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Cell Biology

Autocrine transformation by chimeric signal peptide-basic fibroblast growth factor: Reversal by suramin

(fibroblast growth factor receptor/malignant transformation/K-*fgf*)

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ABSTRACT **NIH 3T3 cells transfected with basic fibroblast growth factor (bFGF) fused to an immunoglobulin signal peptide sequence are transformed *in vitro* and tumorigenic *in vivo*. The transformed phenotype of chimeric signal peptide–bFGF (spbFGF) cells is characterized by an enhanced proliferation rate compared to parental NIH 3T3 cells, density- and anchorage-independent growth, a transformed morphology, and lack of cell adhesion. The rate of spbFGF cell proliferation is not diminished by anti-bFGF neutralizing antibodies. ^{125}I-labeled bFGF receptor cross-linking and binding studies suggest that surface FGF receptors in spbFGF cells are unavailable and down-regulated. The FGF receptors are also down-regulated in K-*fgf*-transformed cells but not in parental 3T3, native bFGF-transfected, and *ras*-transformed NIH 3T3 cells. The addition of suramin to spbFGF and K-*fgf* cells rapidly promotes the up-regulation of FGF receptors. Suramin also induces lowering of the proliferation rate to that of parental cells, anchorage-dependent growth, assembly of cytoskeletal filaments, cellular adhesion, and spreading. These results suggest that spbFGF cells undergo autocrine transformation, possibly by an internal autocrine loop, in which there is constitutive activation of the FGF receptor. Suramin inhibits autocrine transformation, leading to a normal untransformed phenotype.**

Autonomous cell growth and tumorigenicity may result from a constitutive interaction of cellular growth factors with their corresponding receptors (1). Autocrine transformation occurs either when growth factors are secreted and bind to cell surface receptors to form an external autocrine loop or when they interact with their receptors along the secretory pathway to form an internal autocrine loop (2). Both external and internal autocrine stimulation requires the presence of a hydrophobic signal peptide that allows the transforming growth factor to gain access to and to interact with its receptor. Recent observations suggest that internal autocrine stimulation is a major mode of malignant transformation (3, 4). The intracellular site of growth factor–receptor interaction and its relevance to the mechanism of autocrine transformation have not yet been ascertained.

Fibroblast growth factors (FGFs) constitute a family of potent growth and angiogenic factors abundant in normal and malignantly transformed cells (5–7). Unlike most other polypeptide growth factors, basic fibroblast growth factor (bFGF) and acidic fibroblast growth factor (aFGF) are not secreted but are mainly cell-associated, consistent with their lack of signal peptide sequences. The biological activity of FGF is mediated through specific high-affinity saturable cell surface receptors (8, 9). Most if not all cells possess FGF receptors, which are phosphorylated and internalized upon binding of FGF. A 130-kDa high-affinity FGF receptor containing an intracellular tyrosine kinase domain has been purified and cloned (10–12). A low-affinity large-capacity class of binding sites for FGF has also been identified. These are cell surface and extracellular matrix heparan sulfate proteoglycans, which constitute a major pool of cell-associated FGF (21). Despite the abundance of FGF in cells and extracellular matrix *in vitro* (13) and *in vivo* (14), cells with available functional FGF receptors are not transformed.

The oncogenes, *int-2* and *hst*/K-*fgf*, and the FGF-5 gene, have been identified and shown to have a 40–45% homology to bFGF and aFGF (15–17). These oncogenes encode proteins that possess an amino-terminal signal peptide sequence and are secreted. The transforming potential *in vitro* and the occurrence of these oncogenes in tumors and in the developing embryo suggest that these FGF-related oncogenes may be involved in autocrine transformation. Possession of a signal peptide sequence, therefore, appears to be an important factor in determining the transforming potential of members of the FGF family. The bFGF gene can be converted into an oncogene by addition of a 19-amino acid immunoglobulin signal peptide sequence (18). NIH 3T3 cells transfected with this chimeric signal peptide–bFGF (spbFGF) sequence express bFGF and are highly transformed *in vitro* and tumorigenic *in vivo*. In this report we present evidence that spbFGF but not native bFGF-transfected cells are transformed by an autocrine loop accompanied by down-regulation of the FGF receptors. In addition, we show that suramin up-regulates surface bFGF receptors and reverts the transformed phenotype of spbFGF-transformed cells, in terms of altering proliferation rates, morphology, and adhesion.

MATERIALS AND METHODS

Reagents. Recombinant human bFGF was a gift from Takeda Chemical Industries (Osaka, Japan). ^{125}I-labeled bFGF (^{125}I-bFGF; 150 μCi/μg; 1 Ci = 37 GBq) was purchased from R&D Systems (Minneapolis, MN). Suramin (Mobay Chemical, New York) was prepared as a 200 mM suramin stock solution in H_2O and used at concentrations of 0.1–0.4 mM. Neutralizing anti-bFGF antibodies were a generous gift of Patricia D'Amore (Children's Hospital, Boston). Poly(2-hydroxyethyl methacrylate) [Poly(HEMA)] was kindly provided by Judah Folkman (Children's Hospital).

Cells. Isolated clones of NIH 3T3 cells transfected with native bFGF cDNA and with a spbFGF construct have been described (18). No clonal variations, in terms of growth, morphology, or spontaneous transformation, were observed in any of these clones under long-term culture conditions.

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Abbreviations: FGF, fibroblast growth factor; aFGF, bFGF, and spbFGF, acidic, basic, and chimeric signal peptide-fused FGF, respectively; ^{125}I-bFGF, ^{125}I-labeled bFGF; poly(HEMA), poly(2-hydroxyethyl methacrylate); BSA, bovine serum albumin; DSS, disuccinimidyl suberate; PDGF, platelet-derived growth factor.

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NIH 3T3 cells transfected with Ha-*ras* (EJ62Bam6a) (19) were obtained from Robert Weinberg (Whitehead Institute, Boston). NIH 3T3 cells transfected with K-*fgf* (16) were obtained from Claudio Basilico (New York University, New York). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (vol/vol) bovine serum (GIBCO), 2 mM glutamine, penicillin (100 units/ml), streptomycin (100 μg/ml), and glucose (4.5 g/liter). To measure proliferation, cells were plated in 24-well plates at 5×10^3 cells per well and counted daily in duplicate in a Coulter counter (Coulter Electronics). To measure growth in suspension, cells were plated on nonadhesive poly(HEMA)-coated dishes (20). Cell proliferation in 24-well plates was also measured in the presence of suramin (0.2–0.4 mM) or neutralizing anti-bFGF IgG at 50 μg/ml. Anti-bFGF IgG was added 4 hr after seeding of cells and at a concentration that was sufficient to inhibit the mitogenic effects of exogenous bFGF (20 ng/ml), when added simultaneously to BALB/c 3T3 cells. Normal rabbit IgG had no inhibitory effects. For photomicrography, cells were grown in 35-mm dishes (Costar) and photographed (Pan-X film, Eastman Kodak) using a Nikon inverted microscope.

Staining of Actin Filaments. Cells were plated on Lab-Tek 8-chamber microscope slides (Miles) at 1×10^4 cells per well. After 24 hr, the medium was replaced with either fresh medium alone or fresh medium containing 0.4 mM suramin for an additional 24 hr. The cells were fixed with 4% (wt/vol) formaldehyde/isotonic phosphate-buffered saline (PBS) for 1 hr at room temperature, washed three times with PBS, and permeabilized with 0.2% Triton X-100/0.1% bovine serum albumin (BSA: fraction V, Sigma)/PBS for 15 min at room temperature. Rhodamine-labeled phalloidin (Molecular Probes, Junction City, OR) was added at a 1:1000 dilution for 1 hr at room temperature. The cells were washed three times with PBS, mounted with glycerol, examined with a phase-contrast epifluoresence microscope (Zeiss) and photographed with Kodak Tri-X film.

High-Affinity Binding of ^{125}I-bFGF. Confluent cultures (1×10^6 cells per 35-mm dish) were incubated alone or with 0.4 mM suramin for 48 hr and were washed three times with ice-cold PBS and once with BSA (2 mg/ml)/PBS. Cells were incubated for 2 hr at 4°C in DMEM containing BSA (2 mg/ml), 1 mM $MgCl_2$, and saturating concentrations of ^{125}I-bFGF (10 ng/ml, 150 μCi/μg). The cells were washed once with ice-cold PBS/heparin (10 μg/ml)/BSA (2 mg/ml)/0.1 μM KI/1 mM $MgCl_2$ and once with ice-cold PBS. Heparin and KI were included in the buffer to reduce nonspecific and low-affinity binding (13). The cells were then lysed in PBS containing 1% Triton X-100, and cell-associated radioactivity was determined in a γ counter (Beckman, Gamma 5500). Non-specific binding was determined by including a 200-fold excess of unlabeled bFGF in the binding buffer.

Affinity Cross-Linking of ^{125}I-bFGF. Confluent cultures ($3–5 \times 10^6$ cells per 10-cm dish) were washed once with ice-cold PBS and once with BSA (2 mg/ml)/PBS. Cells pretreated with suramin were washed three additional times with ice-cold PBS to remove any trace of suramin, which might inhibit the binding of bFGF to its receptor. Cells were incubated for 2 hr at 4°C in DMEM containing BSA (2 mg/ml), 1 mM $MgCl_2$, 0.1 μM KI, heparin (1 μg/ml; Hepar, Franklin, OH), and ^{125}I-bFGF (3 ng/ml, 150 μCi/mmol). The cells were washed once with ice-cold PBS containing heparin, (10 μg/ml), BSA (2 mg/ml), 0.1 μM KI, and 1 mM $MgCl_2$ and then once with ice-cold PBS. ^{125}I-bFGF was cross-linked by addition of 0.15 mM disuccinimidyl suberate (DSS), prepared from a 15 mM DSS stock solution in dimethyl sulfoxide, for 20 min at room temperature. To quench free-reactive DSS, a large excess of 10 mM Tris·HCl, pH 7.5/150 mM glycine was added for 5 min followed by a wash with ice-cold PBS. For SDS/PAGE analysis of FGF-receptor cross-linking, cells were scraped, centrifuged, and resuspended in 40–60 μl of lysis buffer (50 mM Tris·HCl, pH 8.0/150 mM NaCl/0.5 mM $MgCl_2$/0.5% Nonidet P-40) for 10 min at 4°C. After clarification by centrifugation at maximum speed in an Eppendorf microcentrifuge, the supernatant fractions were diluted to adjust for cell number, mixed with SDS/PAGE sample buffer [100 mM Tris·HCl, pH 6.8/10 mM EDTA/5% (wt/vol) SDS/10% (vol/vol) glycerol/50 mM dithiothreitol/0.05% bromphenol blue], boiled, and analyzed by SDS/PAGE using 0.75-mm-thick 6% polyacrylamide gels. For fluorography, the gels were fixed, dried, and exposed to Kodak X-Omat autoradiography film at −70°C.

RESULTS

Autocrine Transformation by spbFGF and K-*fgf*. NIH 3T3 fibroblasts transfected by bFGF fused to a signal peptide were highly transformed in culture. Their rate of proliferation was markedly enhanced when compared to parental 3T3 cells, and their growth was density-independent (Fig. 1) and anchorage-independent, as shown by their ability to grow on nonadhesive poly(HEMA)-coated dishes (see Fig. 3*B*). Although NIH 3T3 cells transfected with native bFGF cDNA grew at an enhanced proliferation rate and to a higher saturation density, they were density-arrested and anchorage-dependent like parental 3T3 cells (Fig. 1). The proliferation rate of spbFGF cells was not diminished in the presence of neutralizing antibodies to bFGF. These results are consistent with a previous observation that spbFGF-transformed cells do not secrete biologically active bFGF (18).

Further evidence for autocrine transformation of spbFGF cells was obtained by examining the level of their cell surface FGF receptors. The level and size of available cell surface FGF receptors was ascertained by cross-linking of ^{125}I-bFGF to cells (Fig. 2) as well as by high-affinity binding studies (Table 1). Parental 3T3 cells (lane 1) and 3T3 cells expressing native bFGF (lane 2) had FGF receptors with molecular masses of 130 kDa and 150 kDa that were available for cross-linking, in agreement with previous reports (8, 9). In contrast, cross-linkable FGF receptors were not found on the surface of spbFGF cells (lane 3). Cross-linkable FGF receptors were also not available on 3T3 cells transformed by K-*fgf* (lane 4). The apparent decrease in surface FGF receptor levels correlated with a decrease in the binding of radiolabeled bFGF to high-affinity sites on spbFGF-transformed cells (Table 1). Extensive washing of the cells with 2 M NaCl

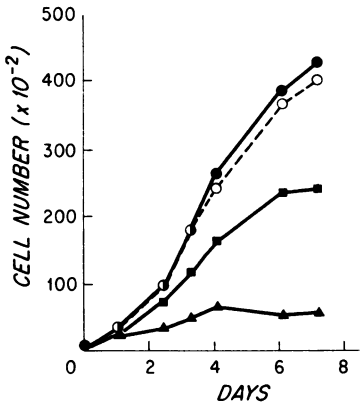

FIG. 1. Growth curves of NIH 3T3 and bFGF-transfected 3T3 cell proliferation. Cells were plated at 2×10^3 cells per well in 24-well dishes and grown for 7 days. At indicated time intervals, the cells were counted. NIH 3T3 cells (triangles), NIH 3T3 cells transfected with native bFGF (squares), NIH 3T3 cells transfected with spbFGF in the presence (open circles) or absence (closed circles) of neutralizing IgG. The values represent mean cell numbers obtained in duplicate in one of three experiments.

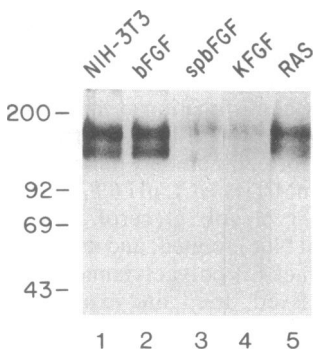

FIG. 2. Cross-linking of ^{125}I-bFGF to high-affinity FGF receptors. Confluent cultures of NIH 3T3 (lane 1) and NIH 3T3 cells transfected with native bFGF (lane 2), spbFGF (lane 3), K-*fgf* (lane 4), or Ha-*ras* (lane 5) were incubated with ^{125}I-bFGF (3 ng/ml) and cross-linked with DSS. The cells were lysed and analyzed by SDS/PAGE and fluorography. Molecular masses in kDa are shown.

buffered at pH 4.0, conditions that dissociate membrane-bound FGF (21), did not uncover any more accessible receptors on spbFGF cells. Interestingly, 3T3 cells transformed by *ras* (lane 5) had available FGF receptors even though these cells synthesize substantial amounts of bFGF (22).

Reversal of Autocrine Transformation of Suramin. Additional evidence for autocrine transformation in spbFGF cells was obtained by use of suramin, a polyanionic drug known to inhibit growth factor–receptor interactions and thereby inhibit autocrine transformation (23–25). Suramin inhibited the mitogenic activity of bFGF on NIH 3T3 cells in a dose-dependent manner (data not shown). At 0.4 mM, suramin reversed the transformed phenotype of spbFGF cells in terms of proliferation rate, morphology, and adhesion. Suramin inhibited the accelerated proliferation rate of spbFGF cells (Fig. 3*A*) and K-*fgf* cells (not shown) compared to that of parental 3T3 cells. On the other hand, suramin did not inhibit the proliferation rate of *ras*-transformed cells, but in fact enhanced it (Fig. 3*A*), suggesting that suramin-sensitive growth factors do not play a major role in *ras*-mediated transformation. The inhibitory effect of suramin on spbFGF and K-*fgf* cell proliferation was completely reversible by either removing the drug or competing it with an excess of bFGF. Suramin also inhibited the ability of spbFGF cells to grow in an anchorage-independent manner on poly(HEMA)-coated dishes (Fig. 3*B*).

Cells transfected with spbFGF underwent profound morphological alterations. Sparse spbFGF cells grew as single cells and at higher densities formed large cell aggregates. Under phase-contrast microscopy, spbFGF cells demonstrated a small, round, and refractile cell body with thin long cytoplasmic extensions (Fig. 4*a*). In the presence of suramin, the cells attached, spread, and acquired a morphology similar to that of parental 3T3 cells (Fig. 4*b*). At high density, cells that did not have room to attach and spread died in the presence of suramin. The same phenotypic reversal of transformation was observed with K-*fgf* cells (Fig. 4 *c* and *d*). Removal of the drug from the culture medium resulted in morphological retransformation of both K-*fgf* and spbFGF transformed cells (data not shown). Suramin had little if any effect on the morphology of *ras*-transformed 3T3 cells (Fig. 4 *e* and *f*), consistent with the lack of inhibition of *ras*-3T3 cell proliferation. The degree of spbFGF cell spreading and morphological alteration was further demonstrated by direct

Table 1. ^{125}I-bFGF binding to spbFGF-transfected and nontransfected cell lines

Binding	Bound bFGF, cpm per 1 × 10^6 cells		
	NIH 3T3 cells	spbFGF cells	
		No addition	+ suramin
Total	3553 ± 380	700 ± 180	2796 ± 290
Nonspecific	899 ± 210	296 ± 130	284 ± 105
High affinity	2654 ± 340	404 ± 150	2512 ± 260

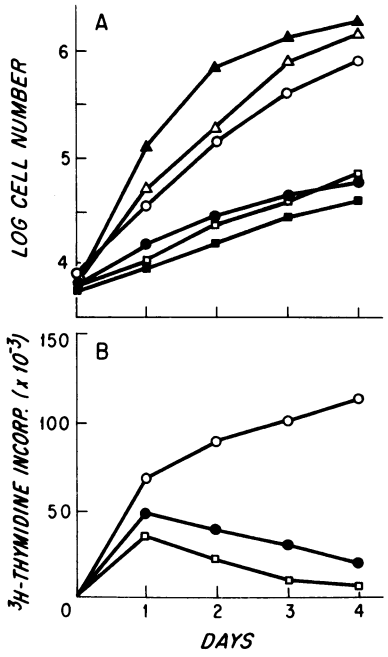

FIG. 3. Effects of suramin on the proliferation of NIH 3T3 and spbFGF-transfected 3T3 cells. (*A*) Growth in 24-well tissue culture plates. Cells were plated at 2 × 10^3 cells per well in the absence (open symbols) or presence (solid symbols) of 0.25 mM suramin and counted in duplicate at indicated intervals. NIH 3T3 cells (squares), spbFGF-transformed 3T3 cells (circles), and *ras*-transformed 3T3 cells (triangles) are indicated. (*B*) Growth on poly(HEMA)-coated dishes. For growth in suspension, cells were plated on poly(HEMA)-coated dishes in the presence or absence of 0.25 mM suramin, and [^{3}H]thymidine incorporation into DNA was measured at indicated intervals. Thymidine incorporation rather than cell number was measured because spbFGF-transformed cells aggregated into large clumps in suspension. NIH 3T3 cells (open squares) and spbFGF-transformed 3T3 cells with (solid circles) or without (open circles) suramin are shown.

fluorescent staining for actin (Fig. 4 *g* and *h*). Suramin induced the reassembly of cytoskeletal actin filaments and focal adhesion points. Typical actin stress fibers were detected as early as 4 hr after addition of suramin and were prominent at 24 hr, when cells were spread maximally (Fig. 4*h*). Suramin had no effect on the morphology of spbFGF and K-*fgf*-transformed cells when applied in the presence of cycloheximide, indicating that the effects of suramin are not posttranslational but require *de novo* protein synthesis.

NIH 3T3 cells transformed by spbFGF were nonadhesive and at high densities grew as large cell aggregates. At all densities spbFGF cells were loosely attached to the substrate and could be removed by simple agitation of the culture dish. Suramin rapidly induced attachment of spbFGF cells, as determined by a quantitative attachment assay (Fig. 5). Within 8 hr, about 50% of the cells were adherent and, within 12–16 hr, virtually all of the cells were as well attached as were the parental 3T3 cells.

Up-Regulation of FGF Receptors Accompanies Reversal of Transformation. The effect of suramin in reversing the transformed phenotype of spbFGF and K-*fgf* cells by blocking autocrine stimulation is consistent with the ability of this drug to uncover high-affinity bFGF binding sites (Table 1) and to up-regulate the FGF receptor (Fig. 6). Time-course analysis revealed that cell surface FGF receptors on spbFGF-transformed cells became available for binding ^{125}I-bFGF within as little as 8 hr in the presence of suramin. At 24 hr, there was an increase in cross-linkable receptors to levels exceeding those seen in NIH 3T3 cells and then a decrease at 48 hr to a level similar to that of parental 3T3 cells. The

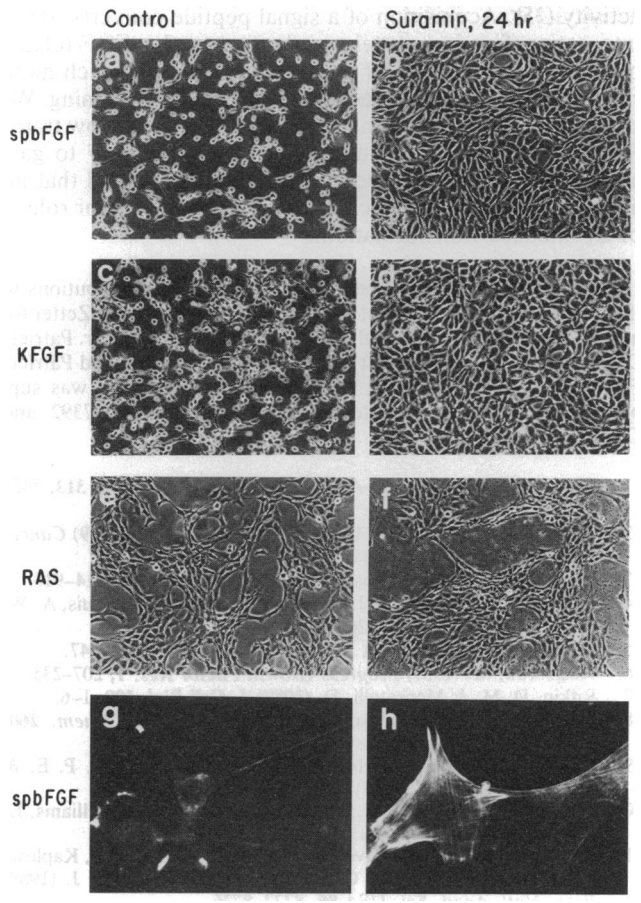

FIG. 4. Effects of suramin on morphology and cytoskeletal organization. spbFGF- (*a* and *b*), K-*fgf*- (*c* and *d*), and Ha-*ras*- (*e* and *f*) transformed cells were plated sparsely in 35-mm dishes and grown for 24 hr. For fluorescent staining of actin filaments with rhodamine-phalloidin (*g* and *h*), spbFGF-transfected cells were plated on microscope slides. The cells were incubated in fresh medium without (*a*, *c*, *e*, and *g*) or with (*b*, *d*, *f*, and *h*) 0.4 mM suramin for 24 hr and then processed for phase-contrast or fluorescent microscopy. (*a–f*, ×60; *g* and *h*, ×400.)

apparent transient overexpression of cross-linked surface FGF-receptor complexes after suramin treatment may result from the mobilization of preexistent FGF receptors from a previously inaccessible pool, supporting the hypothesis that spbFGF-transfected cells are transformed by an internal autocrine loop. The kinetics of receptor binding (Fig. 6), cell

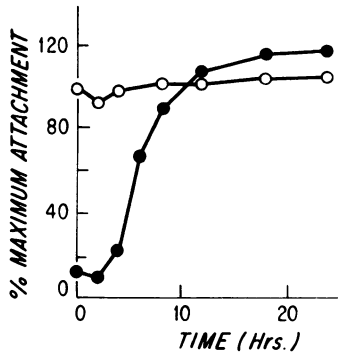

FIG. 5. Effects of suramin on cell attachment. NIH 3T3 (open circles) and spbFGF-transfected 3T3 (solid circles) cells were plated at 5×10^5 cells per well in a 24-well plate. At 24 hr, 0.4 mM suramin was added and the ratio of attached versus unattached cells was measured at the indicated intervals. Adherent cells were defined as those cells that remained attached after three vigorous rinses.

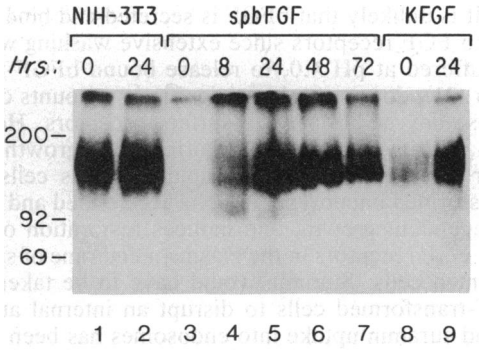

FIG. 6. Affinity cross-linking of ^{125}I-bFGF after suramin treatment. Confluent cultures of NIH 3T3 cells (lanes 1 and 2), spbFGF-transfected 3T3 cells (lanes 3–7), and K-*fgf*-transfected 3T3 cells (lanes 8 and 9) were incubated with 0.4 mM suramin. At the indicated time intervals after addition of suramin, cells were washed extensively, incubated with ^{125}I-bFGF (3 ng/ml), and cross-linked with DSS. The cells were lysed and ^{125}I-bFGF-receptor complexes were analyzed by SDS/PAGE and fluorography. Molecular masses in kDa are indicated.

attachment (Fig. 5), and morphological alteration in the presence of suramin were very similar. These results suggest that spbFGF cells undergo autocrine transformation accompanied by loss of available receptors and adhesiveness, altered morphology, and increased proliferative rate. Suramin inhibits autocrine transformation by possibly blocking bFGF–receptor interaction and as a consequence abolishes the properties associated with the transformed phenotype.

DISCUSSION

NIH 3T3 cells transfected with a construct, in which bFGF cDNA is altered by addition of a signal sequence, undergo autocrine transformation and exhibit morphological and biochemical alterations characteristic of highly transformed cells. spbFGF-transformed cells have an accelerated proliferation rate, are not density arrested, and are capable of anchorage-independent growth. Moreover, spbFGF cells possess few if any functional FGF receptors at the cell surface, supporting the hypothesis that these cells are transformed by constitutive interaction with, and down-regulation of, the FGF receptor. FGF receptors in NIH 3T3 cells transformed by the FGF-related oncogene K-*fgf* are also down-regulated, suggesting that autocrine transformation by K-*fgf* is mediated by the FGF receptor. FGF receptors are not down-regulated in *ras*-transformed cells even though these cells produce substantial amounts of bFGF (22), suggesting that the bFGF produced by *ras*-transformed cells is not involved in autocrine transformation. When injected into syngeneic mice, spbFGF-transformed cells form large and rapidly growing tumors comparable to those formed by *ras*-transformed cells (26). The metastatic and tumorigenic potential of spbFGF-transformed cells may be related to their ability to degrade matrix and to their possession of altered fibronectin receptors—i.e., integrins (unpublished results).

Autocrine transformation has been suggested to occur by either internal or external stimulation. The oncogene v-*sis* encodes a platelet-derived growth factor (PDGF) B homologue that is believed to transform cells by internal activation of an immature form of the PDGF receptor (3). Further evidence for internal autocrine stimulation by v-*sis* is the lack of growth inhibition by neutralizing antibodies to PDGF (27) and reduced surface receptors for PDGF (25). We suggest that spbFGF also transforms cells by way of an internal autocrine loop since these cells do not secrete biologically active bFGF despite the presence of a signal peptide and their proliferation is not inhibited by neutralizing antibodies to

bFGF. It is unlikely that bFGF is secreted and binds immediately to FGF receptors since extensive washing with 2 M NaCl buffered at pH 4.0, to release bound bFGF from its receptor (21), does not release significant amounts of bFGF or expose any additional cell surface receptors. However, suramin, a drug purported to interfere with growth factor–receptor interactions (23–25), rapidly reverts cells to the nontransformed phenotype, inhibits accelerated and anchorage-independent growth, and induces restoration of cross-linkable FGF receptors in the plasma membrane of spbFGF-transformed cells. Suramin would have to be taken up by spbFGF-transformed cells to disrupt an internal autocrine loop, and suramin uptake into endosomes has been demonstrated in v-*sis*-transformed cell lines (25). On the other hand, suramin is a highly charged membrane-impermeable molecule (28). Thus, we cannot completely exclude the possibility that, in spbFGF-transformed cells, FGF–receptor complexes reside in the plasma membrane in a state that is inaccessible to exogenous bFGF and neutralizing antibodies but is accessible to suramin.

bFGF does not appear to be a transforming protein. It is synthesized by many normal cells, such as endothelial cells (13), epithelial cells (29), and chondrocytes (30). NIH 3T3 cells transfected with native bFGF cDNA and expressing 40 times as much bFGF as parental 3T3 cells and 6 times as much as spbFGF-transfected cells are not transformed. Although cells transfected with native bFGF acquire an enhanced proliferation rate and higher saturation density, they are density-arrested *in vitro* and nontumorigenic in syngeneic mice (18). They can produce small slow-growing spontaneously regressing tumors at low frequency in nude mice (26). Cells transfected with native aFGF cDNA (31) show similar properties *in vitro* and *in vivo* to those transfected with native bFGF cDNA. Neither native bFGF- nor aFGF-transfected 3T3 cells show a decrease in cell surface FGF receptors, indicating the absence of autocrine transformation. Extremely high overexpression of bFGF has been reported to result in cell transformation *in vitro* (32). NIH 3T3 cell lines that overexpress bFGF at a very high level, 300- to 1000-fold greater than hepatoma and endothelial cells (among the highest bFGF producers so far described), are morphologically altered, and their FGF receptors are down-regulated. Unlike spbFGF-transformed cells, the conditioned medium of these cells contains high levels of biologically active bFGF. Thus, it is possible that cells transformed by overexpression of bFGF are transformed as a result of continuous external stimulation by released bFGF. Although cells overexpressing bFGF are transformed *in vitro*, it has not been demonstrated that they are tumorigenic *in vivo*. On the other hand, spbFGF cells are highly tumorigenic even though they express a small fraction of the bFGF produced by cells overexpressing bFGF. It is possible that external autocrine stimulation by bFGF released from cells overexpressing bFGF can confer growth advantage *in vitro* but may prove to be inefficient *in vivo* since soluble non-matrix-bound FGF is very labile and rapidly inactivated (33). The instability of secreted bFGF may account for the observation that melanocytes transfected with native bFGF acquired properties *in vitro* similar to those of metastatic melanoma cells but were not tumorigenic *in vivo* (34). Moreover, in a controlled reconstituted skin environment *in vivo*, bFGF transformants reverted to a normal melanotic phenotype with restricted growth, indicating that constitutive production of bFGF by itself is insufficient to make melanocytes tumorigenic.

Possession of a signal sequence may be a prerequisite for a growth factor gene to become a transforming oncogene. For example, the protein encoded by the v-*sis* oncogene contains a signal peptide that is essential for transformation since deletion of this sequence results in loss of transforming activity (35). Acquisition of a signal peptide converts bFGF into a transforming protein analogous to the FGF-related oncogenes, *hst*/K-*fgf* and the gene for FGF-5, which naturally have signal peptide sequences and are transforming. We speculate that the hydrophobic signal sequences allow transforming growth factors such as v-*sis* and spbFGF to gain access to otherwise inaccessible compartments and that inappropriate subcellular localization may play a major role in bFGF-induced transformation.

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1. Sporn, M. B. & Roberts, A. B. (1985) *Nature (London)* **313**, 745–747.
2. Browder, T. M., Dunbar, C. E. & Nienhuis, A. W. (1989) *Cancer Cells* **1**, 9–17.
3. Keating, M. T. & Williams, L. T. (1988) *Science* **239**, 914–916.
4. Browder, T. M., Abrams, J. S., Wong, P. M. C. & Nienhuis, A. W. (1989) *Mol. Cell. Biol.* **9**, 204–213.
5. Folkman, J. & Klagsbrun, M. (1987) *Science* **235**, 442–447.
6. Klagsbrun, M. (1989) *Progress Growth Factor Res.* **1**, 207–235.
7. Rifkin, D. M. & Moscatelli, D. (1989) *J. Cell Biol.* **109**, 1–6.
8. Neufeld, G. & Gospodarowicz, D. (1985) *J. Biol. Chem.* **260**, 13860–13868.
9. Kan, M., Disorbo, D., Hou, J., Hoshi, H., Monsson, P. E. & Mckeehan, W. L. (1988) *J. Biol. Chem.* **264**, 4120–4126.
10. Lee, P. A., Johnson, D. E., Cousens, L., Fried, V. & Williams, L. (1989) *Science* **245**, 57–60.
11. Ruta, M., Burgess, W., Givol, D., Epstein, J., Neiger, N., Kaplow, J., Crumley, G., Dionne, C., Jaye, M. & Schlessinger, J. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 8722–8726.
12. Kornbluth, S., Paulson, K. E. & Hanafusa, H. (1988) *Mol. Cell. Biol.* **8**, 5541–5544.
13. Vlodavsky, I., Folkman, J., Sullivan, R., Friedman, R., Ishai-Michaeli, R., Sasse, J. & Klagsbrun, M. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 2292–2296.
14. Folkman, J., Klagsbrun, M., Sasse, J., Wadzinski, M., Ingber, D. & Vlodavsky, I. (1988) *Am. J. Pathol.* **130**, 393–400.
15. Dickson, C. & Peters, G. (1987) *Nature (London)* **326**, 833.
16. Delli-Bovi, P., Curatola, A. M., Kern, F. G., Greco, A., Ittmann, M. & Basilico, C. (1987) *Cell* **50**, 729–737.
17. Zhan, X., Bates, B., Hu, X. & Goldfarb, M. (1988) *Mol. Cell. Biol.* **8**, 3487–3495.
18. Rogelj, S., Weinberg, R. A., Fanning, P. & Klagsbrun, M. (1988) *Nature (London)* **331**, 173–175.
19. Bernstein, S. C. & Weinberg, R. A. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 1726–1730.
20. Folkman, J. & Moscona, A. (1978) *Nature (London)* **273**, 345–349.
21. Moscatelli, D. (1988) *J. Cell Biol.* **107**, 753–759.
22. Iberg, N., Rogelj, S., Fanning, P. & Klagsbrun, M. (1989) *J. Biol. Chem.* **264**, 19951–19955.
23. Garret, J. S., Coughlin, S. R., Niman, H. L., Tremble, P. M., Giels, G. M. & Williams, L. T. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 7466–7470.
24. Betsholtz, C., Johnson, A., Heldin, C. H. & Westermark, B. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 6440–6444.
25. Huang, S. S. & Huang, J. S. (1988) *J. Biol. Chem.* **263**, 12608–12618.
26. Rogelj, S., Weinberg, R. A., Fanning, P. & Klagsbrun, M. (1989) *J. Cell. Biochem.* **39**, 13–23.
27. Huang, J. S., Huang, S. S. & Deuel, T. F. (1984) *Cell* **39**, 79–87.
28. Wilson, E. J. & Wormall, A. (1949) *Biochem. J.* **45**, 224–231.
29. Schweigerer, L., Ferrara, N., Haaparanta, T., Neufeld, G. & Gospodarowicz, D. (1988) *Exp. Eye Res.* **46**, 71–80.
30. Sullivan, R. & Klagsbrun, M. (1985) *J. Biol. Chem.* **260**, 2399–2403.
31. Jaye, M., Lyall, R. M., Mudd, R., Schlessinger, J. & Sarver, N. (1988) *EMBO J.* **7**, 963–969.
32. Moscatelli, D. & Quarto, N. (1989) *J. Cell Biol.* **109**, 2519–2527.
33. Sommer, A. & Rifkin, D. (1989) *J. Cell Physiol.* **138**, 215–220.
34. Dotto, G. P., Moellmann, G., Ghosh, S., Edwards, M. & Halaban, R. J. (1989) *J. Cell Biol.* **109**, 3115–3128.
35. Hannink, M. & Donoghue, D. J. (1984) *Science* **226**, 1197–1199.