Activated fibroblast growth factor receptor 1 (FGFR1) propagates FGF signals through multiple intracellular pathways via intermediates FRS2, PLCγ, and Ras. Conflicting reports exist concerning the interaction between FGFR1 and Src family kinases. To address the role of c-Src in FGFR1 signaling, we compared proliferative responses of murine embryonic fibroblasts (MEF) deficient in c-Src, Yes, and Fyn to MEF expressing either endogenous levels or overexpressing c-Src. MEF with endogenous c-Src had significantly greater FGF-induced DNA synthesis and proliferation than cells lacking or overexpressing c-Src. This was related directly to c-Src expression by analysis of c-Src-deficient cells transfected with and sorted for varying levels of a c-Src expression vector. This suggests an “optimal” quantity of c-Src expression for FGF-induced proliferation. To determine if this was a general phenomenon for growth factor signaling pathways utilizing c-Src, responses to epidermal growth factor (EGF), platelet-derived growth factor (PDGF), and lysophosphatidic acid (LPA) were examined. As for FGF, responses to EGF were clearly reduced DNA synthesis and proliferation than cells lacking or overexpressing c-Src. The data show that mitogenic pathways activated by FGF-1 and EGF are regulated by c-Src protein levels and appear to differ significantly from those activated by PDGF and LPA.

Fibroblast growth factors (FGFs) comprise a family of 23 polypeptides that induce mitogenic, angiogenic, and chemotactic responses in cells of mesodermal and neuroectodermal origin (1–3). The FGF signaling pathway also plays a significant role in normal development, and increased FGF production is associated with chronic immunologic injury as well as tumor development and metastasis (4–6). Such a diverse array of biological effects occurs through ligand interaction with high affinity cell surface receptors (FGFR1–4) that are structurally similar and exhibit a high degree of sequence homology at the amino acid level (3, 7). The full-length FGFR exhibits three extracellular immunoglobulin-like domains, a single transmembrane domain, and a split intracellular tyrosine kinase domain (7). Ligand binding causes receptor dimerization allowing trans autophosphorylation of intracellular tyrosine residues and activation of intrinsic kinase activity (8).

Activation of FGFR1 results in tyrosine phosphorylation of multiple signaling and adaptor proteins, including FRS2 (FGF receptor substrate 2/SNT-1), Shc, Grb2, Ras/Raf, Crk, phosphatidylinositol 3-kinase, SHP-2, and Src (reviewed in Ref. 9). A constitutive association of the multidocking protein FRS2 at the juxtamembrane segment of FGFR1 occurs independently of receptor activation (10). Interaction of FRS2 with Ras is associated with cell cycle progression (11). Numerous other studies provide evidence that FGF activation of the Ras/MAPK pathway contributes mainly to cellular proliferation and differentiation (10, 12, 13).

Multiple studies have demonstrated activation of the non-receptor Src family tyrosine kinase members in response to activated FGFR1 (14–16). An association of c-Src and FGFR1 has been reported by immunoprecipitation of recombinant receptor (14), however, this finding has not been reported elsewhere. Transient exposure of 3T3 cells to FGF-1 induces continuous activation of the Src kinase pathway, which in turn stimulates increases in the level of c-myc (12). In contrast, Landgren et al. (17) have shown that mutation of FGFR1 tyrosine residue 766 to phenylalanine causes an increase in activation of Src family members, suggesting a regulatory role of protein kinase C in Src activation. The involvement of c-Src in migration of rat bladder carcinoma cells and murine fibroblasts in response to FGF-1 has also been reported (12, 18, 19). Similarly, the direct association of phosphatidylinositol 3-kinase and c-Src in an SH3-dependent manner suggests that these intracellular signaling proteins may act in concert downstream of FGFR1 to coordinate cellular motility (20).

The nine known Src family kinase members exhibit functional redundancy as evident from mice that are homozygous deficient for single versus multiple Src kinases. The predominant abnormality of Src−/− mice is osteopetrosis (21) and mice deficient in other individual Src family kinases exhibit mild phenotypes (reviewed in Ref. 22), whereas multiply deficient animals demonstrate severe phenotypes or lethality (23, 24). With such overlap in function, it is difficult to delineate the exact role of Src family kinases in FGF signaling. Therefore,
the absence of one family member may not eliminate FGFR-driven biological responses if other members are available to compensate.

To assess the role of c-Src in cells with physiologic levels of FGFR1, we examined the effect of varying levels of c-Src expression on FGFR1-induced proliferation of murine embryonic fibroblasts deficient for Yes and Fyn. These fibroblasts express either no c-Src, endogenous levels, or 10-fold excess c-Src (24). Cells with endogenous levels of c-Src exhibited enhanced proliferation to FGFR when compared with c-Src-deficient cells. Unexpectedly, overexpression of c-Src also inhibited FGFR1-proliferative responses. These findings were confirmed using deficient cells that were transfected and sorted by FACS for varying levels of c-Src. Similarly, endogenous levels of c-Src resulted in maximal response to EGF while overexpression was inhibitory. In contrast, cell growth in response to PDGF was minimally affected by c-Src overexpression. The data suggest that these two classes of growth factors differ in their regulation of MEF-proliferative responses by c-Src.

EXPERIMENTAL PROCEDURES

Cell Lines and Culture—Mouse embryonic fibroblasts (MEF) deficient in c-Src, Yes, and Fyn (SFFV) were obtained from the ATCC and kindly provided by Dr. Graham Carpenter, Vanderbilt University. Src −/− (MEF deficient for Yes and Fyn with endogenous c-Src expression) and cSrc (SFFV cells deficient for Yes and Fyn but overexpressing c-Src) cells were obtained from the ATCC. Src4 cells (SFFV cells transfected to express endogenous levels of c-Src protein comparable to Src −/− cells) were kindly provided by Dr. Leslie Cary, Fred Hutchinson Cancer Research Center. These MEF have been described previously and were also determined to be deficient in Lck and Lyn (24). The cells were maintained at 37 °C in DMEM containing 4.5 g/liter glucose and 1.5 g/liter sodium bicarbonate supplemented with 10 mM pyruvate, 10 mM HEPES, 10% FBS, and 500 g/ml G418. For experimentation, SYF and cSrc cells were plated at 0.5 × 10⁶ cells per 100-mm² dish (unless otherwise specified) in 10% FBS/DMEM supplemented with 10 μg/ml heparin and 1 μg/ml recombinant human FGF-1 (R&D Systems) for 10 min (unless otherwise specified) at 37 °C. For detection of phospho-tyrosine proteins, cells were washed with ice-cold PBS containing 100 μM sodium orthovanadate and scraped into boiling 2× SDS sample buffer for sonication. Samples were stored at −20 °C. BALB/c mouse aortic endothelial cells (MAECs) have been described previously (25) and were provided by Dr. Keith Bishop.

RNA Extraction and Reverse Transcriptase-PCR—Total RNA was isolated from fibroblasts using TRI Reagents (Molecular Research Center, Inc., Cincinnati, OH) according to the manufacturer's specifications. First-strand cDNA was synthesized from 2 μg of total RNA using oligo-dT and SUPERSCRIPT RNase H-reverse transcriptase (Invitrogen). Each PCR reaction contained cDNA equivalent to 80 ng of template DNA. FGFR1 transcripts were detected using oligonucleotide PCR primers designed to amplify the transmembrane and kinase domains of hFGFR1 as previously described (4). Sense (5′-GAC AA(A/G) GA(G/A) AG ATG GTG CTG CT-3′) and antisense (5′-GTT (G/A) TA GCA GTA (T/C) TCG CAC CC-3′) primers each exhibited 90% homology to the murine sequences (human/mouse). FGFR2 transcripts were detected using oligonucleotides designed against the transmembrane and kinase domains of hFGFR2. Sense (5′-GTC CAT CAA (T/C)CCA CAC (G/T) CCA CCT CCT G-3′) and antisense (5′-AAT CAT CT CAT CCA CGT TAC G-3′) primers were used as previously described (4) with slight modification of cycling parameters as follows: 94 °C for 1 min, 56 °C for 30 s, and 72 °C for 30 s (35 cycles). All PCR reactions included H₂O only (no DNA) as a negative control. The housekeeping glycereraldehyde-3-phosphate dehydrogenase was used as a positive control of RNA expression and was amplified over 30 cycles using sense (5′-ATG GAC CTC ATC CCA TCA TCT GCC AGG-3′) and antisense (5′-ACA TCT AGA ACC ATC ACG AGG CAC CAG TCT CCC-3′) primers. Amplified products were analyzed by separation on 1% agarose gels containing 0.4 μg of ethidium bromide.

Flow Cytometry with Fr-FGF—FGFR1 expression at the cell surface was detected by FACS analysis using a modified method of enzymatic amplification staining (Flow-Array Systems, Ltd.). Briefly, 3 × 10⁶ cells were washed with Hanks' buffered saline solution and resuspended in PBS containing 0.2% BSA and 2.5 μg/ml heparin. Fr-FGF (28) was added (100 ng/ml), and the cells were rotated at 4 °C for 1 h. Cells were collected by brief microcentrifugation and rapidly washed with PBS containing 250 μg/ml heparin to remove ligand from low affinity cell surface heparan sulfate proteoglycan binding sites. Samples were subsequently incubated with HRP-anti-human IgG (diluted 1:200 in 2% PBS/BSA) for 20 min at 4 °C. The cells were collected by microcentrifugation, washed with PBS, and stained with Flow-Array EAS amplifier and Streptavidin-fluorescein isothiocyanate according to the manufacturer's protocol. Samples were fixed in 1% paraformaldehyde and analyzed by flow cytometry.

Antibodies and Western Immunoblotting—Whole cell lysates (20 μg/lane) from MEF cultures incubated in the presence or absence of FGF-1 (10 ng/ml) were separated by 10% SDS-PAGE and transferred to Immobilon nitrocellulose (Millipore) for protein immunodetection. The anti-Src clone GD11 monoclonal antibody (Upstate Biotechnology) was used according to the manufacturer's recommendations. Briefly, membranes were blocked for 20 min at RT in 3% milk/PBS and antibody was diluted 1:1000 in block solution for membrane incubation overnight at 4 °C. The PY20 monoclonal anti-phospho-tyrosine antibody (Transduction Laboratories) was used at 1:1000 in 1% BSA/TBS-T (10 mM Tris (pH 7.5), 100 mM NaCl, 0.1% Tween 20) for 1 h at RT. Fg (C-15) rabbit polyclonal anti-FGFR1 (Santa Cruz Biotechnology) was diluted 1:5000 in 5% nonfat dry milk/TBS-T for 1 h of membrane incubation at RT. Blots were subsequently incubated with either horseradish peroxidase-conjugated (HRP)-goat anti-mouse IgG (Chemicon International) or HRP-goat anti-rabbit IgG (Southern Biotechnology Associates, Inc.) diluted 1:5000 in the appropriate block solution for 45 min at RT. Protein bands of interest were detected by enhanced chemiluminescence. Membranes were stripped for 30 min at 70 °C using stripping solution (62.5 mM Tris (pH 6.8), 2% SDS, and 100 μM β-mercaptoethanol) for reprobing with relevant antibodies.

Thymidine Incorporation Assay—SYF cells were plated at 2.5 × 10⁴ cells/well in 96-well flat-bottom plates. Src −/− and cSrc cells were plated at 2.5 × 10⁴ cells/well in 96-well plates. Cultures were incubated in 10% FBS/DMEM for 24 h, washed with sterile PBS, and serum-starved in 0.1% FBS/DMEM overnight. Cells were subsequently incubated for 22 h in 0.2% BSA/DMEM with 10 units/ml heparin supplemented with either FGF-1, EGF (R&D Systems), PDGF-B (R&D Systems), or LPA (Sigma) as indicated. [3H]Thymidine was added at 1 μCi/well for the final 17 h of culture, and incorporation was determined by liquid scintillation counting. Experiments were performed four times. For each condition, the mean incorporation of six wells was determined and normalized as a -fold increase over the basal average ± S.F. For experiments comparing various growth factors, cell lines were plated at equal density (1.25 × 10⁵ cells per well) for thymidine incorporation. Data are expressed as mean ± S.E. of six individual wells.

Cellular Proliferation—MEF cells were plated in 24-well dishes at 2.5 × 10⁵ cells/well in 10% FBS/DMEM. After 24 h the media was aspirated and the cells were washed with SF/DMEM. Cultures were inculbated in 0.2% BSA/DMEM supplemented with 10 units/ml heparin in the presence or absence of FGF-1, EGF, PDGF-B (all at 10 ng/ml), or 10% FBS for 5 days. Unless otherwise specified, cells were harvested with trypsin/EDTA, collected by microcentrifugation, and resuspended in 10% FBS/DMEM for visual counting every day. Culture media was replaced at day 3 of stimulation. Data are expressed as mean ± S.E. of three independent experiments.

Transfection of SYF Cells—The pLXSH.mSrc retroviral vector containing mouse non-neuronal Src (mSrc) was made by Dr. Lionel Arnaud and kindly provided by Dr. Leslie Cary, both of the Fred Hutchinson Cancer Research Center (Seattle, WA). The mSrc sequence was excised from pLXSH by BamHI digestion and ligated to the BamHI sites of pRES2-EGFP expression vector (Clontech, kindly provided by Dr. Tomonobu Nii, Japan). The mSrc sequence was cloned into pLXSH vector and grown to 70% confluency in 75-cm² plastic tissue culture flasks and transiently transfected with empty vector or mSrc.EGFP DNA using SuperFect transfection reagent (Qiagen) according to the manufacturer's specifications. Briefly, DNA was incubated with 1.5 SuperFect for 10 min at RT was found to provide optimal EGFP expression and cellular viability and was selected for 3 h. The cultures were then infected with fresh media overnight prior to experimentation. FACS analysis determined transfection efficiency to be ~19% in the vector control population and ~35% in the mSrc.EGFP population (not shown).

Immunofluorescent Detection of c-Src—SYF cells were plated on eth-
FGFR expression has not been characterized in SYF, Src4, or FGFR2. Products were separated by 4-μg/ml ethidium bromide. Glyceraldehyde-3-phosphate dehydrogenase cDNA was amplified as a loading control (bottom). M, molecular weight markers; Y, SYF; 4, Src4; +, Src−; c, cSrc; J, C2-14 Jurkat R1 (FGFR1 control); E, MAEC (FGFR2 control); −, H2O.

FIG. 1. Murine embryonic fibroblasts express FGFR1 mRNA. Total RNA was extracted from fibroblast cultures and reverse-transcribed to cDNA. Primers were designed to amplify regions encoding the transmembrane and kinase domains of either FGFR1 (top) or FGFR2 (middle). Samples were gated for sorting of low and high level EGFP-expressing by FACS analysis comparing transfected cells to the SYF parental line.

RESULTS

Functional FGFR1 Is Expressed at the Cell Surface of MEF—FGFR expression has not been characterized in SYF, Src4, Src−, or cSrc mouse embryonic fibroblasts. Therefore, we examined the MEF for FGFR1 and FGFR2 mRNA expression by reverse transcriptase-PCR. Jurkat C2-14, a T cell tumor line expressing stably transfected FGFR1, was used as a positive control for FGFR1 expression. BALB/c mouse aortic endothelial cells (MAECs), which express only FGFR2, were used as the positive control for FGFR2 expression (25). FGFR1 mRNA was expressed by all MEF (Fig. 1, top) but FGFR2 mRNA was not (Fig. 1, middle). To confirm expression of FGFR1 protein capable of ligand binding, FACS analysis was performed using an anti-FGF fusion protein (26). After incubation with Fe-FGF, the fibroblasts were washed with heparin to remove ligand from low affinity heparan sulfate proteoglycan binding sites on the cell surface. Significant binding of Fe-FGF to high affinity cell surface receptor binding sites was observed for all fibroblasts (Fig. 2A). The mean fluorescence intensity, a reflection of the number of receptors per cell, increased modestly with enhanced c-Src expression.

Derivation of the multiply deficient SYF cell lines required immortalization of the primary cells with SV40 large T antigen (24). c-Src was reintroduced into SYF to produce cSrc, a line with 10-fold greater expression than wild-type cells but which has the same clonal origin as SYF (24). The Src− line was derived from a different embryo and therefore may have clonal differences from SYF and cSrc in addition to differences arising from levels of c-Src protein expression. To overcome this problem, we also examined the Src4 MEF line, which was derived from SYF cells and expresses endogenous levels of c-Src protein. Expression of 60-kDa c-Src was confirmed by immunoblotting with anti-c-Src antibody (Fig. 2B). As expected, c-Src was not detected in SYF cells, was present at comparable levels in Src4 and Src− cells, and was overexpressed in cSrc cells. Expression of the non-glycosylated and glycosylated forms of full-length FGFR1 was confirmed in all cell lines as proteins of 125 and 145 kDa (Fig. 2C). A slight retardation of mobility was observed for the 145-kDa fully glycosylated form with FGF stimulation, reflecting tyrosine phosphorylation predominantly of the cell surface receptor. A protein of 90-kDa

FIG. 2. FGFR1 expressed at the cell surface of MEF are capable of activation by ligand binding. A, detection of cell surface FGFR1 expression by flow cytometry. Shaded tracings are cells incubated with Fe-FGF, anti-human IgG-HRP, and streptavidin-fluorescein isothiocyanate. Open tracings are cells stained in the same way without Fe-FGF. B, Western immunoblotting of SYF, Src4, Src−, and cSrc whole cell lysates (20 μg/lane) in the absence (−) or presence (+) of 10 ng/ml FGF-1 (10 min) using anti-c-Src antibody. C, the same membrane immunoblotted for FGFR1. D, total cellular tyrosine phosphorylation detected by immunoblotting with anti-phosphotyrosine antibody. The arrow denotes enhanced phosphorylation of 90-kDa PRL2 in the presence of FGF-1. The molecular weight marker is indicated at the left. Blots are representative of three independent experiments.
molecular mass was visualized in all FGF-1-stimulated MEF by phosphotyrosine immunoblotting (Fig. 2D, arrow). Molecular size suggests this is FRS2, and its phosphorylation confirms activation of FGFR1. As expected, overexpression of c-Src also caused a marked increase in total tyrosine phosphorylation.

FGF-1-induced Mitogenic Activity Is Regulated by the Level of c-Src Expression—Experiments using neutralizing antibodies or dominant-negative forms of c-Src have shown that, in fibroblasts, mitogenic responses to EGF, PDGF, and CSF-1 depend upon Src kinase activity (27, 28). In contrast, Zhan et al. (14) have reported that FGF-induced proliferation of NIH 3T3 cells was not dependent on c-Src, and Klinghoffer et al. (24) suggest that c-Src, Yes, and Fyn are largely dispensable for PDGF-induced proliferation and signaling, because SYF cells exhibit mitogenic responses to this growth factor. To examine MEF mitogenic response to FGF-1, serum-starved cells were stimulated with FGF-1, and DNA synthesis was measured by [3H]thymidine incorporation. A dose response was observed, and FGF-1 at 1 ng/ml stimulated maximal incorporation in all cell lines (Fig. 3). Src−/− cells, however, demonstrated significantly enhanced incorporation compared with either the deficient SYF cells or the overexpressing cSrc cells. Src4 cells, which have the same clonal derivation as SYF and cSrc but express endogenous levels of c-Src, also exhibited greater thymidine incorporation than SYF or cSrc cells at all concentrations of FGF-1 examined (data not shown). These results suggested that endogenous levels of c-Src expression are optimal for transition of quiescent fibroblasts from G0 to G1 phase of the mitotic cycle in response to FGF-1.

MEF growth was examined to determine the effects of c-Src expression and FGF-1 stimulation on later phases of the mitotic cycle (Fig. 4). SYF, Src−/−, and cSrc cells were plated at equal density in serum and exposed to FGF-1 for 5 days. In the absence of serum or FGF (Fig. 4A, black bars), the number of viable cells ultimately declined. Not surprisingly, the most rapid loss of viability occurred in c-Src-deficient SYF cells. However, in response to FGF, Src−/− cells unexpectedly showed significantly greater proliferation than cells overexpressing cSrc (Fig. 4A, middle versus lower panels). To confirm that these results were not a consequence of the Src−/− cell line being derived from a different embryo (24), the study was repeated...
and included Src4 cells that were derived from the same embryo as SYF and cSrc cells (Fig. 4B). Src4 and Src−− cells expressing endogenous levels of c-Src again demonstrated significantly greater cell growth than either SYF or overexpressing cSrc cells. In contrast, growth in FBS correlated directly with the quantity of c-Src, and no inhibition was seen with overexpression (Fig. 4C).

These data demonstrate that, although c-Src is not essential for FGF-1-stimulated MEF growth, endogenous levels of expression are optimal for maximal proliferation. To quantify this observation and eliminate clonal variability, the deficient SYF parental population. Bars indicate gated populations isolated by sorting. A high level of expression of c-Src suppressed low level c-Src-expressing cells. In contrast, responses to FGF and EGF compared with deficient cells. For both overexpressing cSrc cells, Src4 cells that express endogenous levels of c-Src showed maximal DNA synthesis to all stimuli, but some striking differences were apparent. Restoration of endogenous levels of c-Src resulted in significantly enhanced response to FGF and EGF compared with deficient cells. For both FGF-1 and EGF, the response of overexpressing cSrc cells was not different from deficient cells. In contrast, responses to
required for EGF-, PDGF-, and CSF-1-stimulated entry to the S-phase of the mitotic cycle in fibroblasts (27–29). Similarly, it has been reported that c-Src, Fyn, and Yes are activated at mitosis and play a role in cellular division at the G2-M transition phase (29, 30). Here we show that SYF, Src4, Src−/−, and cSrc MEF express functional FGFR1, but not FGFR2, similar to human fetal fibroblasts (31). All MEF responded to FGF-1 as measured by thymidine incorporation and increased cell number, indicating that FGF-1 can induce proliferation even in the absence of Src family kinases. Surprisingly, however, cells expressing endogenous levels of c-Src showed greater responsiveness to FGF than both Src-deficient and Src-overexpressing cells. This was the case for both clonally derived cell lines and Src-transfected SYF cells, demonstrating that the effect is due to Src and not simply a result of clonal origin.

Our results differ from Liu et al. (19) who reported that Src deficiency did not affect FGF-1-induced proliferation in cells from Src−/− mice compared with Src+/+ mice. The difference likely reflects the compensatory effects of Fyn and Yes that were expressed in Src−/− cells used previously versus the absence of other Src family kinases in the cells used here. In addition, inclusion of high concentrations of insulin in their medium may have activated insulin and IGF receptors thereby stimulating the Ras/Raf/MEK pathway and masking the effects for FGF (reviewed in Ref. 32).

In contrast to the experiments with neutralizing antibodies and dominant-negative Src, Klinghoffer et al. (24) reported that c-Src is dispensable for PDGF- and LPA-induced proliferation. They found no significant difference in thymidine incorporation between deficient SYF cells and overexpressing cSrc cells. The 5-fold increase in total cell number for cSrc versus 3-fold increase for SYF cells in response to PDGF was not specifically addressed, and cells expressing endogenous levels of c-Src were not examined. Our results for PDGF-induced DNA synthesis (Fig. 7A) and proliferation (Fig. 7B) of cSrc versus SYF cells are consistent with those of Klinghoffer. Using cells with endogenous levels of c-Src, we now show that the quantity of c-Src also has little effect on PDGF-induced proliferation. An important caveat is that all of these MEF lines are derived after transformation with SV40 large T antigen. Broome and Courtneidge (33) have reported that MEF responses to PDGF occur due to the independent effect of SV40 large T antigen on c-myc induction rather than c-Src expression. Their study showed data for NIH 3T3 fibroblasts and wild-type MEF expressing an interfering form of Src, and data for Src-deficient or Src-overexpressing cells was not provided.

The unanticipated observation in our experiments was the finding that quantitative differences in c-Src expression have major effects on FGF-1 induced entry into cell cycle and cell growth. MEF with endogenous levels of c-Src showed maximal DNA synthesis and proliferation, whereas cells with 10-fold overexpression of c-Src behaved identically to those that lack c-Src when stimulated by FGF-1. This is in contrast to cells in the absence of growth factor stimulation, where overexpression of c-Src clearly rescues cells from death (Figs. 4A and 7B). This indicates that c-Src has negative as well as positive regulatory effects on FGFR1 signaling and suggests that there is an “optimal” level of c-Src for maximal FGF-induced proliferation. The pronounced negative effect of c-Src overexpression was also observed for EGF but not for PDGF-B, LPA, or serum. This may indicate that there are similar mechanisms for Src regulation of FGF and EGF signaling pathways that differ from PDGF. The mechanisms underlying the negative effects of c-Src overexpression are not addressed by our data. At least for FGFR1, it is unlikely to involve quantitative effects on cell surface receptor density or maximal ERK1/2 activation, be-
cause these were similar in cells with endogenous levels of c-Src and overexpression (Fig. 2A and data not shown).

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REFERENCES
1. Powers, C. J., McLeskey, S. W., and Wellstein, A. (2000) Endocr. Relat. Cancer 7, 165–197
2. Yamashita, T., Yoshioka, M., and Itoh, N. (2000) Biochem. Biophys. Res. Commun. 277, 494–498
3. Basilico, C., and Moscatelli, D. (1992) Adv. Cancer Res. 59, 115–165
4. Zhao, X. M., Frist, W. H., Yeoh, T. K., and Miller, G. G. (1994) J. Clin. Invest. 94, 992–1003
5. Miller, G. G., Davis, S. F., Atkinson, J. B., Chomsky, D. B., Pedroso, P., Reddy, V. S., Drinkwater, D. C., Zhao, X. M., and Pierson, R. N. (1999) Circulation 100, 2396–2399
6. Dickson, C., Spencer-Dene, B., Dillon, C., and Fantl, V. (2000) Breast Cancer Res. 2, 191–196
7. Johnson, D. E., and Williams, L. T. (1993) Adv. Cancer Res. 60, 1–41
8. Jaye, M., Schlessinger, J., and Donne, C. A. (1992) Biochim. Biophys. Acta 1135, 185–199
9. Klint, P., and Claesson-Welsh, L. (1999) Front. Biosci. 4, d165–d177
10. Klint, P., and Claesson-Welsh, L. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 6074–6079
11. Kouhara, H., Hadari, Y. R., Sprvak-Kroizman, T., Schilling, J., Bar-Sagi, D., and Schlessinger, J. (1997) Cell 89, 693–702
12. LaVallee, T. M., Prudovsky, I. A., McMahon, G. A., Hu, X., and Maciag, T. (1998) J. Cell Biol. 141, 1647–1658
13. Klint, P., and Claesson-Welsh, L. (1999) Oncogene 18, 179–185
14. Zhan, X., Plourde, C., Hu, X., Friesel, R., and Maciag, T. (1994) J. Biol. Chem. 269, 20221–20224
15. Landgren, E., Klint, P., Yokote, K., and Claesson-Welsh, L. (1998) Oncogene 17, 283–291
16. Yayon, A., Ma, Y. S., Safran, M., Klagsbrun, M., and Halaban, R. (1997) Oncogene 14, 2999–3005
17. Landgren, E., Blume-Jensen, P., Courtneidge, S. A., and Claesson-Welsh, L. (1995) Oncogene 10, 2027–2035
18. Rodier, J. M., Valles, A. M., Denyelle, M., Thiery, J. P., and Boyer, B. (1995) J. Cell Biol. 131, 20221–20224
19. Liu, J., Huang, C., and Zhan, X. (1999) Oncogene 18, 6700–6706
20. Fincham, V. J., Brunton, V. B., and Frame, M. C. (2000) Mol. Cell. Biol. 20, 6518–6536
21. Soriano, P., Montgomery, C., Geske, R., and Bradley, A. (1991) Cell 64, 693–702
22. Lowell, C. A., and Soriano, P. (1996) Genes Dev. 10, 1845–1857
23. Stein, P. L., Vogel, H., and Soriano, P. (1994) Genes Dev. 8, 1999–2007
24. Klagsbrun, M., Safran, M., and Klagsbrun, M. (1997) Arterioscler. Thromb. Vasc. Biol. 17, 454–464
25. Bastaki, M., Nelli, E. E., Dell’Ern, P., Rusnati, M., Molinari-Tissati, M. P., Parolini, S., Auerbach, R., Ruco, I. P., Possati, L., and Presta, M. (1997) J. Cell Biol. 121, 289–299
26. Tkachuk, F., Cho, Y. H., and Friesel, R. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 3118–3122
27. Tkachuk, F., Cho, Y. H., and Friesel, R. (1994) J. Biol. Chem. 269, 20227–20231
28. Tkachuk, F., Cho, Y. H., and Friesel, R. (1994) Cell 78, 1011–1019
29. Tkachuk, F., Cho, Y. H., and Friesel, R. (1994) Science 265, 1022–1024
30. Tkachuk, F., Cho, Y. H., and Friesel, R. (1994) J. Biol. Chem. 269, 20227–20231
31. Tkachuk, F., Cho, Y. H., and Friesel, R. (1994) Cell 78, 1011–1019
32. Tkachuk, F., Cho, Y. H., and Friesel, R. (1994) Science 265, 1022–1024
33. Tkachuk, F., Cho, Y. H., and Friesel, R. (1994) J. Biol. Chem. 269, 20227–20231
34. Tkachuk, F., Cho, Y. H., and Friesel, R. (1994) Cell 78, 1011–1019
35. Tkachuk, F., Cho, Y. H., and Friesel, R. (1994) Science 265, 1022–1024
36. Tkachuk, F., Cho, Y. H., and Friesel, R. (1994) J. Biol. Chem. 269, 20227–20231
37. Tkachuk, F., Cho, Y. H., and Friesel, R. (1994) Cell 78, 1011–1019
38. Tkachuk, F., Cho, Y. H., and Friesel, R. (1994) Science 265, 1022–1024
39. Tkachuk, F., Cho, Y. H., and Friesel, R. (1994) J. Biol. Chem. 269, 20227–20231
40. Tkachuk, F., Cho, Y. H., and Friesel, R. (1994) Cell 78, 1011–1019
41. Tkachuk, F., Cho, Y. H., and Friesel, R. (1994) Science 265, 1022–1024
42. Tkachuk, F., Cho, Y. H., and Friesel, R. (1994) J. Biol. Chem. 269, 20227–20231
43. Tkachuk, F., Cho, Y. H., and Friesel, R. (1994) Cell 78, 1011–1019
44. Tkachuk, F., Cho, Y. H., and Friesel, R. (1994) Science 265, 1022–1024
45. Tkachuk, F., Cho, Y. H., and Friesel, R. (1994) J. Biol. Chem. 269, 20227–20231
c-Src Regulation of Fibroblast Growth Factor-induced Proliferation in Murine Embryonic Fibroblasts
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