Integrins Uncouple Src-induced Morphological and Oncogenic Transformation\*§

Received for publication, February 5, 2008, and in revised form, March 6, 2008. Published, JBC Papers in Press, March 7, 2008, DOI 10.1074/jbc.M800927200

Stephan Huveneers\*§, Serdar Arslan§, Bob van de Water§, Arnoud Sonnenberg§, and Erik H. J. Danen\*§†

From the Division of Toxicology, Leiden/Amsterdam Center for Drug Research, Leiden University, 2300 RA Leiden, The Netherlands and Division of Cell Biology, The Netherlands Cancer Institute, 1066 CX Amsterdam, The Netherlands

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.

The ubiquitously expressed Src family kinase c-Src is involved in pro-survival and mitogenic signaling cascades (1). Activated mutants of Src, including the oncogenic product of Rous sarcoma virus (v-Src), can induce anchorage- and growth factor-independent growth of cell lines in vitro and tumor formation in vivo (2–4). c-Src has been found to play a critical role in the development of cancer in mice (5, 6), and expression and/or activity of c-Src is frequently increased in human melanoma and carcinomas of the breast, colon, and other epithelia (4, 7, 8). Activation of Ras, phosphatidylinositol 3-kinase, and/or activity of c-Src is frequently increased in human melanoma and carcinomas of the breast, colon, and other epithelia (4, 7, 8). Activation of Ras, phosphatidylinositol 3-kinase, and Stat3 has been implicated in Src-mediated oncogenic transformation (3).

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-SrcV530F), 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 αβ3 augments SrcV5-mediated tumor growth through an interaction at the β3 cytoplasmic tail (22), the αβ3 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.

* This work was supported by Dutch Cancer Society Grant 2003-2858. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† The on-line version of this article (available at http://www.jbc.org) contains supplemental Fig. S1.

‡ To whom correspondence should be addressed: Division of Toxicology, Leiden/Amsterdam Center for Drug Research, Leiden University, Einsteinweg 55, P. O. Box 9502, 2300 RA Leiden, The Netherlands. E-mail: e.danen@lacdr.leidenuniv.nl.
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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 GESrcYF and GDSrcYF 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, β1ex3in, β3ex1α, β3Y747A, and β3Y759A were described (22, 24) and the LZRS-zeo-β1Y783F, Y795F cDNA was provided by Dr. Ed Roos, Netherlands Cancer Institute, Amsterdam, The Netherlands. To compare wild type β3 and β3YYFF, pcDNA3-β3 (25) and pRC/RSV-β3Y747F, Y759F (provided by Dr. Scott Blystone, SUNY Upstate Medical University, Syracuse, NY), plasmids were transiently transfected in parallel using Effectene (Qiagen) and analyzed by immunofluorescence. The following integrin-specific antibodies have been used: anti-human fibronectin (FN; Chemicon), anti-phospho tyrosine (pY99; Santa Cruz Biotechnology), anti-paxillin clone 349 (BD Transduction Laboratories), anti-integrin α5; provided by Dr. Bosco Chan, Robarts Research Institute, London, Canada). Other antibodies were purchased from Calbiochem. Human plasma fibronectin (FN) was conjugated to human plasma FN (Sigma) was added to the plate at 2 μg/ml 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, resuspended in Dulbecco’s modified Eagle’s medium/0.5% bovine serum albumin, 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-biotinylated FN. Cells were fixed in 4% formaldehyde, 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.

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 H₂O₂ 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 orthovanadate, 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. 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 FACS Calibur or sorted on a FACStar plus® (BD Biosciences).

Rho Activity Assays—Cells were plated overnight to subconfluence 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 MgCl₂, 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.

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 (Polysciences, 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 FACS Calibur or sorted on a FACStar plus® (BD Biosciences).

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RESULTS

Morphological Transformation by Src\textsuperscript{YF} Does Not Require β1 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 β1 integrins in Src-mediated morphological transformation, we expressed Src\textsuperscript{YF} in two independent β1-deficient cell lines. As described for Src activation in other cell types (9, 16), expression of Src\textsuperscript{YF} in GE11 and GD25 cells caused disruption of cell-cell contacts and cell scattering (Fig. 1A).

β1 (K20) or β3 (SSA6) coupled to protein A-Sepharose. The beads were resolved in reduced sample buffer and analyzed by SDS-PAGE and Western blotting.

Expression of Src\textsuperscript{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\textsuperscript{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\textsuperscript{YF}-induced morphological transformation can occur in β1 null cells, arguing against a requirement for β1 integrins per se.

Different Aspects of Src\textsuperscript{YF}-mediated Morphological Transformation Can Be Separated; Distinct Roles for β1 and β3 Integrins—Expression of β1 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\textsuperscript{YF} (Fig. 2A). At later times (e.g., 1 h after plating), GEβ1 and GDβ1 cells had all adhered regardless of the absence or presence of Src\textsuperscript{YF}, but in the presence of Src\textsuperscript{YF} cells remained rounded (Fig. 2B). In complete contrast, overexpression of β3 in the β1 null cells led to a similar increase in adhesion and spreading to FN as expression of β1 but this was only minimally affected by Src\textsuperscript{YF}.

FIGURE 1. Morphological transformation by Src\textsuperscript{YF} does not require β1 integrins. A, phase-contrast images of integrin β1-deficient GE11 and GD25 cells with or without stable expression of Src\textsuperscript{YF}. Cells were cultured on an FN-coated coverslip for 2 days to allow cell-cell contact formation. B, immunofluorescent images of GE11 and GD25 cells that were plated overnight on an FN-coated coverslip and subsequently stained with phallolidin to visualize the F-actin cytoskeleton. Podosomes indicated by white arrows are shown enlarged in the insets. Scale bar, 25 or 5 μm (inset).

FIGURE 2. β3 integrin protects against Src\textsuperscript{YF}-induced loss of adhesion and spreading. A, graphs indicate the average percentage ± S.D. of adherent cells 15 min after plating from two independent FN adhesion assays performed in triplicate wells. Asterisks indicate significant difference between average values (t-test, p < 0.01). Expression of various constructs is shown at the bottom of the graph. B, phase contrast images of GE11 and GD25 cells expressing indicated constructs that were plated on an FN-coated surface for 1 h.
Notably, after overnight culture SrcYF/3-expressing cells retained a fusiform or even rounded shape whereas SrcYF/1-expressing cells remained well spread (Fig. 4A). This indicates that SrcYF did not simply delay/1-integrin-mediated spreading but caused a permanent morphological alteration that was not seen in the context of/3. Finally, expression of/1 in GESrcYF cells did not alter the well spread morphology of these cells, indicating that ov/3-mediated protection against SrcYF-induced cell rounding was dominant (supplemental Fig. S1, A and B).

We have reported that expression of/1 integrins in GE11 and GD25 cells stimulates Rho-mediated cytoskeletal contractility and FN matrix assembly, whereas overexpression of/3 in/1 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/1 integrins. However, in the presence of SrcYF, RhoA-GTP levels in/1-expressing cells were dramatically suppressed to levels that were comparable with those in cells lacking/1 (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/1 and/3 integrins affected SrcYF-mediated podosome assembly. Despite the markedly different sensitivities of/1- and/3-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 SrcYF/1 cells often consisted of F-actin dots that were tightly sealed
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Oncogenic and Morphological Transformations Are Separated by Distinct Integrin Domains—We have previously shown that αβ3 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 αβ3 to protect cells against SrcYF-induced rounding (Figs. 2 and 4). Therefore, we expressed a chimeric β1β3 subunit, consisting of a β1 extracellular and transmembrane region fused to the cytoplasmic tail of β3, or an inverse β3β1 subunit integrin in GESrcYF 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, αβ3-mediated protection against SrcYF-induced cell rounding required the β3 extracellular domain: β3β1 failed to support tumor growth but effectively rescued short term cell adhesion and subsequent spreading, whereas the opposite was the case for a β1β3 chimera (Fig. 6, A and C). Like adhesion and spreading, the appearance of podosomes was unaffected by the integrin cytoplasmic tail swap: podosomes in the presence of β1β3 resembled those of β1-expressing cells and were often sealed together, whereas podosomes of β3β1-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 αβ3 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 αβ3-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,Y759F (β3YYFF) subunit did not change SrcYF-mediated morphological transformation when compared with wild type.

together, whereas more dispersed, individual, small F-actin dots were present in SrcYF β3 cells, which may be explained by increased cell spreading (see Fig. 4A, insets). The podosomes that were formed in each of the SrcYF-transformed cell types were dependent on SrcYF kinase activity, because treatment with the Src-selective kinase inhibitor PP2 led to their disassembly (Fig. 4, B and C).

Taken together, these results demonstrate that (i) high levels of β3, 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 GEβYFβ1 and GEβYFβ3 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.

![Figure 5: SrcYF-induced podosomes are proteolytically active irrespective of the integrin type.](https://example.com/figure5.png)

**Figure 5.** SrcYF-induced podosomes are proteolytically active irrespective of the integrin type. A, immunofluorescent images of indicated GE11 cells that were plated overnight on coverslips coated with FITC-labeled FN and stained for paxillin (red). Arrows indicate spots where degradation of FITC-FN occurred. Dotted squares indicate regions that are zoomed in and depicted in the right panel. Scale bars, 25 or 10 μm (Zoom).
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) ανβ3 supports SrcΔF-mediated tumor formation and protects against SrcΔF-induced loss of adhesion and spreading through distinct mechanisms (e.g. experiments using β1ΔF3ΔN and β3Δex1ΔN 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. β1YYFF). In the absence of β1 integrins, β3 does not promote Rho-mediated cytoskeletal contractility (24) and podosomes can be formed without Src-mediated phosphorylation of integrin tails (e.g. β3YYFF).

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 SrcΔF inhibits the ability of β1 integrins to support RhoA-mediated contractility. The kinase activity of SrcΔF is required for podosome formation in SrcΔFβ1- and SrcΔFβ3-expressing cells, and SrcΔF phosphorylates β1 and β3 cytoplasmic domains. However, phosphorylation of β1, but not β3, is important for SrcΔF-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 SrcΔF. Indeed, overexpression of ανβ3 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 ανβ3 to FN (21).

In our studies, ανβ3-mediated adhesion to FN was not affected by the expression of SrcΔF, which may be related to differences between v-Src (which contains multiple additional mutations) and SrcΔF (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 SrcΔF 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.

β3: actin stress fibers were absent and podosomes were formed in the presence of SrcΔF and β3YYFF (Fig. 7C). By contrast, in cells expressing β1, integrin phosphorylation was crucial for SrcΔF-mediated morphological transformation. When a non-phosphorylatable β1ΔY783F,Y795F (β1YYFF) subunit was expressed in GE11 and GD11 cells (supplemental Fig. S1, D and E), SrcΔF-induced podosome formation was completely abolished (Fig. 7, D–F). Instead of podosomes that were formed in SrcΔFβ1 cells, F-actin stress fibers and focal contacts were restored in SrcΔFβ1YYFF cells and eventually these cells became considerably more spread. These results indicate that phosphorylation of β1, but not β3, cytoplasmic tails is important for SrcΔF-mediated morphological transformation. Most likely, phosphorylation is required to suppress Rho-mediated cytoskeletal contractility that is promoted by β1, but not by β3, integrins and would interfere with podosome formation (Fig. 3).
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...
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FIGURE 8. Cross-talk between Sce’ signaling and integrins. A, overview of the different morphological and oncogenic properties of cells expressing Sce’ 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 Sce’ (22). Rho-mediated cytoskeletal contractility is inhibited by Src’ (24) and is a prerequisite for podosome formation. Low levels of 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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Acknowledgments—We thank Drs. Scott Blystone, Bosco Chan, Michael Horton, Frans Ramaekers, Ed Roos, and Sanford Shattil for their generous gifts of reagents.