adhesion to the appropriate ECM (reviewed in References 2 and 7). In smooth muscle cells, EGF- or insulin-like growth factor-1 (IGF-1)–stimulated smooth muscle cells depend on the integrin αβ3,18–20 whereas EGF-stimulated kidney epithelial cells depend on β1 integrins.21 The physiological importance of IGF-1/αβ3 crosstalk in smooth muscle cells is underscored by the reduction in atherosclerotic lesion size and IGF-1 signaling after treatment with αβ3 antagonists.22 Recently, VEGF has been shown to promote the adhesion and migration of cultured endothelial cells via integrins αβ3, αβ5, and β1.23 Interestingly, basic fibroblast growth factor (bFGF), but neither IGF nor PDGF, enhances endothelial cell adhesion and migration in vitro. Integrin αβ3 can also couple with thrombospondin by direct interactions with integrin-associated protein to mediate enhanced cell spreading on vitronectin.24 Thrombospondin and osteopontin can bind IGF-1 binding protein-5 to regulate IGF-1–induced cell growth.25 Models of shear stress in endothelial cells indicate that integrins and growth factor receptors can be mechanosensors to transduce mechanical stimuli into chemical signals via intracellular signaling pathways.26,27 Taken together, these findings suggest that although a wide variety of cell types may depend on integrin growth factor receptor crosstalk for integrin-mediated cell adhesion and migration, discrete growth factor receptors may be required to provide cell type–specific biological responses.

Integrin αβ5, but not αβ3, Requires Growth Factor Stimulation for Integrin-Mediated Cell Migration In Vitro and Metastasis In Vivo

Although integrins αβ3 and αβ5 mediate cell adhesion to a wide variety of ECM proteins including vitronectin, proteolyzed collagen, osteopontin, and other ECM proteins,28 the functional differences between these integrins remain poorly understood. Cell adhesion and migration studies have identified a critical role for crosstalk between growth factor receptors with the integrin αβ3, but not αβ5, during adhesion and migration on vitronectin.29 For example, in human pancreatic carcinoma cells, integrin αβ5-bearing cells (lacking αβ3) depend on EGF or insulin prestimulation for adhesion and migration on vitronectin. In contrast, αβ3-bearing cells (lacking αβ5) adhere and migrate on vitronectin in the absence of growth factor prestimulation.29 Furthermore, adhesion and migration of either αβ3- or αβ5-expressing cells on collagen via β3 integrins are independent of EGF stimulation. In support of these findings, vitronectin-mediated adhesion and migration of a melanoma cell line expressing αβ5 required IGF or insulin prestimulation, whereas cells expressing αβ3 supported growth factor–independent adhesion and migration.30 Additional evidence for a model in which the αβ5-mediated cell migration depends on growth factor prestimulation is provided in studies of IGF-stimulated human breast carcinoma cells expressing αβ3 but not αβ5.31 In these cells, IGF-induced migration is inhibited by anti-αβ3 antibodies, but not by anti-β3 antibodies. Although the molecular basis for the integrin specificity between αβ3 and αβ5 remains unknown, analysis of the intracellular signaling pathways downstream of each integrin is likely to provide clues.

In addition to the role for growth factor stimulation in integrin-mediated cell adhesion and proliferation, growth factor stimulation is also important in several in vivo models of tumor cell invasion and metastasis.30 For example, in both chick and mouse models, αβ5-bearing melanoma cells depend on ex vivo prestimulation with IGF for metastasis, whereas αβ3-bearing melanoma cells metastasize in the absence of growth factor pretreatment.30 These findings parallel the growth factor dependence for the adhesion and migration of pancreatic carcinoma and melanoma cells expressing either integrin αβ5 or αβ3, and suggest that in these...
models, the integrin αβ can selectively mediate important biological responses in vivo. These results, along with the following examples, provide a molecular basis for the regulation of vascular responses by specific combinations of integrins and growth factor receptors.

**Direct Biochemical Evidence for Crosstalk Between Integrins and Growth Factor Receptors**

Coimmunoprecipitation of growth factor receptors with integrins has been an important approach to identify biochemical interactions between these receptors in cultured cells. For example, αβ has been found to associate with the PDGF receptor (PDGFR) or the VEGF receptor-2 (VEGF-2),14,16,17,32 as well as IRS-1, a cytoplasmic signal transduction mediator of insulin and IGF receptors33 (Tables 1 and 2). Additional examples of growth factor receptor integrin crosstalk include the association of integrins αβ and αβ1 with ErB-2 receptor in human breast carcinoma cells after EGF or insulin stimulation.34 ErB-2 is widely expressed in breast carcinomas, forming heterodimers with the EGF receptor (EGFR) in vivo. An integrin-activating anti--α integrin antibody promotes αβ association with ErB-2 correlating with enhanced cell proliferation and invasion.34 In contrast, other integrins such as αβ1, αβ1, and αβ1 do not associate with ErB-2 in these cells. The capacity for growth factor stimulation to synergize with ECM inputs and promote the crosstalk between integrins and growth factor receptors in multiple cell types may be the result of coclustering of these receptors on the surface of the cell in focal adhesions or in association with the actin cytoskeleton.

Gene delivery of mutant cDNAs of integrins, growth factor receptors, and/or downstream signaling intermediates and/or targeted gene deletion in intact animal models have provided clues to the molecular basis of the crosstalk between integrins and growth factor responses in cultured cells35.36 (Table 2). For example, crosstalk between the integrin αβ and VEGF-2 can occur at the level of intracellular signaling molecules associated with focal adhesions. In primary endothelial cells and in blood vessels, the nonreceptor tyrosine kinase, Src, regulates the association of FAK with the cytoplasmic tail of integrin αβ (B.P. Eliceiri and D.A. Cheresh, unpublished data, 2001). In this model, the VEGF-mediated vascular responses depend on the ligation of integrin αβ. Furthermore, angiogenic growth factors such as bFGF and VEGF promote endothelial cell adhesion and migration mediated by αβ integrins that depend on PI3-kinase,23 an important Src substrate, which can also associate with FAK.37–39 Recent reports indicate that FAK may bridge the crosstalk between EGFR,40 or ephrin receptors,41 during integrin-mediated responses. The capacity for specific cell types to coordinate individual integrins and/or growth factor receptors may be an important mechanism to provide specificity to the regulation of the activity of intracellular signaling pathways.

**bFGF-Induced Angiogenesis Depends on Integrin αβ Ligation**

Angiogenesis induced by growth factors or tumor cells involves multiple interactions between the ECM and vascular endothelial cells. Dynamic remodeling of the ECM surrounding blood vessels facilitates several steps during angiogenesis, including matrix degradation and deposition of new ECM components. Of the wide spectrum of integrin subunit combinations that are expressed on the surface of cells, the αβ integrin has been identified as having an especially interesting expression pattern among vascular cells during angiogenesis and vascular remodeling events. For example, during bFGF-induced angiogenesis, expression of integrin β1 is upregulated.42 The upregulation of αβ during angiogenesis suggests that this integrin may have an important function during angiogenesis. Disruption of integrin αβ ligation with antibody (LM609) or cyclic peptide antagonists of αβ prevents blood vessel formation in the chick chorioallantoic membrane (CAM), quail embryo, rabbit cornea, mouse retina, and human skin transplanted onto severe combined immunodeficiency mice42–46 (see Table 3). Angiogenesis induced by αβ-negative human tumor cells can also be blocked with αβ antagonists. These antagonists prevent the growth of new blood vessels without detectably influencing the preexisting blood vessels. The inhibition of blood vessels supporting tumors not only blocks tumor growth but induces tumor regression.47 Histological examination of tumors treated with the αβ antagonists reveal few if any viable tumor cells or detectable blood vessels.47 Cytokine- or tumor cell–stimulated blood vessels treated with the αβ antagonists undergo programmed cell death (apoptosis) in response to administration of the antagonists.47 These findings suggest that the integrin αβ provides specific cell survival signals that facilitate vascular cell proliferation during bFGF-induced angiogenesis. Further examination of this model reveals that inputs from the ECM (ie, αβ ligation) regulate growth

**TABLE 1. Evidence for Crosstalk Between Growth Factor Receptors and Integrins: Direct Growth Factor Receptor Associations**

<table>
<thead>
<tr>
<th>Growth Factor Receptor</th>
<th>Integrins</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDGF</td>
<td>αβ</td>
<td>14, 16</td>
</tr>
<tr>
<td>VEGF-2</td>
<td>αβ</td>
<td>16, 17</td>
</tr>
<tr>
<td>IR</td>
<td>αβ</td>
<td>14</td>
</tr>
<tr>
<td>ErB-2</td>
<td>αβ</td>
<td>34</td>
</tr>
<tr>
<td>IRS-1 (cytoplasmic mediator for IR and IGF)</td>
<td>αβ</td>
<td>33</td>
</tr>
</tbody>
</table>

**TABLE 2. Evidence for Crosstalk Between Growth Factor Receptors and Integrins: Integrin-Mediated Growth Factor Responses**

<table>
<thead>
<tr>
<th>Growth Factor</th>
<th>Integrins</th>
<th>Response</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDGF</td>
<td>αβ</td>
<td>Proliferation, migration</td>
<td>14</td>
</tr>
<tr>
<td>bFGF</td>
<td>αβ, αβ</td>
<td>Angiogenesis, migration</td>
<td>42, 44, 45, 53</td>
</tr>
<tr>
<td>VEGF</td>
<td>αβ</td>
<td>Angiogenesis</td>
<td>45</td>
</tr>
<tr>
<td>VEGF</td>
<td>αβ, αβ, αβ, αβ</td>
<td>Adhesion, migration</td>
<td>23</td>
</tr>
<tr>
<td>VEGF</td>
<td>αβ, αβ, αβ, αβ</td>
<td>Angiogenesis, migration</td>
<td>51, 52</td>
</tr>
<tr>
<td>EGF</td>
<td>αβ</td>
<td>Migration, metastasis</td>
<td>29, 30, 31</td>
</tr>
<tr>
<td>EGF</td>
<td>αβ, αβ, αβ</td>
<td>Proliferation</td>
<td>18, 21</td>
</tr>
<tr>
<td>IGF/insulin</td>
<td>αβ</td>
<td>Migration, metastasis</td>
<td>29, 30, 31</td>
</tr>
</tbody>
</table>
Studies suggest that highly regulated mechanisms exist to restrict the extent of angiogenic sprouting. Integrin activation is thought to play a role in these mechanisms, as angiogenic growth factors such as bFGF and VEGF have been shown to induce angiogenesis through signaling cascades. BFGF- and VEGF-induced angiogenesis are each inhibited by antagonists of the distinct yet functionally related \( \alpha_5 \) integrins, \( \alpha_\beta_1 \) and \( \alpha_\beta_3 \), respectively. Both in studies of the rabbit corneal eye pocket and in the chick CAM angiogenesis assays, anti-\( \alpha_\beta_1 \) monoclonal antibody antagonists block bFGF-induced angiogenesis, whereas anti-\( \alpha_\beta_3 \) antagonists block VEGF-induced angiogenesis. Furthermore, inhibition of the PKC pathway blocks VEGF-induced angiogenesis specifically but does not affect bFGF-induced angiogenesis. Although anti-\( \alpha_\beta_1 \) antagonists do not affect bFGF-induced neovascularization, anti-\( \alpha_\beta_3 \) antagonists can inhibit up to 50% of VEGF-induced angiogenesis. This observation is consistent with findings that VEGF can promote \( \alpha_\beta_1 \)- and \( \alpha_\beta_3 \)-mediated endothelial cell adhesion and migration in vitro. Although VEGFR-2 associates with \( \alpha_\beta_1 \),\(^{16}\) ligands for other growth factor receptors that have been shown to associate with \( \alpha_\beta_1 \) do not promote vitronectin-mediated endothelial cell migration.\(^{24}\) Integrins containing \( \beta_1 \) subunits have also been implicated during growth factor–induced angiogenesis. For example, antagonists of \( \alpha_\beta_1 \) block VEGF-induced angiogenesis, whereas \( \alpha_\beta_3 \)-mediated endothelial cell migration and angiogenesis depend on the ligation state of \( \alpha_\beta_3 \) to fibronectin.\(^{53}\) In addition to a role for \( \beta_1 \) integrins in angiogenesis, mice lacking fibronectin or \( \alpha_\beta_1 \) die early in embryogenesis from extraembryonic and vascular defects, indicating an important role for \( \alpha_\beta_1 \) during vasculogenesis.\(^{54,55}\) In support of this, \( \alpha_5 \)-null embryoid bodies have delayed and reduced formation of endothelial structures.\(^{56}\) Antagonists of \( \alpha_\beta_1 \) or \( \alpha_\beta_3 \), block bFGF–but not VEGF-induced angiogenesis, suggesting that \( \alpha_\beta_1 \) and \( \alpha_\beta_3 \) may regulate a similar pathway of angiogenesis.\(^{53}\) In combination, these findings suggest that like the \( \alpha_5 \) integrin subunit,\(^{57,58}\) the \( \alpha_\beta_3 \) subunit is important for blood vessel development during mouse embryogenesis as well as during angiogenesis. Several reports provide additional evidence that ligation of integrins \( \alpha_\beta_1 \) and \( \alpha_\beta_3 \) during cell adhesion are important mechanisms of integrin crosstalk.\(^{58–61}\)

The understanding of the specific integrin-mediated signaling requirements after growth factor stimulation remains an important goal. For example, recent evidence suggests that whereas the PI3-kinase/Akt/PTEN (a 3’-inositol lipid phosphatase) pathway is required for both VEGF- and serum-induced responses in cultured endothelial cells,\(^{53}\) the Src pathway is critical for VEGF–but not bFGF-induced angiogenesis in vivo.\(^{63}\) Src is activated downstream of both integrins and growth factor receptors and can promote the activation of a wide range of biochemical pathways. However, gene delivery of kinase-deleted mutants of Src or expression of C-terminal Src kinase block VEGF–but not bFGF-induced angiogenesis in chick and mouse angiogenesis models.\(^{63}\) These results suggest a selective requirement for Src family kinases (SFKs) in the VEGF pathway, although the molecular mechanisms remain unknown.

### Role of \( \alpha_5 \) Integrins During Angiogenesis and Embryonic Development

The development and characterization of several knockout mouse models relevant to vascular biology have provided

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**TABLE 3. Evidence of Role for \( \alpha_5 \) Integrins in Angiogenesis and Development: \( \alpha_5 \) Integrin Inhibitor Studies**

<table>
<thead>
<tr>
<th>Chick</th>
<th>Mouse</th>
<th>Rabbit</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blocks growth factor–induced angiogenesis on CAM</td>
<td>Inhibits retinal vessel outgrowth, human tumor xenografts, human/SCID chimera tumors, and skin Matrigel angiogenesis</td>
<td>Inhibits corneal micropocket angiogenesis, arthritic knee angiogenesis, and VX-2 carcinoma growth</td>
<td>Stable disease and/or tumor shrinkage</td>
</tr>
<tr>
<td>Causes regression of human tumors on CAM</td>
<td></td>
<td></td>
<td>74</td>
</tr>
</tbody>
</table>

*\( \alpha_5 \) Integrin antagonists were antibodies, peptides, and organics. SCID indicates severe combined immunodeficiency.

**TABLE 4. Evidence of Role for \( \alpha_5 \) Integrins in Angiogenesis and Development: Integrin Knockout Phenotypes in Mice**

<table>
<thead>
<tr>
<th>Integrin</th>
<th>Phenotype</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \alpha_5 )</td>
<td>Lethal as a result of defective brain and intestinal blood vessels</td>
<td>57</td>
</tr>
<tr>
<td>( \beta_3 )</td>
<td>No vascular defect; suggests an integrin other than ( \alpha_\beta_3 ) is required for central nervous system and gastrointestinal blood vessel development</td>
<td>68</td>
</tr>
<tr>
<td>( \beta_3 )</td>
<td>Normal vascular bed, with defect in VEGF-induced vascular permeability</td>
<td>69*</td>
</tr>
<tr>
<td>( \alpha_5 )</td>
<td>Lethal as a result of extraembryonic and embryonic blood vessel defects</td>
<td>55</td>
</tr>
</tbody>
</table>


Two Angiogenic Pathways Are Characterized by Distinct \( \alpha_5 \) Integrins

Angiogenic growth factors such as bFGF and VEGF have been shown to induce angiogenesis through signaling cas-
important insights into the importance of integrins, growth factor receptors, and their downstream signaling targets during vascular development. For example, mice lacking the \( \alpha_v \) integrin, and therefore lacking \( \alpha_v\beta_3 \) and \( \alpha_v\beta_5 \) integrins, have extensive blood vessel defects in the brain and intestinal tract. \( \alpha_v \)-null mice are lethal, with 80% of these mice dying midgestation and the remaining 20% dying within 1 day of birth. Many of the other blood vessels in these mice appear to develop normally, suggesting that mechanisms of compensation may exist in these tissues during mouse embryogenesis. In contrast, mice lacking only integrin \( \beta_3 \) or \( \beta_5 \) form normal blood vessels during development. Nevertheless, \( \beta_3 \)-deficient mice have a bleeding disorder consistent with a role for \( \beta_3 \) integrin in platelets (Table 4). The conditional and/or tissue-specific deletion of integrin subunits, \( \alpha_v \), \( \beta_3 \), or \( \beta_5 \), should provide additional insight into the biological role of these subunits during mouse embryonic development. Although knockout mouse models are important to determine the role of integrin subunits during development, inhibitor studies in several adult animal models demonstrate that the specific inhibition of \( \alpha_v\beta_3 \) and/or \( \alpha_v\beta_5 \) integrins blocks pathological tumor growth/metastasis and angiogenesis \(^{44,47} \) (see Tables 3 and 4). Therefore, one intriguing hypothesis is that mice lacking one of these integrin subunits may have the capacity to compensate during embryogenesis by the upregulation of parallel pathways during embryogenesis. Whereas knockout mouse models address the role of a specific integrin during embryogenesis, \(^{64,66,70} \) inhibitor studies have provided the most data on the role of specific integrins in pathological processes in adults. \(^{71} \) The clinical importance of understanding the molecular basis of compensation pathways is underscored by the fact that patients with Glanzmann thrombasthenia with genetic defects of integrin \( \beta_3 \) have otherwise apparently normal blood vessels. \(^{72,73} \) Nevertheless, data from clinical trials indicate that treatment of cancer patients with an \( \alpha_v\beta_3 \) integrin antagonist results in stable disease and/or tumor shrinkage in a majority of the cases evaluated. \(^{74} \)

Conclusions from knockout mouse studies and the evidence for the role of \( \alpha_v \) integrins in angiogenesis are summarized in Tables 3 and 4.

Although the precise role of \( \alpha_v\beta_3 \) in vasculogenesis and angiogenesis remains to be fully elucidated, recent data from Stupack et al \(^{75} \) suggest that the ligation of integrin \( \alpha_v\beta_3 \) may function as a biosensor to regulate endothelial cell survival. Elements of other growth factor receptor/integrin signaling pathways may also function to compensate for defects in parallel pathways during development. Evidence for two parallel pathways of growth factor–induced angiogenesis exist in which bFGF-induced angiogenesis depends on integrin \( \alpha_v\beta_3 \) and VEGF-induced angiogenesis depends mainly on integrin \( \alpha_v\beta_5 \). \(^{45} \) Therefore, mice lacking integrin \( \beta_3 \) may upregulate components of the VEGF/\( \alpha_v\beta_5 \) pathway by increasing integrin \( \beta_3 \) expression and/or VEGF/VEGFR-2 expression. Conversely, mice lacking \( \beta_3 \) integrin could upregulate components of the bFGF/\( \alpha_v\beta_3 \) pathway or otherwise harbor defects in VEGF signaling. Indeed, recent work suggests that although \( \beta_3 \)-deficient mice develop normal blood vessels, these mice have specific VEGF-induced vascular permeability (VP) defects (B.P. Eliceiri, D. Sheppard, and D.A. Cheresh, unpublished data, 2001).

### Analysis of Integrin and Growth Factor Signaling Crosstalk in Knockout Mouse Models

The SFKs constitute an important class of nonreceptor tyrosine kinases that are activated by growth factor receptors and integrins. \(^{2,4,76-81} \) SFKs are likely candidates to promote crosstalk between growth factor receptors and integrins because of the apparent overlap in expression of related SFKs in the same cell types. Src kinase activity is essential for a wide spectrum of cell biological processes including cell proliferation, survival, spreading, invasion, and angiogenesis. \(^{79,81,82} \) For example, Src can synergize with EGFR to promote cell proliferation, \(^{83} \) as well as the PDGFR to induce integrin-dependent cell adhesion and migration. \(^{64} \) Knockout mice lacking individual or combinations of multiple SFKs have provided an important physiological basis for mechanisms of compensation among related SFKs using traditional knockout mouse strategies. \(^{85} \) Although the capacity for SFKs to support compensation in the absence of another SFK(s) has been previously reviewed, \(^{86} \) recent emerging evidence suggests that there may be additional functions of SFKs that are physiologically important during pathological disease. For example, mice lacking Src are osteopetrotic \(^{87,88} \) and lack normal VEGF-induced VP responses, \(^{63,89} \) but otherwise develop normal blood vessels. An important consequence of the VEGF-induced VP defect in Src-deficient mice is that these mice have reduced neuronal damage after stroke. \(^{89} \) Mice lacking another related SFK such as Yes also have defective VEGF-induced VP, although Fyn-deficient mice have apparently normal blood vessel development and VP responses. \(^{63} \) However, Fyn-deficient mice do have specific defects in neuronal development. \(^{86} \) Triple-knockout mice lacking Src, Fyn, and Yes are embryonic lethal, a condition in which the embryos have neural tube defects and blood-filled islands. \(^{90} \) Therefore, although mice lacking individual SFKs can support relatively normal development, together this group of SFKs is required for development. The analysis of blood vessel development during embryogenesis is complemented by the comparison with the vascular responses to pathological conditions (ie, cerebral ischemia, angiogenesis, or vascular permeability) in normal animals. The functional requirement for individual components of integrin-mediated signal transduction pathways can be facilitated by the combination of knockout mouse models, conditional/tissue-specific gene targeting, and inhibitor studies. These examples suggest that the plasticity of a complex growth factor receptor/integrin signaling network in the vascular endothelium can adapt to the selection pressures during development in mice lacking single-integrin subunits or individual members of a related family of tyrosine kinases.

### Conclusions

Further study of the basic cell biological mechanisms underlying growth factor receptors and integrin crosstalk will continue to provide insight into approaches to target tumor growth/metastasis and angiogenesis. The elucidation of the molecular basis of angiogenesis remains a challenge because of the complex interactions between the ECM and cells, which must be temporally and spatially coordinated. For example, examination of the signaling events transduced by cell adhesion molecules to the
smooth muscle and endothelial cells may reveal mechanisms in which cells can process cytokine or growth factor stimuli to impact changes in intracellular phosphorylation cascades, gene expression levels, and ECM-associated enzymatic activities. The coordinated response of inputs from the ECM and growth factors may direct the processes of cell invasion, migration, proliferation, and differentiation in vivo.

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