Expression of activated mutants of c-Src in epithelial cells can induce tumorigenicity. In addition to such oncogenic transformation, the cells undergo a dramatic morphological transformation: cell-cell contacts are disrupted, spreading on extracellular matrix proteins is suppressed, actin stress fibers and focal contacts are lost, and podosomes are formed. We have previously shown that integrin αβ3 strongly supports Src-mediated oncogenic transformation through an interaction at the β3 cytoplasmic tail. Our current findings demonstrate that this interaction does not affect Src-mediated morphological alterations, thus separating oncogenic from morphological transformation. Moreover, β1 and β3 integrins differently affect the various aspects of Src-induced morphological transformation. High levels of β3, but not β1, integrins can prevent Src-induced cell rounding although stress fiber disassembly and podosome formation still occur. Studies using chimeric integrin subunits demonstrate that this protection requires the β3 extracellular domain. Finally, like tumor formation, podosome assembly occurs independent of β3 phosphorylation. Instead, phosphorylation of β1 is required to suppress Rho-mediated contractility in order to assemble podosomes. Thus, integrins regulate Src-mediated oncogenic transformation and various aspects of morphological transformation through dissociative pathways.

In addition to its role in mitogenic signaling, c-Src is a critical regulator of both cadherin- and integrin-mediated adhesion structures (9, 10). While low levels of c-Src kinase activity or kinase-independent functions of c-Src can support the formation of cell-cell or cell-matrix adhesions (11–13), c-Src kinase activation typically stimulates the disassembly of these structures (14, 15). Indeed, expression of activated mutants of Src in epithelial cells induces scattering, loss of cytoskeletal contractility, weak adhesion, cell rounding, and the formation of highly dynamic cell-matrix adhesions termed podosomes that are considered to be hotspots for invasion and matrix remodeling (9, 16–18).

It is not clear to what extent the signaling pathways activated by Src that are involved in oncogenic transformation overlap with those involved in the morphological transformation. Moreover, the different aspects of Src-induced morphological transformation may be connected (e.g. they may all be explained to some extent by loss of actomyosin contractility) or may involve activation of distinct signaling processes (e.g. separable alterations at cell-cell junctions, within the cytoskeletal contractility machinery, and at cell-matrix adhesions). In cell-matrix adhesions, integrins can serve as direct phosphorylation substrates of v-Src, which suppresses integrin function and weakens cell-matrix adhesion. Phosphorylation of the cytoplasmic domain of β1 integrins was shown to be critical for v-Src-mediated morphological transformation (19). Others have found that v-Src phosphorylates and reduces the affinity of β3, but not of β1, integrins, and instead an indirect mechanism that disrupts β1 integrin-mediated cell adhesion was proposed (20, 21).

To clarify how different integrins regulate the various aspects of Src-mediated morphological transformation and how this relates to oncogenic transformation, we have expressed a c-Src mutant that is constitutively in an open, primed conformation (c-Src[Y530F], here referred to as SrcV5), in the context of wild type, chimeric, and mutant β1 and β3 integrin subunits in two independent β1-deficient cell lines. While overexpression of αvβ3 augments SrcV5-mediated tumor growth through an interaction at the β3 cytoplasmic tail (22), the αvβ3 extracellular domain protects against SrcV5-induced cell rounding. Moreover, like tumor formation SrcV5-induced podosome assembly occurs independent of β3 phosphorylation. Instead, phosphorylation of β1 is required to suppress Rho-mediated contractility in order to assemble podosomes. Thus, integrins uncouple SrcV5-mediated oncogenic transformation and various aspects of morphological transformation.
**Experimental Procedures**

**Cell Lines, Plasmids, Antibodies, and Other Materials**—The β1-deficient GE11 and GD25 cells were previously described (23). Cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum and antibiotics. To ensure identical expression of SrcYF in all cell lines, we first generated GE/SrcYF and GD/SrcYF single cell clones and subsequently expressed the wild type, mutant, and chimeric integrin subunits using retroviral transduction and bulk sorting (22). Retroviral expression constructs for β1, β3, β1<sup>C379A</sup>, β3<sup>C379A</sup>, β3<sup>Y759A</sup>, and β3<sup>Y759F</sup> were described (22, 24) and the LZRS-geo-β1<sup>V178DF</sup>,<sup>V178SF</sup> cDNA was provided by Dr. Ed Roos, Netherlands Cancer Institute, Amsterdam, The Netherlands. To compare wild type β3 and β3<sup>YFF</sup>, pcDNA3-β3 (25) and pRC/RSV-β3<sup>Y747F</sup>,<sup>V759F</sup> (provided by Dr. Scott Blystone, SUNY Upstate Medical University, Syracuse, NY), plasmids were transiently transfected in parallel using Effectene (Qiagen), and anti-tubulin clone B-5-1-2 (Sigma), Santa Cruz Biotechnology), anti-phospho tyrosine (pY99; Santa Cruz Biotechnology), polyclonal anti-Myc (A-14; Maastricht, The Netherlands), RhoA clone 26C4 (Santa Cruz Biotechnology), anti-vimentin (C20 and N20 (Santa Cruz Biotechnology), anti-murine β1 and α5 (clones MB1.2 and BMA5, respectively; provided by Dr. Bosco Chan, Robarts Research Institute, London, Canada). Other antibodies were anti-paxillin clone 349 (BD Transduction Laboratories), anti-β3 SSA6 (provided by Dr. Sanford Shattil, University of California, San Diego, CA), 23C6 (provided by Dr. Michael Horton, University College London, UK), C20 and N20 (Santa Cruz Biotechnology), anti-murine β1 and α5 (clones MB1.2 and BMA5, respectively; provided by Dr. Bosco Chan, Robarts Research Institute, London, Canada). Other antibodies were anti-paxillin clone 349 (BD Transduction Laboratories), anti-RhoA clone 26C4 (Santa Cruz Biotechnology), anti-vimentin clone K36 (provided by Dr. Frans Ramaekers, University of Maastricht, The Netherlands), polyclonal anti-Myc (A-14; Santa Cruz Biotechnology), anti-phospho tyrosine (pY99; Santa Cruz Biotechnology), and anti-tubulin clone B-5-1-2 (Sigma). Src-selective inhibitor PP2 and the inactive PP3 analogue were purchased from Calbiochem. Human plasma fibronectin (FN) was prepared as described previously (24). Fluorescein isothiocyanate (FITC; Sigma) was conjugated to human plasma FN using bovine serum FN in PBS overnight at 4 °C, blocked with 2% heat-denatured bovine serum albumin for 2 h at 37 °C, and washed once with PBS. Cells were trypsinized, collected in culture medium, washed once with PBS, and resuspended in Dulbecco’s modified Eagle’s medium/0.5% bovine serum albumin, and added to the plate at 2 × 10<sup>4</sup> cells/well. After 15 min of incubation at 37 °C, unattached cells were removed by rinsing the wells with PBS; the remaining attached cells were lysed and stained overnight at 37 °C in 3.75 mm p-nitrophenyl N-acetyl-β-d-glucosaminide/0.05 m sodium citrate/0.25% Triton X-100. Stopbuffer (50 mm glycine, pH 10.4, 5 mm EDTA) was added, and the A<sub>405</sub> was determined in triplicate wells and related to the A<sub>405</sub> measured in wells in which all 2 × 10<sup>4</sup> cells were stained to calculate the percentage of adhered cells.

2 The abbreviations used are: FN, fibronectin; FITC, fluorescein isothiocyanate; PBS, phosphate-buffered saline.

**Immunofluorescence and Flow Cytometry**—For immunofluorescence, cells were fixed in 4% formaldehyde, permeabilized in 0.4% Triton X-100, blocked with 2% bovine serum albumin, and incubated with anti-paxillin antibody or anti-human β3 (23C6), followed by Alexa-488-conjugated secondary antibody, rhodamine-phalloidin or TOPRO-3 staining (Molecular Probes). Preparations were mounted in Poly Aquamount (Poly-sciences, Inc.) and analyzed using a Bio-Rad Radiance 2100 confocal system. Images were obtained using a × 40 or × 60 oil objective and imported in Adobe Photoshop. For flow cytometry and cell sorting, cells were trypsinized, collected in culture medium, washed with PBS, and incubated with primary antibodies in PBS containing 2% serum for 1 h at 4 °C. Cells were then washed in PBS, incubated with phycoerythrin- or allophycocyanin-conjugated secondary antibodies for 1 h at 4 °C, washed in PBS, and analyzed on a FACSCalibur or sorted on a FACStar plus® (BD Biosciences).

**Rho Activity Assays**—Cells were plated overnight to subconfluency before lysis in Nonidet P-40 lysis buffer (0.5% Nonidet P-40, 50 mm Tris-HCl, pH 7.4, 150 mm NaCl, 5 mm MgCl<sub>2</sub>, 10% glycerol, supplemented with a protease inhibitor mix (Sigma-Aldrich)), and lysates were clarified by centrifugation at 14,000 rpm for 20 min at 4 °C. A 1% aliquot was removed for determination of total quantities of RhoA. Clarified lysates were then incubated for 45 min at 4 °C with a glutathione S-transferase fusion protein of the Rho-binding domain of the Rho effector protein Rhotekin. Complexes were bound to glutathione-conjugated beads and washed three times in Nonidet P-40 lysis buffer. The samples were analyzed by SDS-PAGE and Western blotting.

**FN Matrix Assembly Assays**—To visualize FN matrix assembly, cells were plated on FN-coated coverslips for 4 h and subsequently incubated for an additional 20 h in medium containing 10% FN-depleted serum supplemented with 10 μg/ml biotinylated FN. Cells were fixed in 4% formaldehyde, blocked with 2% bovine serum albumin, and stained with streptavidin-Texas Red. Subsequently, coverslips were permeabilized in 0.4% Triton X-100 and stained with TOPRO-3. For biochemical analysis of FN matrix assembly cells were labeled with biotinylated FN as described above and lysed in DOC buffer (1% sodium deoxycholate, 20 mm Tris-HCl, pH 8.5, 2 mm N-ethylmaleimide, 2 mm iodoacetic acid, 2 mm EDTA, and 2 mm phenylmethylsulfonyl fluoride). Lysates were passed through a 23-gauge needle, and deoxycholate-insoluble material was collected by centrifugation at 14,000 rpm for 20 min at 4 °C. The pellet was washed once with DOC buffer, resolved in reduced sample buffer, and analyzed by SDS-PAGE and Western blotting.

**Integrin Immunoprecipitations**—Prior to immunoprecipitation some cells were stimulated with 3 mm H<sub>2</sub>O<sub>2</sub> and 1 mm sodium orthovanadate for 20 min to maximize phosphorylation. Cells were lysed for 15 min at 4 °C in lysis buffer (1% Nonidet P-40, 50 mm Tris-HCl, pH 7.4, 150 mm NaCl, 1 mm sodium vanadate, 0.5 mm sodium fluoride, and protease inhibitor mixture (Sigma-Aldrich)). Lysates were clarified by centrifugation at 14,000 rpm for 15 min at 4 °C and precleared with protein A-Sepharose (Amersham Biosciences) for 2 h at 4 °C. Proteins were immunoprecipitated overnight at 4 °C with antibodies to

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The abbreviations used are: FN, fibronectin; FITC, fluorescein isothiocyanate; PBS, phosphate-buffered saline.
\( \beta_1 \) (K20) or \( \beta_3 \) (SSA6) coupled to protein A-Sepharose. The beads were resolved in reduced sample buffer and analyzed by SDS-PAGE and Western blotting.

**RESULTS**

Morphological Transformation by Src\(^ {YF} \) Does Not Require \( \beta_1 \) Integrins—Src activation causes a dramatic change in cellular morphology by interfering with adhesion and cytoskeletal organization, processes in which integrin signaling plays a critical role. To investigate the role of \( \beta_1 \) integrins in Src-mediated morphological transformation, we expressed Src\(^ {YF} \) in two independent \( \beta_1 \)-deficient cell lines. As described for Src activation in other cell types (9, 16), expression of Src\(^ {YF} \) in GE11 and GD25 cells caused disruption of cell-cell contacts and cell scattering (Fig. 1A).

Expression of Src\(^ {YF} \) also caused a dramatic reorganization of the actin cytoskeleton: F-actin bundles and ruffles disappeared and, instead, actin clusters were formed that resemble podosomes (Fig. 1B). Initial adhesion (e.g. 15 min) of GE11 and GD25 cells to FN is weak (Fig. 2A), but at later time points (1 h) they do fully adhere and spread (Fig. 2B). Expression of Src\(^ {YF} \) interfered with this spreading, causing a rounded or fusiform phenotype, which was maintained after overnight culture (Figs. 1B and 2B). These experiments show that all aspects of Src\(^ {YF} \)-induced morphological transformation can occur in \( \beta_1 \) null cells, arguing against a requirement for \( \beta_1 \) integrins per se.

Different Aspects of Src\(^ {YF} \)-Mediated Morphological Transformation Can Be Separated; Distinct Roles for \( \beta_1 \) and \( \beta_3 \) Integrins—Expression of \( \beta_1 \) in GE11 and GD25 cells led to a strong increase in cell adhesion to FN (~70% of the cells attached at 15 min after plating) that was suppressed by Src\(^ {YF} \) (Fig. 2A). At later times (e.g. 1 h after plating), GE\( \beta_1 \) and GD\( \beta_1 \) cells had all adhered regardless of the absence or presence of Src\(^ {YF} \), but in the presence of Src\(^ {YF} \) cells remained rounded (Fig. 2B). In complete contrast, overexpression of \( \beta_3 \) in the \( \beta_1 \) null cells led to a similar increase in adhesion and spreading to FN as expression of \( \beta_1 \) but this was only minimally affected by Src\(^ {YF} \).
Notably, after overnight culture SrcYFβ1-expressing cells retained a fusiform or even rounded shape whereas SrcYFβ3-expressing cells remained well spread (Fig. 4A). This indicates that SrcYF did not simply delay β1-integrin-mediated spreading but caused a permanent morphological alteration that was not seen in the context of αβ3. Finally, expression of β1 in GESrcYFβ3 cells did not alter the well spread morphology of these cells, indicating that αβ3-mediated protection against SrcYF-induced cell rounding was dominant (supplemental Fig. S1, A and B).

We have reported that expression of β1 integrins in GE11 and GD25 cells stimulates Rho-mediated cytoskeletal contractility and FN matrix assembly, whereas overexpression of β3 in β1 null cells is unable to do so (24). We wondered whether higher levels of Rho-mediated cytoskeletal contractility could also explain the inhibition of cell spreading in the SrcYF-transformed cells expressing β1 integrins. However, in the presence of SrcYF, RhoA-GTP levels in β1-expressing cells were dramatically suppressed to levels that were comparable with those in cells lacking β1 (Fig. 3A). Moreover, FN matrix assembly, a process that requires Rho-mediated contractility, was strongly reduced upon introduction of SrcYF (Fig. 3, B and C).

Subsequently, we investigated whether β1 and β3 integrins affected SrcYF-mediated podosome assembly. Despite the markedly different sensitivities of β1- and β3-mediated adhesion and spreading to suppression by SrcYF (Fig. 2), loss of F-actin stress fibers and conversion of focal adhesions into podosomes was seen in each case (Fig. 4A). Podosomes of SrcYFβ1 cells often consisted of F-actin dots that were tightly sealed (Fig. 4, A and B).
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Oncogenic and Morphological Transformations Are Separated by Distinct Integrin Domains—We have previously shown that α3β1 strongly supports SrcYF-mediated tumorigenesis through an interaction between the β3 cytoplasmic domain and the Src homology 3 domain (22). We examined whether this was related to the capacity of α3β3 to protect cells against SrcYF-induced rounding (Figs. 2 and 4). Therefore, we expressed a chimeric β1α3β3 subunit, consisting of a β1 extracellular and transmembrane region fused to the cytoplasmic tail of β3, or an inverse β3α3β1 integrin in GEα3β1 cells (supplemental Fig. S1C). Using these chimeric integrins we demonstrated that the β3 cytoplasmic domain was required and sufficient for the stimulation of SrcYF-mediated tumor growth (Fig. 6C, left graph, and Ref. 22). In complete contrast, α3β3-mediated protection against SrcYF-induced cell rounding required the β3 extracellular domain: β3α3β1 failed to support tumor growth but effectively rescued short term cell adhesion and subsequent spreading, whereas the opposite was the case for a β1α3β3 chimera (Fig. 6F, A and C). Like adhesion and spreading, the appearance of podosomes was unaffected by the integrin cytoplasmic tail swap: podosomes in the presence of β1α3β3 resembled those of β3-expressing cells and were often sealed together, whereas podosomes of β3α3β1-expressing cells were comparable with those expressing β3, consisting mainly of dispersed small F-actin dots (Fig. 6B). These results demonstrate that (i) high levels of α3β3 support SrcYF-mediated tumor formation and protect against SrcYF-induced loss of adhesion and spreading through distinct mechanisms and (ii) SrcYF-mediated oncogenic and morphological transformation can be separated.

Podosome Formation Requires SrcYF-mediated Phosphorylation of the β1 Cytoplasmic Tail to Suppress Cytoskeletal Contractility—Integrin cytoplasmic tails serve as direct phosphorylation substrates of v-Src, which impairs their adhesive function (19, 21). Analysis of immunoprecipitated integrin β subunits demonstrated that β1 and β3 can both be tyrosine-phosphorylated by SrcYF (Fig. 7A), although phosphorylation was very low compared with maximal levels reached with pervanadate (Fig. 7B). Using single tyrosine point mutants we have found that phosphorylation of either of the two tyrosines in the β3 cytoplasmic tail is not required for α3β3-mediated support of tumor growth (22). We observed that these mutations also did not affect morphological transformation by SrcYF (data not shown). Moreover, expression of a non-phosphorylatable β3Y747F,Y770F (β3YYFF) subunit did not change SrcYF-mediated morphological transformation when compared with wild type expression (22). Together, these results demonstrate that (i) high levels of α3β3, but not β1, integrins protect SrcYF-transformed cells from rounding up and (ii) two typical aspects of SrcYF-induced morphological transformation, cell rounding and podosome formation, are distinct processes and are differently affected by the integrin expression profile.

SrcYF-induced Podosomes Are Proteolytically Active Irrespective of the Integrin Type—Formation of podosomes is a morphological hallmark of Src transformation, and these adhesions are thought to be hotspots for invasion and proteolytic remodeling of the extracellular matrix (17, 18). We next tested whether the integrin expression profile affected the proteolytic activity of these podosomes. No matrix degradation was observed to be associated with focal contacts in GEβ1 and GEβ3 cells in the absence of SrcYF when plated on immobilized FITC-labeled FN (Fig. 5). By contrast, podosomes formed in GEβ1 and GEβ3 cells were both able to degrade FITC-FN. Proteolytic activity was often evident at sites outside cell borders, indicating that cells had moved along these sites (Fig. 5, arrowheads). Thus, podosomes in SrcYF-transformed cells are proteolytically active, irrespective of the integrin composition.
DISCUSSION

In summary (see Fig. 8), we show that (i) Src-mediated oncogenic and morphological transformations are distinct processes; (ii) podosome formation and cell rounding are independent aspects of Src-mediated morphological transformation (e.g. all cells expressing high levels of integrin subunits containing β3 extracellular domain contain podosomes but remain well spread); (iii) αvβ3 supports SrcYF-mediated tumor formation and protects against SrcYF-induced loss of adhesion and spreading through distinct mechanisms (e.g. experiments using β1ex3in and β3ex1in chimeras indicate that the β3 cytoplasmic domain supports Src-mediated tumor growth whereas the β3 extracellular domain protects against Src-induced cell rounding); and (iv) Src-induced podosome assembly in the presence of β1 requires phosphorylation of the integrin cytoplasmic domain to reduce cytoskeletal contractility (e.g. β1YVVF). In the absence of β1 integrins, β3 does not promote Rho-mediated cytoskeletal contractility and podosomes can be formed without Src-mediated phosphorylation of integrin tails (e.g. β3YVVF).

Disruption of cytoskeletal contractility is one of the key events during Src-induced morphological transformation that enables reorganization of the actin cytoskeleton in order to assemble podosomes. Relaxation of the actin cytoskeleton requires inactivation of RhoA, and indeed expression of constitutively activated RhoA suppresses loss of stress fibers and podosome formation induced by v-Src (26). On the other hand, complete inhibition of RhoA also perturbs podosomes, indicating that local RhoA activity might still be required (27). We find that SrcYF inhibits the ability of β1 integrins to support RhoA-mediated contractility. The kinase activity of SrcYF is required for podosome formation in SrcYFβ1- and SrcYFβ3-expressing cells, and SrcYF phosphorylates β1 and β3 cytoplasmic domains. However, phosphorylation of β1, but not β3, is important for SrcYF-mediated morphological transformation. In line with a previous report (28), mutation of the tyrosines in the β1 cytoplasmic tail restored focal adhesions and cell spreading. Our findings suggest that this is due to restored cytoskeletal contractility that prevents the transformation from focal contacts to podosomes in the presence of SrcYF. Indeed, overexpression of αvβ3 fails to promote Rho-mediated cytoskeletal contractility in β1-null cells (24), explaining why corresponding mutations in the β3 subunit do not affect Src-mediated morphological transformation. Notably, in osteosarcoma cells phosphorylation of β3 by v-Src reduces the binding strength of αvβ3 to FN (21). In our studies, αvβ3-mediated adhesion to FN was not affected by the expression of SrcYF, which may be related to differences between v-Src (which contains multiple additional mutations) and SrcYF (which may closely resemble c-Src in human cancer cells where its interaction with overexpressed receptor tyrosine kinases or mutations in the C terminus can lead to enhanced priming) or to the moderate SrcYF expression and integrin phosphorylation levels that we reach in GE11 and GD25 cells. Nevertheless, these levels are sufficient to cause all the aspects of morphological transformation and lead to rounding of β1-expressing cells.
Our study dissociates SrcYF-mediated oncogenic from morphological transformation and shows that different aspects of morphological transformation (e.g. podosome formation and cell rounding) involve separable, independent pathways. These findings are corroborated by studies in which mitogenic activity, morphological alterations, and the anchorage independence of cells expressing mutants of v-Src were compared. It was shown that the amino-terminal domain of v-Src is important for determining cell morphology, whereas the kinase domain is essential for all three parameters (29). Also, when expressed at

![Image](http://www.jbc.org/)

**FIGURE 7. Effect of phosphorylation of β1 and β3 integrins by SrcYF.** A, Western blot analysis of tyrosine-phosphorylated or total amounts of immunoprecipitated β1 (left) or β3 (right) integrins from GE11 cells expressing the indicated constructs. B, Western blot analysis of tyrosine-phosphorylated or total amounts of immunoprecipitated β1 (left) or β3 (right) integrins from GE11 cells expressing the indicated constructs. Prior to lysis, cells were left untreated or stimulated with pervanadate for 15 min. C, immunofluorescent images of GESrcYF cells transiently transfected with human β3 or β3YYFF cDNAs. Cells were stained for human β3 integrin (green) and phalloidin (red). Arrows indicate transfected cells. D, immunofluorescent images of GE11 and GD25 cells stably expressing the indicated β1 constructs stained with phalloidin (red) and paxillin (green) at different time points of spreading on FN. E, higher magnifications of the 1-h and overnight time points of D are depicted. Scale bar, 25 μm. F, graph shows a quantification of the percentage ± S.D. of podosome-containing cells after overnight spreading in at least five different fields of two independent experiments as in D. Asterisks indicate significant difference between average values (t-test, p < 0.01).
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|  | SrcYF-induced | Loss of Stress fibers | Podosomes | Cell rounding | Tumor growth |
|---|---|---|---|---|---|
| β3 low | β1 null | n.a | + | + | - |
| β3 high | β1 null | + | + | - | + |
| β3/β1 high | β1 null | + | + | - | n.d. |
| β3/β1 high | β1 null | + | + | - | + |
| β3/β1 high | β1 high | + | + | - | + |
| β3 low | β1 high | + | + | - | + |
| β3 low | β1/β3 high | - | - | - | n.d. |
| β3 low | β1/β3 high | + | + | - | + |

FIGURE 8. Cross-talk between SrcYF signaling and integrins. A, overview of the different morphological and oncogenic properties of cells expressing SrcYF and the indicated integrin subunits used in this study and previous work (22). B, model illustrating how integrins modulate three separable aspects of Src transformation: tumor growth, podosome formation, and cell rounding. There is cross-talk of integrins with Src signaling through different mechanisms: a functional interaction of the β3 cytoplasmic domain with the Src homology 3 domain supports tumor growth through activation of SrcYF, Stat3, focal adhesion kinase (FAK), and increased proliferation and survival (22), whereas the extracellular domain of β3 protects against Src-induced cell rounding. Src-induced podosome formation occurs independent of the expression of either β1 or β3 integrins, but the β1 cytoplasmic tail must be phosphorylated by SrcYF to inhibit Rho-mediated cytoskeletal contractility that is promoted by α5β1 (24), which is a prerequisite for podosome assembly.

very low levels in Madin-Darby canine kidney cells, v-Src elicited disruption of zonula adherences, which was dissociable from oncogenic transformation, as determined by anchorage-independent growth capacity and proliferation (30). Attempts to transform c-Myc-deficient fibroblasts with v-Src resulted in morphological transformation but failed to induce DNA synthesis and proliferation (31). All together, these studies show that signaling downstream of Src can occur through multiple independent pathways. Our current work indicates that the integrin expression profile differentially modulates all these aspects of Src transformation.

In human cancer increased expression and activity of c-Src contributes to tumor development through stimulation of mitogenic signaling pathways in which c-Src normally plays a regulatory role (10, 32). In addition, reorganization of the actin cytoskeleton, cell-cell, and cell-matrix adhesions upon Src activation may contribute to tumor invasion and metastasis (4, 9). Interestingly, changes in the expression profile of integrins often occur with tumor formation and during later steps of tumor progression. Increased expression levels of αvβ3 are associated with growth and progression of various cancers (33). For example, high levels of αvβ3 promote the conversion from radial to vertical growth phase in human melanoma (34, 35), a cancer type in which c-Src activity is frequently increased (4). Our findings suggest that such changes in integrin expression can have a dramatic impact on Src-mediated effects on growth and/or invasion of tumors. Cooperation between integrin αvβ3 and c-Src may be important for tumor growth, whereas shifts in the relative expression of β1 and β3 integrins might be important to control tumor cell adhesion and spreading during cancer progression.

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