C-Src Kinase Activity Is Required for Hepatocyte Growth Factor-induced Motility and Anchorage-independent Growth of Mammary Carcinoma Cells*

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Overexpression and amplification of hepatocyte growth factor (HGF) receptor (Met) have been detected in many types of human cancers, suggesting a critical role for Met in growth and development of malignant cells. However, the molecular mechanism by which Met contributes to tumorigenesis is not well known. The tyrosine kinase C-Src has been implicated as a modulator of cell proliferation, spreading, and migration; these functions are also regulated by Met. To explore whether C-Src kinase is involved in HGF-induced cell growth, a mouse mammary carcinoma cell line (SP1) that co-expresses HGF and Met and a nonmalignant epithelial cell line (MvILu) that expresses Met but not HGF were used. In this study, we have shown that C-Src kinase activity is constitutively elevated in SP1 cells and is induced in response to HGF in MvILu cells. In addition, C-Src kinase associates with Met following stimulation with HGF. The enhanced activity of C-Src kinase also correlates with its ability to associate with Met. Expression of a dominant negative double mutant of C-Src (SRC-RF), lacking both kinase activity (K295R) and a regulatory tyrosine residue (Y527F), in SP1 cells significantly reduced C-Src kinase activity and strongly blocked HGF-induced motility and colony growth in soft agar. In contrast, expression of the dominant negative C-Src mutant had no effect on HGF-induced cell proliferation on plastic. Taken together, our data strongly suggest that HGF-induced association of C-Src with Met and C-Src activation play a critical role in HGF-induced cell motility and anchorage-independent growth of mammary carcinomas and further support the notion that the presence of paracrine and autocrine HGF loops contributes significantly to the transformed phenotype of carcinoma cells.

Evidence supports a role of hepatocyte growth factor (HGF)1 and its receptor, the product of the met protooncogene, in both normal (1, 2) and malignant (3–5) epithelial cell development. In addition, a majority of human breast cancers show increased expression of HGF and Met (6–8), and this high level of HGF expression correlates with recurrence and poor patient survival (9). Met is also overexpressed in several other human cancers, including ovarian (10), melanoma (11), colon carcinomas (12), and osteosarcomas (13). Collectively, these observations suggest that activation of Met by overexpression, gene amplification, or establishment of an HGF autocrine loop may contribute to growth and development of mammary carcinomas. Previous studies demonstrated that co-expression of HGF and Met (4, 14), as well as expression of a constitutively active Met (Tpr-Met) in NIH3T3 fibroblasts (15, 16) directly leads to cell transformation and tumorigenicity. However, the molecular mechanism by which HGF binding to its receptor elicits cell transformation is not fully understood.

A number of cytoplasmic signaling proteins, such as phosphatidylinositol (PI) 3-kinase, Grb2, Shc, Ras, and C-Src, have been shown to be involved in Met-dependent signal transduction pathways (17, 18). It is important to establish which of these signaling proteins regulate Met-dependent steps in tumor progression, because different signaling proteins may regulate various HGF-induced cellular functions, including mitogenic, motogenic, and morphogenic signals in target cells (18–22). The HGF-mediated signaling pathway is further complicated by the observation that the majority of SH2-containing cytoplasmic effectors bind to a single multifunctional docking site on the cytoplasmic domain of Met, whereas a second site is required for Grb2 binding (17, 18). Recent findings using a mutational approach demonstrated that different HGF-induced effects are regulated by these separate Met binding sites for cytoplasmic transducers (23–25) and that complementation in trans between these two binding sites is required for the invasive-metastatic phenotype (25). However, to study the role of specific SH2-containing cytoplasmic effectors in HGF receptor function, approaches to target individual cytoplasmic effectors are required. Recently, we (26) and others (27) have demonstrated that PI-3-kinase activity is required for HGF-induced mitogenic (26) and motogenic functions (27). These findings strongly argue that PI-3-kinase may play an important role in HGF-mediated growth of mammary carcinomas.

The tyrosine kinase C-Src is activated in response to HGF (17, 18) and other growth factors such as platelet-derived growth factor (PDGF) (28–30), fibroblast growth factor (31),...
and epidermal growth factor (32). c-Src kinase activity is known to modulate cell proliferation (33, 34), spreading (35, 36), and migration (36–38) in many cell types; these functions are also regulated by HGF (19–23). c-Src kinase activity is increased 4-fold in human breast cancer (39, 40) and is also elevated in Neu-induced mouse mammary carcinomas in transgenic mice (41, 42). Activation of c-Src tyrosine kinase in transgenic mice induces mammary epithelial hyperplasia and is required, but is not sufficient, for induction of mammary tumors in polyoma virus middle T-transgenic mice (42, 43). Altogether, these observations support the notion that increased c-Src kinase activity in mammary carcinomas plays an important role in mammary tumor growth and development. However, the role of c-Src kinase in HGF-induced functions in mammary carcinoma cells is not clearly known.

To analyze whether c-Src kinase is involved in HGF-induced mammary carcinoma cell growth, we used a mouse mammary carcinoma cell line, SP1, which expresses HGF and tyrosine-phosphorylated Met, thereby generating an autocrine HGF loop in these cells (44). Our current results demonstrate that c-Src kinase activity is elevated in SP1 cells, compared with nonmalignant Mv1Lu epithelial cells. The increased activity of c-Src kinase correlates with its ability to associate with tyrosine-phosphorylated Met. We therefore examined the effect of expressing a dominant negative mutant form of c-Src on c-Src kinase activity and HGF-induced cell motility and anchorage-independent growth of SP1 carcinoma cells. Taken together, our findings show that c-Src kinase activation plays a significant role in HGF-induced cell motility and anchorage-independent growth, characteristics of the transformed phenotype.

EXPERIMENTAL PROCEDURES

Antibodies—Rabbit anti-sheep IgG conjugated to horseradish peroxidase was from Jackson ImmunoResearch Laboratories (Westgrove, PN). Mouse anti-phosphotyrosine (PY20) monoclonal antibody was purchased from Transduction Laboratories (Lexington, KT). Rabbit anti-c-Src IgG, anti-Met (mouse) IgG, and anti-PLC-γ1 IgG were obtained from Santa Cruz Biotechnology (San Diego, CA).

Cell Lines—Mv1Lu cells are members of a minor lung epithelial cell line obtained from ATCC (Rockville, MA). Maintenance medium for Mv1Lu cells was Dulbecco’s modified Eagle’s medium (Life Technologies, Inc.) supplemented with 10% FBS. The SP1 tumor cell line is derived from a spontaneous poorly metastatic murine mammary intraductal adenocarcinoma and expresses HGF and Met. The cell line is derived from a spontaneous poorly metastatic murine mammary carcinoma cell line, SP1, which expresses HGF and tyrosine-phosphorylated Met. The cell line is derived from a spontaneous poorly metastatic murine mammary carcinoma cell line, SP1, which expresses HGF and tyrosine-phosphorylated Met. The cell line is derived from a spontaneous poorly metastatic murine mammary carcinoma cell line, SP1, which expresses HGF and tyrosine-phosphorylated Met. Cell line is derived from a spontaneous poorly metastatic murine mammary carcinoma cell line, SP1, which expresses HGF and tyrosine-phosphorylated Met. Cell line is derived from a spontaneous poorly metastatic murine mammary carcinoma cell line, SP1, which expresses HGF and tyrosine-phosphorylated Met.

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Immunoprecipitation and Western Blotting—Cells were grown to confluence and serum-starved for 24 h. Cells were rinsed with cold phosphate-buffered saline three times and lysed in a lysis buffer containing 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Nonidet P-40, 1 mM Na3VO4, 50 mM NaF, 2 mM EDTA, 2 μg/ml aprotinin, 2 μg/ml leupeptin, and 1 mM phenylmethylsulfonyl fluoride. Lysates were centrifuged for 10 min at 14,000 rpm in an IEC/Micromax centrifuge at 4 °C. Protein content of supernatants was determined using a bicinchoninic acid protein assay (Pierce). Equal protein amounts from each cell lysate were incubated with the indicated antibodies at 4 °C for 2 h or overnight. Immunoprecipitates were collected on protein A-Sepharose (Amersham Pharmacia Biotech), washed three times with lysis buffer, separated by SDS-PAGE, and transferred to a nitrocellulose membrane. The membrane was blocked for 15 min with 5% skimmed milk in Tris-HCl, pH 7.5, 150 mM NaCl, 0.1% Tween 20, and probed for 1 h with the indicated antibodies. The membrane was washed three times for 5 min each with TBST buffer, incubated with horseradish peroxidase-labeled secondary anti-rabbit or anti-mouse antibodies for 15 min, and washed three times with TBST for 10 min each time. Immune complexes were detected using ECL (Amersham).
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Fig. 1. c-Src kinase activity is elevated in SP1 carcinoma cells compared with Mv1Lu epithelial cells. Cell lysates were prepared from serum-starved Mv1Lu and SP1 cells treated without (-) or with (+) HGF (40 ng/ml) for 10 min and were immunoprecipitated with anti-c-Src IgG. Immunoprecipitates were subjected to an in vitro kinase assay using enolase as a substrate, and kinase activity was measured as described under “Experimental Procedures.” A, autoradiogram showing 32P-labeled enolase. B, quantitation of autoradiogram using PhosphorImager. Results are expressed as the percentage of cpm in untreated Mv1Lu cells (100%), normalized to the amount of c-Src protein in C. The means ± range of two experiments are shown. Similar results were obtained using the c-Src kinase family-specific cdc2 peptide as substrate (data not shown). C, Western blot analysis of immunoprecipitates in A, probed with anti-c-Src IgG.

Fig. 2. c-Src kinase binds to tyrosine-phosphorylated Met. Cell lysates derived from serum-starved Mv1Lu cells treated without (-) or with (+) HGF (40 ng/ml) for 15 min were immunoprecipitated with anti-c-Src IgG (A) or anti-Met IgG (B). The immune complexes were separated by 8% SDS-PAGE and immunoblotted with anti-Met IgG (A) or anti-c-Src IgG (B). Protein molecular mass standards are shown on the right. This experiment was done twice with similar results.

80 °C under a vacuum. Autoradiograms were produced and quantitated using a Storm PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

In some experiments (see Fig. 3), c-Src kinase activity was assayed according to Cheng et al. (52) using the c-Src tyrosine kinase family-specific cdc2 peptide substrate. Anti-c-Src or anti-Met immunoprecipitates prepared as above were incubated with 40 µl of a reaction buffer (100 mM Tris-HCl, pH 7.0, 0.4 mM EGTA, 0.4 mM Na3VO4, 40 mM Mg(OAc)2), 5 µl of cdc2 peptide (Life Technologies, Inc., 250 µM/assay), 5 µl of cold ATP (25 µM), and 2.5 µCi of [γ-32P]ATP. A control consisting of immunoprecipitation with anti-Met IgG under more stringent conditions with RIPA buffer to prevent co-precipitation of other proteins (see “Experimental Procedures”). The immunoprecipitates were used in an in vitro c-Src kinase assay with the c-Src kinase family-specific cdc2 peptide substrate. The amount of radiolabeled cdc2 substrate was determined and plotted as c-Src kinase activity (cpm/well) as in Fig. 3. B, equal amounts of cell lysates derived from serum-starved SP1 cells were immunoprecipitated with anti-Met antibody or anti-c-Src antibody under more stringent conditions with RIPA buffer to prevent co-precipitation of other proteins (see “Experimental Procedures”). The immunoprecipitates were used in an in vitro c-Src kinase assay with the c-Src kinase family-specific cdc2 peptide substrate. As a control, a reaction containing no protein (None) was carried out concurrently. Results are plotted as c-Src kinase activity (cpm/well) as in A. Anti-Met immunoprecipitates under these more stringent conditions showed no significant phosphorylation of the cdc2 substrate.
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RESULTS

Detection of Elevated c-Src Tyrosine Kinase Activity in SP1 Carcinoma Cells—SP1 carcinoma cells express HGF and tyrosine-phosphorylated Met, consistent with an HGF autocrine loop in these cells (44). To test the possibility that activation of c-Src kinase may be involved in Met-induced signaling pathways, we measured the kinase activity of c-Src in SP1 carcinoma cells and an HGF-sensitive epithelial cell line, Mv1Lu. c-Src kinase activity was measured by the capacity of c-Src immunoprecipitates from these cells to tyrosine phosphorylate the substrate, enolase. c-Src immunoprecipitates from serum-starved SP1 cells showed a pronounced elevated kinase activity, which increased only slightly following treatment with exogenous HGF (Fig. 1). In contrast, c-Src kinase activity in Mv1Lu cells was highly dependent on stimulation of cells with exogenous HGF (Fig. 1). The levels of c-Src kinase activity observed correlated with the constitutive tyrosine phosphorylation of Met (44) and in vitro Met kinase activity (data not shown) in SP1 cells, and the HGF-induced tyrosine phosphorylation of Met in Mv1Lu cells (Ref. 26 and data not shown).

Association of c-Src Kinase Protein and Activity with Activated Met—It is conceivable that the high level of c-Src kinase activity in SP1 cells, could have resulted from interaction of c-Src with activated Met due to an autocrine HGF loop in these cells (44). To test for interaction of c-Src kinase family proteins with activated versus nonactivated Met, we first examined the association of c-Src with Met in Mv1Lu cells that express Met but not HGF. Serum-starved Mv1Lu cells were incubated alone or with HGF, and cell lysates were immunoprecipitated with anti-Met IgG or anti-c-Src IgG. Protein precipitates were electrophoresed and subjected to Western blotting with anti-c-Src IgG or anti-Met IgG, respectively. As shown in Fig. 2 (A and B), an increased amount of c-Src protein was recovered from anti-Met immunoprecipitates and vice versa in cell lysates from HGF-treated Mv1Lu cells compared with untreated Mv1Lu cells. We also showed that association of c-Src kinase with Met occurred via the SH2 domain of c-Src and correlated with tyrosine phosphorylation of Met (data not shown). It should be noted that a trace amount of c-Src protein was detected in lysates of unstimulated cells immunoprecipitated with anti-Met IgG and blotted with anti-c-Src IgG, possibly due to incomplete starvation of these cells before HGF stimulation (Fig. 2B).

To determine whether elevated activity of c-Src kinase in SP1 cells correlates with its ability to associate with Met, serum-starved SP1 cells were immunoprecipitated with anti-Met IgG or anti-c-Src IgG, and immunoprecipitates were tested for the ability to tyrosine phosphorylate the c-Src kinase family-specific cdc2 peptide substrate (52). As shown in Fig. 3A (bars I and II), similar amounts of c-Src kinase protein and activity were recovered from immunoprecipitates of both anti-Met and anti-c-Src antibodies. In contrast, immunoprecipitates from anti-Met IgG under more stringent conditions with RIPA buffer where c-Src is not co-precipitated resulted in no signifi-
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Fig. 5. Expression of dominant negative mutant SRC-RF does not alter Met protein levels or activity and downstream signaling. A, SP1 cells transfected with SRC-RF or SRC or untreated SP1 cells were prestarved overnight and lysed as described in the legend to Fig. 1. Equal amounts of protein from each lysate were concentrated on Microcon 10 filters (Amicon Inc., Beverly, MA) and analyzed by Western blotting with anti-Met IgG (top panel). The blot was stripped and reprobed with anti-phosphotyrosine antibody (middle panel). Cell lysates were also immunoprecipitated with anti-Met IgG, and immunoprecipitates were subjected to an in vitro Met kinase assay as described under “Experimental Procedures.” The autoradiogram depicting 32P-labeled anti-Met IgG. Immuno-precipitates were subjected to a 7% SDS-PAGE and transferred to nitrocel- lulose. The blot was probed with anti-PCL-gamma IgG (top panel) before being stripped and reprobed with anti-phosphotyrosine antibody (bottom panel). This experiment was done twice with similar results. IP, immunoprecipitation; IB, immunoblot.

significant phosphorylation of cdc2 peptide (Fig. 3B), confirming the specificity of the cdc2 peptide as a substrate for c-Src (52). Thus a significant portion of c-Src kinase activity is associated with activated Met in SP1 cells. To further evaluate the contribution of c-Src association with Met, the supernatant from the immuno-precipitate of anti-Met IgG was immunoprecipitated for a second time with anti-c-Src IgG and subjected to the in vitro c-Src kinase assay. As shown in Fig. 3A (bar III), some c-Src kinase activity was detected in the Met-depleted SP1 cell lysate; however, it was with much lower activity, corresponding to the reduced amount of c-Src protein present. Immunoprecipitation of SP1 cell lysates with higher concentrations of anti-Met IgG and subsequently with anti-c-Src IgG showed a similar result (data not shown). These results demonstrate that the majority of c-Src kinase activity correlates with its ability to associate with Met in SP1 cells.

c-Src Kinase Activity Is Required for Colony Growth in Agar, but Not Cell Proliferation on Plastic—SP1 cells exhibit paracrine stimulation by HGF of colony growth in agar and proliferation on plastic (45, 49). To determine whether c-Src kinase activity is required for HGF-induced proliferation or colony growth in agar, an expression vector (SRC-RF) containing cDNA encoding a dominant negative double mutant of c-src with loss-of-function mutations in the kinase domain (K295R) and a regulatory tyrosine residue (Y527F) (47) was stably transfected into SP1 cells. A control consisted of cells transfected with the same vector expressing wild type c-src (SRC). Uncloned (pooled) transfected cells were selected in G418-containing medium and assessed for c-Src kinase activity and HGF-induced functions. For in vitro c-Src kinase assays, immunoprecipitation with anti-c-Src IgG was carried out at antibody excess, indicating that the majority of wild type c-Src protein was present in immunoprecipitates. The results showed that c-Src kinase activity was strongly reduced in SRC-RF transfected SP1 cells compared with SRC transfected or untransfected cells incubated alone, or following stimulation with 40 ng/ml HGF or 7% FBS (Fig. 4). However, tyrosine phosphorylation of Met or an unrelated signaling molecule PLC-gamma 1 and in vitro autophosphorylation of Met remained unaffected in SRC-RF- or SRC-transfected SP1 cells, compared with untransfected cells (Fig. 5). These results demonstrate
cells were prestarved overnight, and each cell line was plated at 10^4 cells/well in 24-well plates in 0.25% FBS without or with HGF at the concentrations indicated. Controls consisted of cultures with 7% FBS. DNA synthesis was measured as described under “Experimental Procedures.” Results are expressed as relative mean [3H]thymidine incorporation compared with control (no HGF) (mean cpm/well ± S.D. of triplicates). This result is representative of four experiments.

specificity of the inhibitory effect of SRC-RF on c-Src kinase activity.

Expression of the dominant negative SRC-RF mutant in SP1 cells significantly inhibited FBS- and HGF-induced colony formation in soft agar, compared with SRC-transfected or untransfected SP1 cells (Fig. 6). Similarly, a subclone of SP1 cells expressing SRC-RF showed a marked reduction in colony formation, compared with a wild type SRC-transfected subclone or untransfected SP1 cells (data not shown). In contrast, SRC-RF-transfected SP1 cells showed no difference in HGF-induced or serum-induced proliferation on plastic, compared with SRC-transfected or untransfected SP1 cells (Fig. 7). Thus reduction of c-Src kinase activity in SRC-RF-transfected cells abrogated HGF- or serum-induced colony growth in soft agar but had no effect on cell proliferation on plastic.

c-Src Kinase Activity Is Required for HGF-induced Cell Motility—Because c-Src kinase activity has been shown to modulate cell motility in several cell types (36–38), we examined the role of c-Src kinase in HGF-induced cell motility in SP1 cells. Our results showed that HGF strongly stimulated motility of SP1 cells through collagen-coated porous membranes in a paracrine manner. HGF-induced motility was significantly reduced in SP1 cells transfected with dominant negative mutant SRC-RF, compared with SRC-transfected or untransfected cells (Figs. 8 and 9). Similar results were obtained using a wound healing assay (data not shown). These results are consistent with a role of c-Src kinase in HGF-induced cell motility.

DISCUSSION

We (6) and others (7, 8) have previously shown that HGF and Met mRNA are strongly co-expressed in invasive carcinomas in human breast cancer. These findings suggest that signals transduced by activated Met confer survival and growth advantage to carcinoma cells during progression to metastasis. This concept is further supported by the observation that cells transfected with an activated version of met (tpm-met) acquire invasive and metastatic properties (15, 16). Unlike most other receptor tyrosine kinases, Met shows one high affinity binding site for the majority of SH2-containing cytoplasmic effectors, suggesting that these proteins bind Met in a competitive manner (23–25). Therefore, to study the role of specific SH2-containing cytoplasmic effectors in HGF receptor function, approaches to target individual cytoplasmic effectors are required.

To analyze downstream effector molecules in HGF-induced tumorigenic properties of mammary carcinoma cells, we have studied a mouse mammary carcinoma, SP1, which co-expresses HGF and Met (44). However, depending on culture conditions, both paracrine and autocrine effects of HGF have been observed in SP1 cells (44, 45, 49). In monolayer cultures, autocrine phosphorylation of Met at tyrosine in SP1 cells without addition of exogenous HGF was observed (44). In contrast, tyrosine phosphorylation of Met was reduced in suspended SP1 cells and can be restored by addition of exogenous HGF. These observations suggest that the base level of Met activation may be influenced by extracellular environmental conditions, such as cell adhesion to various substrata (53), cell density effects on HGF expression and secretion (54), or proteolytic processing of pro-HGF to the biologically active form (55). In the present report, paracrine stimulation with exogenous HGF was required for optimal cell proliferation, motility, and colony growth in agar under serum-starved conditions. Previous studies showed that PI 3-kinase activity is elevated in SP1 cells and that its activity is required for HGF-induced proliferation in monolayer culture. Treatment of SP1 cells with wortmannin, a potent inhibitor of PI 3-kinase (56), or transfection of a dominant negative mutant of the p85 subunit of PI 3-kinase into these cells (26) inhibited HGF-induced cell proliferation in monolayer culture.

In the present report, we show that c-Src kinase activity is elevated in SP1 mammary carcinoma cells compared with nonmalignant Mv1Lu epithelial cells and is associated with Met. The elevated level of c-Src kinase activity in SP1 cells and its association with Met strongly suggest that this signaling mol-
ecule may be involved in intracellular events triggered by HGF. This observation prompted us to test whether expression of a dominant negative mutant form of c-Src influences growth of SP1 cells. Expression of a dominant negative form of c-Src (SRC-RF) in SP1 cells showed no significant effect on HGF-induced cell proliferation on plastic but markedly inhibited HGF- or serum-induced colony growth in soft agar. Thus activation of c-Src kinase is essential for colony formation in agar by SP1 cells but appears not to be required for cell proliferation on plastic.

Other laboratories have reported variable effects of c-Src kinase on cell growth. In support of our observations, Demali and Kazlauskas (57) have shown that a mutant form of PDGF β-receptor that cannot bind or activate, c-Src, retains the ability to stimulate growth of fibroblasts on plastic or in agar in response to PDGF. In contrast, Courtneidge and co-workers (33, 58) have shown that microinjection of a kinase dead mutant c-Src or neutralizing antibodies that inhibit basal and stimulated c-Src kinase activity inhibited PDGF-dependent DNA synthesis in fibroblasts. Similarly, constitutive expression of c-Src mutants inhibited PDGF and epidermal growth factor-induced mitogenesis of mouse embryonal fibroblasts lacking c-Src (34). The apparent differences in the role of c-Src kinase in the above systems could be due to different levels of residual basal activity of c-Src kinase or the developmental and malignant status of the cells used. Our observation that anchorage-independent growth but not proliferation on plastic is inhibited in cells expressing dominant negative SRC-RF suggests that the reduced level of c-Src kinase activity in SRC-RF expressing SP1 cells is insufficient to support anchorage-independent growth, whereas proliferation on plastic remains unaffected. c-Src-independent signaling mechanisms may also promote HGF-induced proliferation of SP1 cells on plastic.

We have also shown that SP1 cells transfected with the dominant negative SRC-RF mutant showed reduced cell motility in response to HGF compared with SRC-transfected or untransfected SP1 cells. Thus c-Src kinase activity is required for HGF-induced cell motility in SP1 carcinoma cells, although complementary signaling molecules may also be involved. This observation reflects recent reports that c-Src kinase activity is required for epithelial cell scattering (38–40, 50) and organi-
zation of the cortical cytoskeleton (50). In addition, Richardson et al. (59) have shown that co-expression of c-Src in cells expressing the dominant negative C-terminal domain of focal adhesion kinase can reconstitute cell spreading and motility and induces tyrosine phosphorylation of paxillin. Together, these observations raise the possibility that HGF-induced c-Src kinase activity may regulate cell motility through the cytoskeletal complex. This possibility is currently being investigated.

There is now growing evidence that the c-Src family protein-
tyrosine kinases are involved in signal transduction pathways that result in cell growth, adhesion, and differentiation. c-Src kinase activity is required for cell proliferation induced by platelet-derived growth factor, colony-stimulating factor-1, and epidermal growth factor (51, 60), and increased c-Src kinase activity is associated with many cancers. These observations support the notion that increased c-Src kinase activity in mammary carcinomas may play an important role in mammary tumor growth and development. Our findings involving transfection of a dominant negative c-Src kinase-defective mutant into SP1 cells represent the first direct demonstration of a requirement for c-Src kinase activity in HGF-induced cell mo-

Fig. 9. Photomicrographs of migrating SP1 cells transfected with SRC or SRC-RF or untransfected SP1 cells following HGF stimulation. SP1 cells untransfected (A and B) and transfected with SRC (C and D) or SRC-RF (E and F) were serum-starved overnight and assessed for cell motility without (−) or with (+) HGF (20 ng/ml) as described in the legend to Fig. 8. After removing non-migrating cells on the upper side of the membrane, membranes were mounted onto glass slides, and migrating cells were photographed using a Leitz microscope with phase contrast illumination. Photographs correspond to the groups shown in Fig. 8. Original magnification, 250×.
tility and anchorage-independent growth of carcinoma cells, although interactions with other signaling molecules may also be involved. These data strongly suggest that HGF-induced activation of c-Src kinase with Met and its activation are important in growth and transformation of mammary carcinomas and further argue that paracrine and autocrine HGF loops play a significant role in the transformed phenotype of some mammary carcinomas.

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