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Differential Growth Factor Expression in Transformed Mouse NIH-3T3 Cells

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The expression of growth factor-specific mRNA transcripts and the presence of biologically active growth factors in the conditioned medium and in the cell extracts from mouse NIH-3T3 cells transformed by different oncogenes (Ki-*ras*, *mos*, *src*, *fms*, *fes*, *met*, and *trk*), by a DNA tumor virus (SV40), or by a chemical carcinogen (N-nitrosomethylurea) were studied. In contrast to NIH-3T3 cells or simian virus 40 (SV40)-transformed 3T3 cells, all the other transformed NIH-3T3 cell lines expressed a 4.5 kb transforming growth factor-α (TGFα)-specific mRNA transcript and secreted immunoreactive and biologically active TGFα ranging from 100 to 225 ng/10^8 cells/48 h. In addition, in the transformed cell lines that were secreting elevated levels of biologically active TGFα, there was a 75–95% reduction in the total number of epidermal growth factor receptors on these cells. A 2.6 kb TGFβ mRNA transcript and TGFβ protein in the conditioned medium (30–140 ng/10^8 cells/48 h) was also detected in those lines expressing TGFα. Basic fibroblast growth factor-like activity (11–50 ng/10^8 cells) was detected in the cell lysates from NIH-3T3 cells transformed with N-nitrosomethylurea or with *trk*, where expression of specific 6.9 and 3.9 kb mRNA transcripts for basic fibroblast growth factor could also be found. B chain (c-*sis*) expression of platelet-derived growth factor was present only in *trk*-transformed NIH-3T3 cells in which specific c-*sis* 6.5 and 4.6 kb transcripts were identified. In contrast, platelet-derived growth factor A chain expression of 2.9 and 2.3 kb transcripts was found in *ras*-, *met*-, *mos*-, and *fms*-transformed NIH-3T3 cells. These results suggest that the expression of different sets of growth factors is controlled in part by structurally distinct groups of transforming genes.

Key words: oncogenes, neoplastic transformation, transforming growth factor-α, transforming growth factor-β, basic fibroblast growth factor, platelet-derived growth factor

Retroviral oncogenes and cellular protooncogenes can code for proteins that are growth factors (e.g., c-*cis*/B chain of platelet-derived growth factor [PDGF]), growth factor receptors (e.g., c-*erb*B/epidermal growth factor [EGF] receptor; c-*fms*/colony

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stimulating factor-1 receptor) or proteins that are involved in the intracellular signal transduction pathway(s) for growth factors (e.g., c-*myc* and c-*fos*) [1–3]. Alternatively, oncogenes can indirectly enhance the production and the secretion of a variety of endogenous cellular growth factors, such as transforming growth factor-α (TGFα), transforming growth factor-β (TGFβ), insulin-like growth factor I (IGF-I), and platelet-derived growth factor (PDGF). These growth factors may be facilitating the uncontrolled proliferation of transformed cells through potential autocrine and/or paracrine pathway(s) [3–5]. For example, different mouse and rat fibroblast cell lines such as 3T3 (NIH, BALB/c, or Swiss), Rat-1, and normal rat kidney (NRK) cells that have been transformed by retroviral oncogenes or by activated cellular protooncogenes, including *ras, mos, abl, fes, fms,* and *src,* exhibit an increased secretion of TGFα and/or TGFβ [6–13]. These or other growth factors may be mediating the biological effects of specific oncogenes or groups of oncogenes. Introduction of expression vector plasmids containing the cDNAs of either epidermal growth factor (EGF), TGFα, or basic fibroblast growth factor (bFGF) into rodent fibroblast cell lines can lead to the in vitro transformation, and in some instances the in vivo tumorigenicity, of these cells [14–18]. Finally, different growth factors can function in a concerted manner to elicit the reversible phenotypic transformation of nontransformed indicator cells in vitro [10,19–21].

The purpose of this study was to determine whether the transformation of the same clonal cell line (mouse NIH-3T3 fibroblasts) by seven structurally distinct oncogenes (Ki-*ras, src, mos, fes, fms, met,* and *trk*), by a DNA tumor virus (simian virus 40; SV40), or by a chemical carcinogen (N-nitrosomethylurea [NMU]) could affect the expression and production of the same set or different sets of cellular growth factors, such as TGFα, TGFβ, bFGF, and the A and B chains of PDGF. In this respect, previous studies have compared only the relative levels of secretion of various growth factors and/or the expression of growth factor-specific mRNA transcripts following oncogene transformation in fibroblast cell lines of different species [7–9,13]. To gain some insight into the possible mechanism(s) or pathways by which different oncogenes may be regulating cellular proliferation through their ability to modulate the expression of growth factors and/or their receptors, it may be important to conduct such comparative studies in vitro on cells of the same genetic background.

MATERIALS AND METHODS

Cell Cultures

Mouse NIH-3T3 clone 7 cells were kindly provided by Dr. Douglas Lowy, National Cancer Institute (Bethesda, MD). Ki-*ras*-NIH-3T3, *mos*-NIH-3T3, *src*-NIH-3T3, *fms*-NIH-3T3, *fes*-NIH-3T3, *sis*-NIH-3T3, *trk*-NIH-3T3, and *met*-NIH-3T3 cells were derived from NIH-3T3 cells following infection with retroviruses containing the appropriate viral oncogenes or following transfection with plasmids containing the activated cellular protooncogenes *met* and *trk* as previously described [22]. C11 cells are a clone of nontransformed flat cellular revertants isolated from Ki-*ras*-transformed NIH-3T3 cells [23]. SV40-NIH-3T3 are NIH-3T3 cells infected and transformed with the SV40 DNA tumor virus [22]. NMU-NIH-3T3 are NIH-3T3 cells transformed by treatment with the chemical carcinogen NMU [22]. All cell lines were grown in Dulbecco's modified Eagle's medium (DMEM) (GIBCO, Grand Island, NY), supplemented with 10% fetal bovine serum containing 4 mM glutamine, 20 mM Hepes (pH

7.4), streptomycin (100 μg/ml), and penicillin (100 U/ml) in a humidified atmosphere of 95% air and 5% CO_2 at 37°C.

Preparation of Conditioned Media (CM) for TGFα and TGFβ Analysis

Cells were grown to 50–60% confluency in serum-containing DMEM medium in 150 cm^2 tissue culture flasks, washed twice with serum-free medium, and cultured in PC-1 serum-free medium (Ventrex, Portland, ME) for an additional 48 hr period. For TGFα determinations, CM was acidified with 0.4 N HCl and concentrated on a C_{18} Sep-pak reverse-phase minicolumn (Waters, Inc., Rochester, MN) as previously described [24]. For TGFβ determinations, aliquots of CM were transiently acidified by treatment with 45 mM HCl for 5 min at 23°C to activate any latent TGFβ activity, followed by neutralization with 45 mM NaOH and 1 M Hepes (pH 7.0) as previously described [25].

Preparation of Cell Lysates for Analysis of bFGF-Like Activity

Cells were grown to near confluence in 150 cm^2 tissue culture flasks in DMEM containing 10% fetal calf serum. Cells were scraped in 0.15 M NaCl (pH 7.4) containing 10 μg/ml of leupeptin and then centrifuged (100*g*, 10 min). The cell pellets were resuspended in 5 ml of 0.6 M NaCl containing 10 mM Tris HCl (pH 7.0) and pulse sonicated for 3 min at 4°C. The sonicated cell lysates were subsequently centrifuged (50,000*g*, 30 min, 4°C) and applied to heparin-Sepharose columns (1.0 ml) that had previously been equilibrated in 0.6 M NaCl containing 10 mM Tris HCl (pH 7.0). The columns were sequentially washed with 6 ml of 0.6, 1.0, and 3.0 M NaCl solutions containing 10 mM Tris HCl (pH 7.0) as previously described [26]. The 3.0 M NaCl eluates from the columns, which is where authentic bFGF elutes, were then dialyzed against 0.15 M NaCl (pH 7.4).

Radioimmunoassay (RIA) and Radioreceptor Assay (RRA) for TGFα

The levels of immunoreactive TGFα were determined using a liquid-phase competitive RIA with a polyclonal rabbit antirat TGFα antiserum that is specific for TGFα and fails to recognize mouse or human EGF as previously described [24]. The labeled rat synthetic ^{125}I-TGFα, rabbit anti-TGFα antiserum, and other reagents were purchased from Biotope, Inc. (Seattle, WA). EGF receptor-competing activity was analyzed as previously described by using monolayer cultures of 184A1N4 cells, a human mammary epithelial cell line that possesses approximately 10^6 EGF receptor sites/cell [27,28]. The amount of TGFα equivalent units in the samples was calculated by comparison to the competition curves produced by unlabeled human TGFα (Bachem, Torrance, CA) with 1 ng/ml mouse ^{125}I-EGF (specific activity 100 μCi/μg; Amersham, Arlington Heights, IL).

^{125}I-EGF Binding Assay

EGF binding assays were performed on cells in monolayer cultures using mouse ^{125}I-EGF as previously described [24,27]. The number of EGF binding sites and the dissociation constants (K_d) for binding were determined by Scatchard analysis of the specific binding isotherms over a concentration of 0.5–25 ng/ml ^{125}I-EGF as previously described [27].

Bioassay for TGFβ Activity

Inhibition of ^{3}H-thymidine incorporation into the DNA of CCL-64 mink lung epithelial cells was utilized as a bioassay for measuring the presence of TGFβ activity as previously described [25]. Different concentrations of porcine TGFβ-1 (R & D Systems, Minneapolis, MN) were used as standard.

Bioassay and RRA for bFGF-Like Activity

Aliquots of the dialyzed 3.0 M NaCl fractions from the cell lysates were assayed for their ability to stimulate the anchorage-independent growth of human SW-13 small cell adrenal cortical tumor cells in soft agar as previously described [29]. SW-13 cells are specifically sensitive to growth factors that possess bFGF-like activity [30]. The amount of bFGF-like activity in the samples was calculated by comparison to the number of soft agar colonies that was induced by different concentrations of bovine recombinant bFGF (Amgen Biologicals, Thousand Oaks, CA) after 2 weeks following seeding of 2×10^4 SW-13 cells in agar. In addition, bFGF activity in the dialyzed 3.0 M NaCl aliquots was quantitated in a bFGF RRA by measuring the amount of activity in the samples that specifically competed with the binding of bovine ^{125}I-bFGF (2 ng/ml, specific activity 148 μCi/μg; R & D Systems) to bFGF receptors on baby hamster kidney (BHK) cells compared to the amount of competition produced by different concentrations of unlabeled bovine bFGF as previously described [31].

Isolation of Poly-(A)$^+$ RNA and Northern Blot Analysis

Equivalent amounts of poly-(A)$^+$ RNA isolated from NIH-3T3 cells and from their transformed derivatives were electrophoresed through a denaturating 1.2% agarose–2.2 M formaldehyde gel. Ethidium bromide staining of the gel showed that each lane contained an equivalent amount of RNA. The gels were then transferred to nitrocellulose [32] and hybridized sequentially, as previously described [24], to the following ^{32}P-labeled nick-translated cDNA probes: a 406 bp *Eco*RI-*Apa*I restriction fragment derived from a human TGFα cDNA clone, pTGF-C1 [33]; a 1,050 bp human TGFβ cDNA probe [34]; a 770 bp human β-actin cDNA probe (Oncor, Gaithersburg, MD); a 1,400 bp *Eco*RI fragment of the bovine bFGF cDNA probe [35]; a 1,300 bp *Eco*RI restriction fragment derived from a human PDGF A chain cDNA clone, PDGF-A-D1 [36]; and a 2,700 bp *Bst*EII restriction fragment of the human PDGF B chain cDNA clone, pSM-1 [37].

RESULTS

Production of TGFα and Expression of EGF Receptors in Transformed NIH-3T3 Cells

TGFα is a 50-amino-acid peptide that is functionally related to EGF, although it exhibits only a 30–40% amino acid sequence homology with EGF [5]. TGFα binds to and interacts through the EGF receptor, the product of the c-*erb*B protooncogene [1]. TGFα is produced by a number of human and rodent tumor cell lines and has been circumstantially implicated in the autocrine growth of these cells [2,3,5]. To determine whether transformation of NIH-3T3 cells by different oncogenes or by other transforming agents such as a DNA tumor virus or a chemical carcinogen can lead to the enhanced production of TGFα, CM was collected from NIH-3T3 cells transformed by v-Ki-*ras*,

v-*mos*, v-*fes*, v-*fms*, v-*src*, activated c-*met* and c-*trk*, NMU, and the SV40 DNA tumor virus. All these transformed NIH-3T3 cell lines exhibited cloning efficiencies in soft agar of 5–12% [22]. Aliquots of CM were assayed in a TGFα-specific RIA and in an EGF/TGFα competing RRA. As shown in Figure 1, NIH-3T3 cells secrete very low levels of immunoreactive and biologically active TGFα (15–25 ng/10^8 cells/48 h). In contrast, cells transformed by Ki-*ras*, *mos*, *fes*, *fms*, *src*, *met*, *trk*, and NMU secrete five- to tenfold higher levels of TGFα protein into their CM (100–225 ng/10^8 cells/48 h). In fact, there is a reasonable concordance between the levels of immunoreactive TGFα and the levels of biologically active TGFα in the CM obtained from a majority of the cell lines as determined by RIA and RRA, respectively. In contrast to the other transformed cell lines, SV40-transformed NIH-3T3 cells were not producing significantly higher levels of TGFα than the parental NIH-3T3 cells. However, C11 cells, a flat Ki-*ras* cellular revertant, produced amounts of TGFα equivalent to those produced by the fully transformed Ki-*ras* NIH-3T3 cells. This latter observation agrees with previous data demonstrating the presence of TGFα-like activity in the CM from several flat cellular Ki-*ras* revertant cell lines [23].

Poly-$(A)^+$-selected RNA that was obtained from these cell lines was analyzed by Northern blot hybridization for the expression of specific TGFα mRNA transcripts. As is shown in Figure 2A, a 4.5 kb mRNA species was detected in the same cell lines that were secreting TGFα protein into their CM. The levels of expression of TGFα mRNA varied from high (*trk*, NMU, *src*) to moderate (C11, *fes*, *mos*). Little to no expression could be detected in SV40-transformed cells and in the parental NIH-3T3 cells. The differences in the level of TGFα mRNA expression among the cell lines was not due to intrinsic variations in RNA loading or to transfer, since relatively equivalent amounts of β-actin mRNA were detected using a specific labeled β-actin cDNA insert (Fig. 2F).

One common feature associated with cells transformed by different oncogenic retroviruses is a decreased expression of EGF receptors [9,23,24,27,38,39]. Figure 3 shows the EGF binding isotherms of NIH-3T3 cells and of their transformed counterparts. As determined by Scatchard analysis, NIH-3T3 clone 7 cells express approxi-

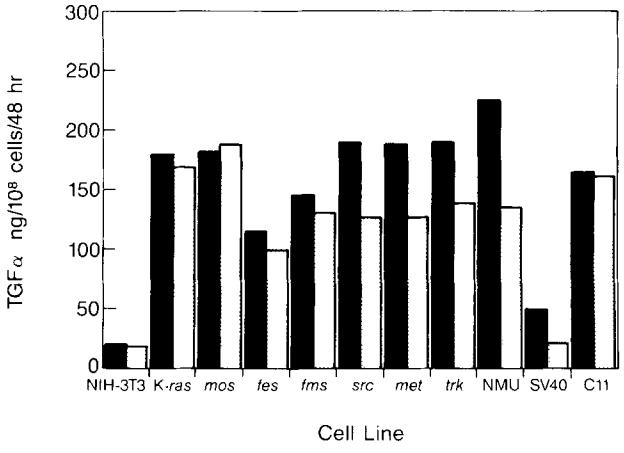

Fig. 1. TGFα activity in the CM from NIH-3T3 cells and transformed NIH-3T3 cell lines. Concentrated CM was assayed in a TGFα-specific RIA (solid bars) and in a EGF/TGFα RRA (stippled bars). Values are the average of quadruplicate determinations. The variation between the individual samples was less than 5%.

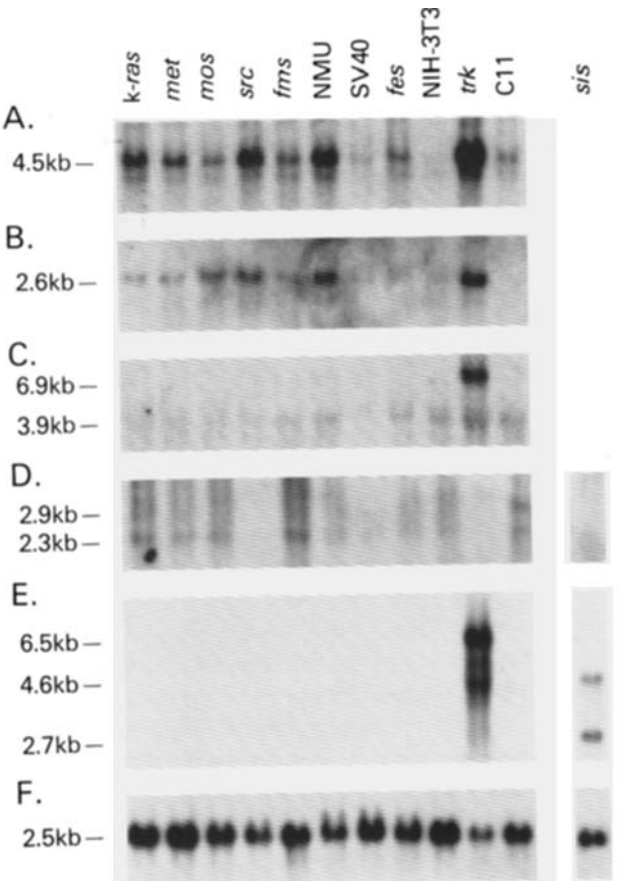

Fig. 2. Northern blot analysis of poly-(A)$^+$ RNA (10 μg/lane) isolated from NIH-3T3 cells and from their transformed derivatives. Northern blots were sequentially hybridized with a ^{32}P-labeled human TGFα cDNA probe (**A**), human TGFβ cDNA probe (**B**), bovine bFGF cDNA probe (**C**), human PDGF A chain cDNA probe (**D**), human PDGF B chain cDNA probe (**E**), and human β-actin cDNA probe (**F**).

mately 35,000 EGF receptor sites/cell with a low- and a high-affinity class of binding sites, having a K_d of 3.1×10^{-10} M and of 6.2×10^{-11} M, respectively. SV40-transformed NIH-3T3 cells show an approximately 25% reduction in the total number of EGF binding sites/cell, with no change in the affinity of the high- and the low-affinity classes of EGF binding sites. In contrast, NIH-3T3 cells transformed by Ki-*ras*, *mos*, *fes*, *fms*, *src*, *met*, *trk*, NMU, and *sis*, as well as the flat cellular Ki-*ras* revertant C11, exhibit little or no specific binding of ^{125}I-EGF over a wide concentration range (0.5–25 ng/ml). In fact, there is a 75–95% reduction of ^{125}I-EGF binding at 25 ng/ml to these different transformed cells compared to the parental NIH-3T3 cells (Fig. 3, inset).

Expression and Production of Other Growth Factors in Transformed NIH-3T3 Cells

The phenotypic and reversible transformation of some rodent fibroblast cell lines, such as NRK cells, in the presence of serum requires the cooperative interaction among several growth factors, including TGFα, TGFβ, and PDGF [3,4,20,21]. In the absence

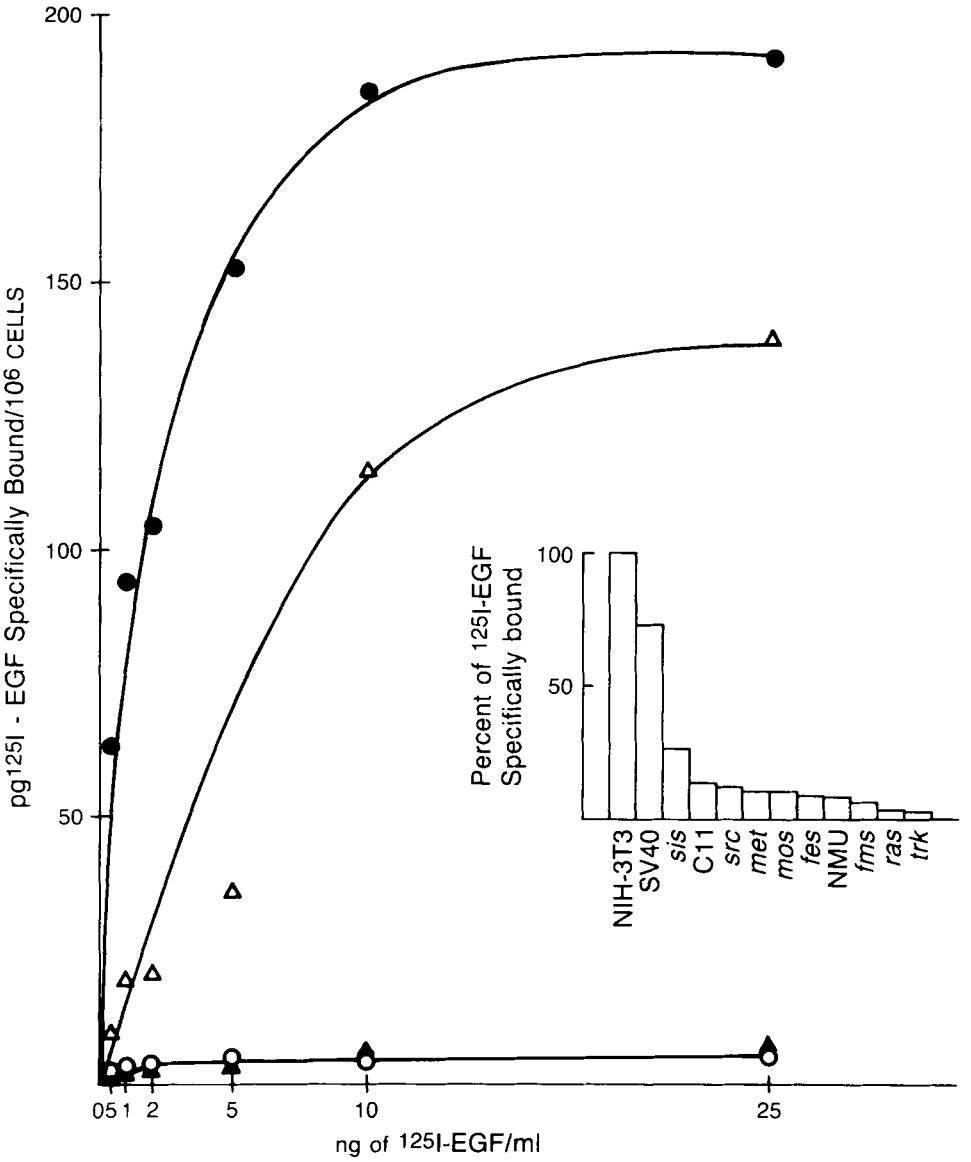

Fig. 3. Binding of different concentrations of ^{125}I-EGF to NIH-3T3 cells (●), SV40-NIH-3T3 cells (△), Ki-*ras*-NIH-3T3 cells (▲), and *trk*-NIH-3T3 cells (○). **Inset:** Percentage of ^{125}I-EGF specifically bound to transformed NIH-3T3 cell lines at a concentration of 25 ng/ml ^{125}I-EGF compared to parental NIH-3T3 cells. Values are the average of duplicate determinations. The variation between the individual samples was less than 5%.

of serum or in plasma-supplemented medium, bFGF can potentiate the effects of TGFα, TGFβ, and PDGF on inducing the anchorage-independent growth of rat NRK cells and of mouse AKR-2B cells in vitro [20]. In addition, several spontaneous rodent and human tumor cell lines produce increased amounts of TGFβ, PDGF, and in some instances bFGF [3,9,10,40].

To determine whether the transformed NIH-3T3 cell lines are also differentially expressing TGFβ to the same extent as TGFα, CM from these cells was analyzed for the presence of TGFβ activity, following acid treatment to activate any potential latent growth factor [10,25]. The inhibition of ^{3}H-thymidine incorporation into DNA of CCL-64 mink lung epithelial cells was used as a bioassay for the quantitation of TGFβ activity present in the CM [25]. Nontransformed NIH-3T3 cells secrete approximately 10–15 ng/10^8 cells/48 h of TGFβ into their CM (Fig. 4). Following transformation with Ki-*ras, mos, fes, src, met, trk,* NMU, or SV40, NIH-3T3 cells secrete two- to ninefold higher levels of TGFβ (30–140 ng/10^8 cells/48 h) than nontransformed cells. In addition, C11 cells secrete a comparable amount of TGFβ activity as Ki-*ras*-transformed NIH-3T3 cells, whereas little or no increase in the secretion of TGFβ activity could be found in *fms*-transformed NIH-3T3 cells. Figure 2B shows the presence of a specific TGFβ 2.6 kb mRNA transcript in NIH-3T3 cells transformed by Ki-*ras, met, mos, src,* NMU, *fes,* and *trk,* with the highest levels of expression being detected in *trk*-, *src*-, and NMU-transformed cells. These results are in reasonable agreement with the relative levels of TGFβ activity found in the CM obtained from these cell lines. However, although TGFβ activity could be detected in the CM from *fes*- and SV40-transformed NIH-3T3 cells and from C11 cells, no increase in the levels of TGFβ mRNA expression could be detected in these cells compared to parental NIH-3T3 cells. This may be due in part to an enhanced secretion and/or activation of latent TGFβ protein in these transformed cell lines rather than to an actual increase in TGFβ production.

bFGF is a potent mitogen for a variety of mesenchymal and smooth muscle cells and stimulates chemotaxis and proliferation of capillary endothelial cells, suggesting that it may play an important role in tumor angiogenesis [41]. bFGF is generally associated with the extracellular matrix and is not found in soluble form in the CM, presumably because it lacks a hydrophobic N-terminal signal or leader sequence [31,41]. The presence of bFGF-like activity was determined in the CM or cell lysates by the ability of these samples to stimulate the anchorage-independent growth of SW-13 cells in soft agar, a human adrenal cortical tumor cell line, and by the ability to compete

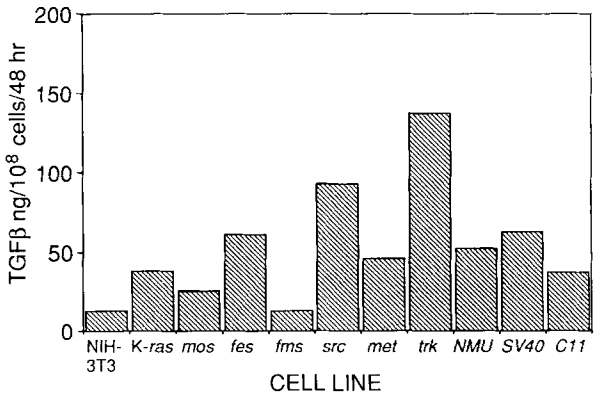

Fig. 4. TGFβ activity in the CM from NIH-3T3 cells and transformed NIH-3T3 cell lines. CM were acid-treated to activate any latent TGFβ activity and were analyzed in the CCL-64 growth inhibition bioassay. Values represent the average of two different experiments in triplicate. The variation between the individual experiments was less than 10%.

specifically with ^{125}I-bFGF for binding to bFGF receptors on BHK cells in a RRA [30, 31]. No bFGF-like activity, as measured in either assay, could be detected in the CM form in any of the cell lines. However, as is shown in Figure 5, varying amounts of bFGF-like activity, which bind to and elute from a heparin-sepharose affinity column under high-salt conditions, could be observed in the cell lysates that were prepared from Ki-*ras*-, *mos*-, *fes*-, *fms*-, *trk*-, and NMU-transformed NIH-3T3 cells and from C11 cells in the range of 3–50 ng/10^8 cells. The highest levels of bFGF-like activity were found in *trk*- and NMU-transformed NIH-3T3 cells and in the C11 cells. No appreciable bFGF-like activity was present in the cell lysates that were obtained from the parental NIH-3T3 cells and from the *src*-, *met*-, and SV40-transformed cell lines. Essentially identical results were obtained using a bFGF RRA (data not shown). Northern blot analysis for the presence of bFGF mRNA transcripts demonstrated the presence of a 3.9 kb species in the majority of the cell lines, with the highest levels of expression found in *fms*-, NMU-, and *trk*-transformed NIH-3T3 cells and in C11 cells (Fig. 2C). Furthermore, a prominent 6.9 kb bFGF mRNA species could be detected in *trk*-transformed NIH-3T3 cells.

PDGF consists of A and B chain heterodimers or homodimers [2,3,36]. The A and B chains are encoded by separate genes that are differentially regulated in various cell lines [36]. In fact, the B chain of PDGF is the product of the c-*sis* protooncogene [1,37]. As is shown in Figure 2E, v-*sis*-transformed NIH-3T3 cells express a 2.7 kb and a 4.6 kb mRNA transcript for the B chain of PDGF, whereas no PDGF A chain mRNA expression could be detected in the same cells (Fig. 2D). None of the transformed cell lines except *trk*-transformed NIH-3T3 cells expressed PDGF B chain mRNA transcripts (Fig. 2E). In *trk*-transformed NIH-3T3 cells, a minor 2.7 kb and a major 4.6 kb transcript could be observed. Furthermore, an additional major PDGF B chain transcript at 6.5 kb could be found in these cells. Whereas PDGF B chain mRNA expression is restricted to *trk*-transformed NIH-3T3 cells, PDGF A chain mRNA expression is not

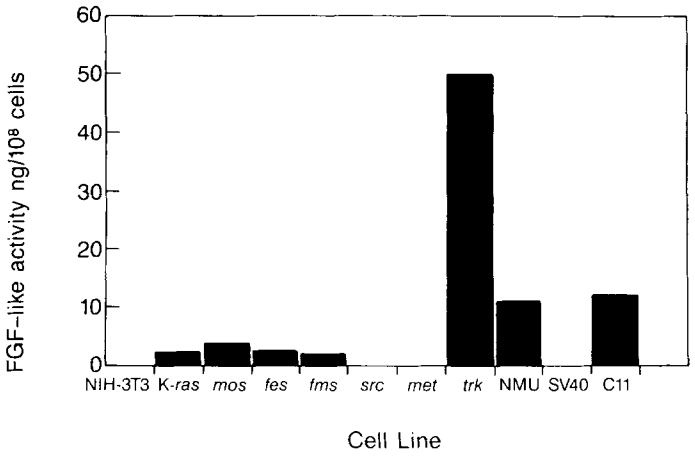

Fig. 5. bFGF-like activity present in the cell lysates prepared from NIH-3T3 cells and their transformed derivatives. Dialyzed aliquots of 3 M NaCl fractions after heparin-Sepharose chromatography were assayed for the ability to stimulate the anchorage-independent growth of SW-13 cells in soft agar. Values are the average of two different experiments in duplicate. The variation between the individual experiments was less than 10%.

detected in these cells but is detected in Ki-*ras*-, *met*-, *mos*-, and *fms*-transformed NIH-3T3 cells and in C11 Ki-*ras* revertants as a 2.6 kb and a 2.9 kb mRNA species (Fig. 2D).

DISCUSSION

Previous studies have shown that the transformation of different rodent cell lines with various transforming agents, such as activated cellular or retroviral oncogenes, can lead to the enhanced production of cellular growth factors [3–13,42,43]. However, none of these studies has systematically compared the effects of transformation of the same cell line by a variety of different oncogenes with respect to the spectrum of growth factors that may be produced. The present study demonstrates that transformation of the same clonal cell type, NIH-3T3 cells, clone 7, with different retroviral oncogenes (Ki-*ras, mos, fes, fms,* or *src*), with activated cellular protooncogenes (*met* or *trk*), with a DNA tumor virus (SV40), or with a chemical carcinogen (NMU) results in the enhanced expression of specific combinations of growth factors, including TGFα, TGFβ, bFGF, and the A or B chains of PDGF. In most of the transformed NIH-3T3 cell lines, the increased levels of biologically active growth factors, such as TGFα, TGFβ, and to a lesser extent bFGF, are paralleled by a corresponding increase in the level of expression of mRNA transcripts for these respective growth factors.

In all the transformed cell lines, with the possible exception of SV40-transformed NIH-3T3 cells, there is an elevation in the level of production of immunoreactive and biologically active TGFα that can be detected in the concentrated CM from these cells. It is noteworthy that the enhanced production of TGFα occurs in NIH-3T3 cells transformed with oncogenes that are structurally distinct yet can be categorized into several major functional families, including those that encode for tyrosine kinases (*fes, fms, src, met,* and *trk*), for serine/threonine kinases (*mos*), and for guanine nucleotide binding proteins (Ki-*ras*). This would suggest that these oncogenes and possibly some carcinogens such as NMU may be regulating cell growth by convergence at some point on common pathways that involves the indirect regulation of TGFα production and the control of EGF receptor expression. This may be the case since the increased secretion of TGFα is concurrently reflected by a reduction in or a total absence of unoccupied cell surface EGF receptors on these same cells. In fact, there appears to be a reasonable correlation with respect to the amount of TGFα that is being secreted by these transformed cells and the degree of EGF receptor reduction. There may be a functional relationship between these two events, since it is possible that the TGFα being secreted is occupying the EGF receptors on these cells, thereby leading to a chronic down-regulation as has been suggested for *ras*-, *mos*-, and *fes*-transformed NRK cells or rat embryo fibroblasts [5,9,23,38]. Alternatively, there may be a reduction in the affinity of the EGF receptor for its ligand(s) or a decrease in EGF receptor protein and/or mRNA expression in these cells that can occur independently of any change in TGFα production [39].

TGFβ is another growth factor whose production is known to be enhanced following the transformation of rat and mouse fibroblasts with oncogenes such as *ras* and *mos* [9,13]. In addition, TGFβ can also cooperate with TGFα and, under certain conditions, with other growth factors, such as PDGF, IGF-II, and bFGF to induce the soft agar growth of nontransformed rodent fibroblasts in vitro, suggesting that the synthesis of TGFβ in some transformed cells may be coordinately controlled with the expression of other growth factors [4,10,19,20]. This appears to be the case for the

majority of NIH-3T3 cells that are transformed with agents that enhance the production of TGFα. For example, NIH-3T3 cells that are transformed with *src*, *trk*, or NMU secrete both TGFα and TGFβ protein and also express high levels of mRNA for these two growth factors. However, there are exceptions, as in the case of *fms*-transformed NIH-3T3 cells in which TGFα CM levels are high, but TGFβ levels in the CM are not significantly different from the nontransformed NIH-3T3 cells. It should also be stressed that TGFβ activity in the CM from all of the cell lines was ascertained after acid treatment. There may be differences between these various transformed cell lines and nontransformed NIH-3T3 cells with respect to the relative proportions of TGFβ protein that are being secreted in active vs. latent forms.

For other growth factors, such as bFGF and the A and B chains of PDGF, the expression of mRNA transcripts and/or bioactivity for these growth factors appears to be more restrictive. Activity related to bFGF can be readily detected at various levels in the cell lysates prepared from six of the nine transformed cell lines. In contrast, only *trk*-transformed NIH-3T3 cells are expressing mRNA transcripts for the B chain of PDGF, whereas these same cells fail to express PDGF A chain mRNA. The *trk*-transformed cells also appear to be very high producers of most of the growth factors that were screened in this study, such as TGFα, TGFβ, bFGF, and probably B chain homodimers of PDGF. Collectively, these results suggest that transformation of the same cell type by different modalities can lead to the enhanced production of common sets of growth factors such as TGFα and TGFβ. However, the overexpression of other growth factors such as bFGF and various species of PDGF such as AA, BB, or AB homodimers and heterodimers, respectively, may be more limited to cells transformed by different subpopulations of oncogenes. Presently, it is not clear whether these patterns of growth factor expression following transformation with different oncogenes are functionally significant. It is certainly possible, as has been demonstrated with PDGF in *sis*-transformed fibroblasts and with bombesin- or gastrin-releasing peptide in small cell lung carcinoma cells [1–3], that these growth factors act in a collective fashion to stimulate the growth of these cells through an autocrine and/or paracrine mechanism. In addition, these growth factors may be involved in mediating specific stages of the metastatic process such as angiogenesis and invasion, two steps in which growth factors may function as chemoattractants [3–5]. In this respect, NIH-3T3 that have been transformed by Ha-*ras*, *src*, *fes*, *fms*, and *mos* exhibit a metastatic phenotype, demonstrating that structurally diverse oncogenes can affect tumor progression, possibly through a common pathway [44]. This pathway may involve components associated with the cytoskeleton, since changes in these proteins may lead to alterations in cell shape and motility that are generally observed in transformed cells and that may contribute to the onset of the metastatic phenotype. For example, NIH-3T3 cells that have been transformed with Ha-*ras*, Ki-*ras*, *src*, *fes*, *fms*, and *mos* show markedly reduced expression of two muscle-related forms of tropomyosin that are normally found in nontransformed fibroblasts [45]. Derangements in microfilament formation due to the deficient production and utilization of the same isoforms of tropomyosin that are suppressed by different retroviral oncogenes are also produced by TGFα in NIH-3T3 cells and in normal rat kidney fibroblasts, suggesting that TGFα is one growth factor that may be mediating some of the effects of these different oncogenes [46]. A comparative approach, as in this study, with cells of the same genetic background may lead to the identification of common cellular pathways involving various growth factors

that might be shared between different oncogenes and that might be involved in the initiation or the maintenance of cellular transformation and subsequent tumor metastasis.

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