Identification of the Differentiation-associated p93 Tyrosine Protein Kinase of HL-60 Leukemia Cells as the Product of the Human c-fes Locus and Its Expression in Myelomonocytic Cells*

(Received for publication, June 6, 1988)

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A differentiation-associated 93-kDa tyrosine kinase (p93) was purified previously from the human promyelocytic leukemia cell line HL-60. The present study conclusively identifies p93 as the c-fes proto-oncogene product and shows that expression of p93tyr and its associated tyrosine kinase activity are marked in mature granulocytes, monocytes, and human myeloid leukemia cell lines. Antisera to peptides obtained by expression of p93 cDNA fragments in Escherichia coli reacted strongly with p93 purified from HL-60 cells. Western blots using one of these antisera demonstrated high levels of p93 protein in normal human granulocytes and monocytes, as well as the cell lines KG-1, THP-1, HEL, and U-937, all of which can be induced to differentiate along the myelomonocytic pathway. Conversely, in cell lines resistant to myeloid differentiation, p93 expression was either very low or absent. Expression of immunoreactive p93tyr in these cell lines showed a strong positive correlation with p93tyr tyrosine kinase activity, which was measured in cell extracts using a nonnaturating gel assay. Finally, the expression of p93tyr, its tyrosine kinase activity, and the binding of 125I-granulocyte-macrophage colony-stimulating factor (GM-CSF) were all coordinately increased in HL-60 cells treated with the granulocytic differentiation inducer dimethyl sulfoxide, while all three parameters were low in untreated or differentiation-resistant HL-60 cells. These results suggest that expression of p93tyr tyrosine kinase activity may be an essential component of myeloid differentiation and responsiveness to granulocyte-macrophage colony-stimulating factor.

Tyrosine-specific protein kinase activity is a property shared by many transforming viral oncoproteins, their normal cellular homologs, and a variety of growth factor receptors, suggesting that tyrosine phosphorylation of critical cellular targets may influence growth and differentiation (reviewed in Ref. 1). In several instances, proto-oncogenes show strong sequence homology with growth factor receptor-kinase complexes (2, 3), providing an even stronger link between tyrosine kinase activity and cellular growth regulation. For example, the cellular homolog of the v-fms transforming oncogene is the receptor for macrophage colony-stimulating factor, a hematopoietic growth factor essential for proliferation and differentiation of mature macrophages (3, 4). Treatment of HL-60 promyelocytic leukemia cells (5) with the macrophage inducer TPA1 leads to increases in c-fms transcription (6) and accumulation of the macrophage colony-stimulating factor receptor-kinase (7), along with acquisition of characteristics of the mature phenotype. These results suggest that expression of ligand-regulated tyrosine kinase activity is an essential component of macrophage differentiation. Conversely, cell-surface expression of the retroviral form of this kinase (gp140-55) may contribute to malignant transformation (8).

Induction of terminal differentiation in HL-60 cells by a variety of compounds in addition to TPA is also associated with increases in tyrosine kinase activity (9–11). Two major differentiation-associated tyrosine kinases have recently been purified to homogeneity from this cell line, following treatment with the granulocytic inducer MeSO (12). These kinases have apparent molecular masses of 60 and 93 kDa, and both were immunoprecipitated with monoclonal antibodies raised against the kinase domain of p60tyr. In addition, the 93-kDa form (p93) was shown to be antigenically related to the v-fps/5-transforming oncogene product (13, 14), suggesting that p93 is the product of the human c-fes locus (15). p93 kinase activity is also markedly increased following treatment of HL-60 cells with the granulocytic inducer retinoic acid and the monocytic inducers, 1,25-dihydroxyvitamin D3, interferon-γ, and tumor necrosis factor-α (16–19), indicating a role for p93 tyrosine kinase activity in myelomonocytic differentiation. This study unequivocally confirms the identity of p93 as the c-fes proto-oncogene product, and demonstrates that expression of p93tyr and its attendant tyrosine kinase activity are associated with mature granulocytes, monocytes, and leukemia cell lines capable of myeloid differentiation.

EXPERIMENTAL PROCEDURES

Materials—[γ-32P]ATP was purchased from Du Pont-New England Nuclear. Tyrosine-agarose, poly(glut,tyr)6, BSA, and MeSO were obtained from Sigma. ABP-derivatized tyrosine-agarose (ABP-agarose) was synthesized by coupling diazotized ABP to tyrosine-agarose according to the method of Landt et al. (20).

Cell Culture—HL-60, U-937, HEL, K-562, and THP-1 cells were obtained from the American Type Culture Collection (ATCC), Rockville, Maryland. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

†Recipient of a Pharmacology Research Associate Training (PRAT) Fellowship from the National Institute of General Medical Sciences.

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1The abbreviations used are: TPA, 12-O-tetradecanoylphorbol-13-acetate; GM-CSF, granulocyte-macrophage colony-stimulating factor; ABP, 4-aminobenzylphosphonic acid; Hepes, 4-(2-hydroxyethyl)-1-piperazineneethanesulfonic acid; SDS, sodium dodecyl sulfate; BSA, bovine serum albumin; EGTA, ethylenediaminetetraacetic acid.
villie, MD, and were grown in RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum, 50 mM Hepes, pH 7.4, 1 mM sodium pyruvate, nonessential amino acids, 100 units/ml penicillin, and 100 µg/ml streptomycin. KG-1 and KG-1a cells were also obtained from the ATCC and grown in Iscove's modified Dulbecco's medium supplemented as above except with 15% fetal calf serum. The TPA-resistant HL-60/MezSO line and the TPA-resistant line were maintained in 0.8% MezSO. TPA and HL-60/MezSO were supplied by Dr. Steven Grant, Medical College of Virginia, Richmond, VA, and were grown in the same media as the parent cell line; the MezSO-resistant line was maintained in 0.8% MezSO. All cells were subcultured twice weekly, and maintained at a density of 10^6-10^7 cells/ml. Normal human granulocytes and monocytes were isolated using Mono-Poly Resolving Medium (Flow Laboratories, McLean, VA). HL-60 cells were treated with 1.6% MezSO for 5 days to induce granulocytic differentiation.

Preparation of Cell Extracts—Cells (0.5-1.0 x 10^7) were collected by centrifugation and washed twice in Hank's balanced salt solution containing 20 mM EDTA without Mg^2+ or Ca^2+. The cell pellet was sonicated for 5 s in 0.5 ml of 50 mM Tris-HCl, pH 7.5, containing 2 mM EGTA, 10 mM dithiothreitol, 0.1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, 50 µg/ml aprotinin, 200 µg/ml leupeptin, and 400 µg/ml soybean trypsin inhibitor, and centrifuged at 15,000 x g at 4°C for 20 min. The supernatant was removed, and the pellet was re-extracted with an identical buffer containing 1% Triton X-100. Protein concentrations were determined using a Coomassie Blue-based reagent (Fierce) and BSA as standard.

Tyrosine-Agarose Chromatography—Proteins present in 1.0% TPA extracts were further fractionated by tyrosine-agarose chromatography (21). Extracts containing 0.5 mg of protein were applied to 1.0 ml tyrosine-agarose columns pre-equilibrated with Buffer A (40 mM Hepes-NaOH, pH 7.2, containing 1 mM dithiothreitol, 10% glycerol, 0.5% Triton X-100, 0.2 mM phenylmethylsulfonyl fluoride, 10 µg/ml aprotinin, 30 µg/ml leupeptin, 40 µg/ml soybean trypsin inhibitor) containing 0.8 M (NH_4)_2SO_4. The columns were washed with 5.0 ml of the same buffer and then eluted with 2.0 ml of Buffer A containing 0.2 M (NH_4)_2SO_4. Aliquots (1.0 ml) of the wash and elution fractions were concentrated using Centricon 30 microconcentrators (Amicon Corp., Danvers, MA), diluted in Buffer A without (NH_4)_2SO_4, and re-extracted to a final volume of 40 µl prior to the nondenaturing gel assay for tyrosine kinase activity or further chromatography on ABP-agarose.

ABP-Agarose Chromatography—Tyrosine kinase activity that eluted from tyrosine-agarose was analyzed further by ABP-agarose chromatography. Equal volumes of the concentrated elution fractions from tyrosine-agarose were applied to ABP-agarose columns (2.0 ml) pre-equilibrated in Buffer A containing 150 mM NaCl. The columns were washed with 4.0 ml of the same buffer, and eluted with Buffer A containing 150 mM NaCl and 40 mM phenyl phosphate. The wash and elution fractions were concentrated to a final volume of 40 µl, and aliquots (1.0 µl) were assayed for tyrosine kinase activity using the nondenaturing gel assay.

Nondenaturing Gel Assay for Tyrosine Kinase Activity—Analysis of tyrosine kinase protein activity present in crude cell extracts and column fractions was conducted using a nondenaturing gel assay system described in detail elsewhere (17). Briefly, protein samples were subjected to electrophoresis on 4.5% polyacrylamide mini-gels ( Hoefer Scientific, San Francisco, CA) at 4°C. Following electrophoresis, the gels were incubated with Mg^2+, Mn^2+, and [γ-32P]ATP in the presence and absence of gly[b(1,5)A(1,5)]p, a synthetic polymer substrate in which tyrosine acts as sole phosphate acceptor. Following incubation at 37°C for 30 min, the gels were washed extensively in 5% trichloroacetic acid containing 10 mM sodium pyrophosphate, dried, and the kinase activity localized by autoradiography. Enzyme activity was quantified by counting the radio labeled bands from the gels and determining the amount of 32P present by liquid scintillation counting. Data are expressed as the increase in dpm observed in the presence of poly[b(1,5)A(1,5)].

Preparation of Anti-c-fes Antibodies—Rabbit antiserum to recombinant c-fes peptides were generously provided by Dr. Dennis J. Slamon, UCLA School of Medicine, Los Angeles, CA. Details of the molecular cloning of cDNA fragments, as well as expression of these fragments in E. coli and generation of polyclonal antiserum will be described elsewhere.

Immunoblotting—Total cell extracts were prepared in buffer containing 1.0% Triton X-100 (see above), and 0.1 mg aliquots were fractionated on tyrosine-agarsose as described above. Aliquots of pro-teins (10 µg) present in both the wash and elution fractions were resolved on 7.5% sodium dodecyl sulfate-polyacrylamide mini-gels using the discontinuous buffer system of Laemmli (22). Proteins were transferred to nitrocellulose membranes by electrotransfer using the Genie electrophoretic blower (Idean Scientific, Coralville OR). After transfer, the membranes were incubated for 1 h in TBS/HCl, pH 7.4, 150 mM NaCl, containing 3% BSA. The membranes were then incubated with the primary antibody (diluted 1:1000 in TBS/3% BSA) for 1 h, washed three times in TBS, and incubated for 1 h with goat anti-rabbit IgG alkaline phosphatase conjugate, diluted 1:7,500 with TBS/3% BSA. Bands were visualized using the color development solution supplied with the secondary antibody (Protoblot, Promega Biotec, Madison, WI). Alternatively, levels of immunoreactive proteins reactive proteins were quantitated using 125I-protein A (Amersham Corp.). Following incubation with the primary antibody, membranes were incubated for 1 h with iodinated protein A (10 µg/ml) in TBS/3% BSA. Membranes were then washed extensively with TBS, and bands were localized by autoradiography. Radiolabeled bands were then excised from the blot, and the amount of label present was determined by γ-counting.

Binding of 125I-GM-CSF to Myeloid Cells—125I-GM-CSF was the generous gift of Dr. Judith C. Gasson, UCLA School of Medicine, Los Angeles, CA. Preparation and iodination of recombinant human GM-CSF, as well as the details of the whole-cell binding assay have been described in detail elsewhere (23). Briefly, cells (5 x 10^6) were washed once in Hank's salt solution and resuspended in 0.4 ml of binding buffer (Iscove's modified Dulbecco's medium containing 25 mM Hepes and 2 mg/ml BSA) containing 0.5 mM 125I-GM-CSF and incubated for 2 h at 22°C. Identical assays were run in the presence of a 50-fold excess of unlabeled GM-CSF to define nonspecific binding. After incubation, the cells were washed in 0.75 ml of ice-cold binding buffer containing 75% fetal calf serum, and the amount of 125I-GM-CSF bound was determined by γ-counting. Data are expressed as the difference in dpm observed in the presence and absence of unlabeled GM-CSF. Under these conditions, specific binding represents 80% of total binding, and greater than 90% of the GM-CSF receptors are labeled.

RESULTS AND DISCUSSION

Identification of Purified HL-60 p93 Tyrosine Kinase as the Human c-fes Gene Product by Western Blot Analysis—A differentiation-associated tyrosine protein kinase of apparent molecular weight 93 kDa (p93) was previously purified to homogeneity from the human promyelocytic leukemia cell line HL-60 (12). The purified enzyme was immunoprecipitated by monoclonal antibodies raised against v-fps/fes peptides, suggesting that p93 was related to the human c-fes gene product, which is similarly a 93-kDa tyrosine kinase (15, 24, 25). To rigorously establish the identity of p93, rabbit polyclonal antibodies raised against a family of c-fes peptides obtained by expression of various restriction fragments of c-fes cDNA were used to probe purified p93 on Western blots (Fig. 1). All of these antisera reacted strongly with the purified kinase. Since these antibodies were raised against peptides spanning almost the entire c-fes amino acid sequence, this result establishes that p93 is identical to the c-fes protooncogene product. The differences in reactivity observed on the Western blot are related to the varying antigenicity of the c-fes peptides; note that antibodies to the largest fragment (no. 61) reacted most strongly with p93.

Quantitation of p93"fes" Expression in Human Granulocytes, Monocytes, and Leukemia Cell Lines by Western Blot Analysis—Total proteins from human granulocytes and monocytes as well as various human leukemia cell lines were extracted with buffer containing 1% Triton X-100 and fractionated by tyrosine-agarose chromatography. Proteins eluted from the tyrosine-agarose columns were probed with purified 125I-protein A (Amersham Corp.) and re-extracted with an identical buffer containing 1% Triton X-100. Protein concentrations were determined using a Coomassie Blue-based reagent (Fierce) and BSA as standard.

Preparation of Anti-c-fes Antibodies—Rabbit antiserum to recombinant c-fes peptides were generously provided by Dr. Dennis J. Slamon, UCLA School of Medicine, Los Angeles, CA. Details of the molecular cloning of cDNA fragments, as well as expression of these fragments in E. coli and generation of polyclonal antiserum will be described elsewhere.

Immunoblotting—Total cell extracts were prepared in buffer containing 1.0% Triton X-100 (see above), and 0.1 mg aliquots were fractionated on tyrosine-agarsose as described above. Aliquots of pro-
were quantitated using $^{125}$I-protein A in place of goat anti-rabbit-alkaline phosphatase conjugate; p93$^{c-fes}$ was localized on the blot by autoradiography, and the bands were excised and quantitated by $\gamma$-counting. The relative levels of immunoreactive p93$^{c-fes}$ determined in this manner are shown in Fig. 3 and were elevated in myelomonocytic cells. Normal human granulocytes and monocytes exhibited very high levels of p93$^{c-fes}$, which were slightly greater than the level in the cell lines KG-1 (26) and HL-60 (5), which are predominantly myeloblasts and promyelocytes, respectively. THP-1 monocytes (27) expressed somewhat lower levels of p93$^{c-fes}$ than HL-60 or KG-1 cells. HEL cells (28), an early blast stage cell line that can be induced to differentiate along the monocyte/macrophage pathway, also expressed p93$^{c-fes}$ at moderate levels. K-562 cells (29) are also undifferentiated myeloblasts, but differentiate mainly along the erythroid pathway. This cell line exhibits very little p93$^{c-fes}$. A number of variant cell lines resistant to inducers of myelomonocytic differentiation also showed greatly reduced expression of p93$^{c-fes}$. These include KG-1a (30), a differentiation-resistant variant of KG-1 with characteristics of early myeloblasts, and two variants of HL-60 which are resistant to either granulocytic differentiation by Me$_2$SO (HL-60/Me$_2$SO) or TPA-induced differentiation to macrophage-like cells (HL-60/TPA). These results suggest that expression of p93$^{c-fes}$ is an important component of terminal myelomonocytic differentiation and are consistent with earlier reports that c-fes expression appears to be limited to cells of this lineage (24, 25).

In addition to immunoreactive p93$^{c-fes}$, several of the cell lines also expressed a lower molecular mass protein of approximately 50 kDa that cross-reacted with the anti-c-fes antiserum (Fig. 2). Although the identity of this protein is unknown, it may be derived from c-fes by either alternate splicing at the mRNA level, as has been observed for the tyrosine kinase products of the c-abl (31) and c-fgr (32) oncogenes, or by proteolysis.

Analysis of Tyrosine Kinase Activity in Myeloid Cells by Nondenaturing Gel Electrophoresis—Previous studies of tyrosine kinases in this laboratory have employed a nondenaturing polyacrylamide gel assay, which permits direct quantitation of individual kinases without interference from proteases or phosphotyrosine phosphatases (17). Crude membrane fractions assayed in this way revealed two major tyrosine kinases in differentiated HL-60 cells, which were purified to homogeneity (12). The high mobility form was shown by immunoprecipitation to be related to p60$^{c-src}$, while the low mobility form as shown in this study is indistinguishable from the p93$^{c-fes}$ proto-oncogene product (see above). Therefore, this assay system was used to screen all of the cell lines which expressed immunoreactive p93$^{c-fes}$.

Since the majority of the p93$^{c-fes}$ tyrosine kinase activity in Me$_2$SO-differentiated HL-60 cells is associated with the membrane fraction, protein extracts were prepared from each cell

Fig. 1. Western blots of p93 tyrosine kinase purified from HL-60 cells differentiated with the granulocytic inducer Me$_2$SO. Equal amounts of purified kinase (500 ng) were electrophoresed on 7.5% SDS-polyacrylamide gels and transferred to nitrocellulose membranes as described under “Experimental Procedures.” Following transfer, nitrocellulose strips were probed with rabbit antisera raised against cloned c-jun peptides; these peptides (arbitrarily numbered 61, 69, 71, and 75) were produced by expressing the c-fes cDNA restriction fragments shown in E. coli.

Fig. 2. Identification of p93$^{c-fes}$ in human granulocytes, monocytes, and leukemia cell lines by Western blot analysis. Cell extracts of leukemia cell lines and normal peripheral leukocytes were fractionated by tyrosine-agarose chromatography and separated on 7.5% sodium dodecyl sulfate-polyacrylamide gels. Proteins were transferred to nitrocellulose membranes and probed for immunoreactive p93$^{c-fes}$ using antiserum 61 (see Fig. 1) as described under “Experimental Procedures.” The blot shown was obtained from fractions eluted from tyrosine-agarose. Similar blots of the wash fractions contained no immunoreactive p93$^{c-fes}$. The abbreviation used here and in Figs. 3-7: DMSO, Me$_2$SO.

Fig. 3. Quantitation of immunoreactive p93$^{c-fes}$ levels by Western blot analysis. Equal quantities (10 µg) of cellular proteins extracted in buffer containing 1% Triton were fractionated by tyrosine-agarose chromatography as described under “Experimental Procedures.” The eluted fractions were concentrated, and equal aliquots were separated on 7.5% sodium dodecyl sulfate-polyacrylamide gels. Proteins were transferred to nitrocellulose, and probed with p93$^{c-fes}$ antiserum 61 and $^{125}$I-protein A. Radiolabeled bands were visualized by autoradiography, excised from the blot, and quantitated by $\gamma$-counting.

Fig. 4. Image of a blot showing the expression of p93$^{c-fes}$ in various cell lines. The blot was probed with antiserum 61 and autoradiographed. The cell lines shown, from left to right, are U-871, K-562, HEL, KG-1a, KG-1, HL-60/TPA, THP-1, monocyte, granulocyte, and HL-60.
line in buffer containing 1% Triton X-100 and separated on nondenaturing gels. An autoradiogram of this gel is shown in Fig. 4. All of the cells which exhibited immunoreactive p93\textsuperscript{c-fes} contained a low mobility tyrosine kinase activity (arrow) of identical electrophoretic mobility to the kinase observed in MepSO-treated HL-60 cells, suggesting that this kinase activity is catalyzed by p93\textsuperscript{c-fes} in all of the cell lines.

To obtain a quantitative measure of tyrosine kinase activity and of its subcellular distribution, separate sets of nondenaturing gels were run on cell extracts prepared sequentially with buffer containing 0.1% Triton (predominantly soluble proteins) and 1.0% Triton (membrane proteins), and tyrosine kinase activity was quantified from the radiolabeled bands. The results are shown in Fig. 5 and are expressed as the increase in dpm incorporated in the presence of the substrate.

In general, the total amount of tyrosine-specific phosphorylation observed paralleled the levels of p93\textsuperscript{c-fes} on the Western blots. The differentiation-competent cell lines KG-1, THP-1, and U-937 (33), and HEL showed elevated tyrosine kinase activity relative to the differentiation-resistant cell line KG-1a and the erythroleukemia K-562. In addition, mature granulocytes and monocytes also exhibited high levels of this tyrosine kinase activity, whereas the differentiation-resistant variants of HL-60 cells which did not express immunoreactive protein had no detectable kinase activity on nondenaturing gels (34).

Although all of the p93\textsuperscript{c-fes} tyrosine kinase activity is associated with the membrane fraction in MepSO-differentiated HL-60 cells, this is not the case in every cell line. For example, KG-1, THP-1, and U-937 cells also have significant activity associated with the soluble protein fraction. It is possible that induction of differentiation may be associated with the translocation of p93\textsuperscript{c-fes} to the cell membrane. Such a hypothesis is consistent with the suggestion that this tyrosine kinase may modulate signal transduction from a growth hormone receptor on the cell surface.

One notable exception to this correlation between immunoreactive c-fes and tyrosine kinase activity was observed in untreated HL-60 cell. Although these cells express moderate levels of immunoreactive protein, the corresponding kinase levels are low, suggesting that mechanisms independent of c-fes expression may regulate c-fes tyrosine kinase activity in this cell line. One possibility is autophosphorylation, which has been shown to increase the activity of p140\textsuperscript{c-fes}, a transforming viral homolog of p93\textsuperscript{c-fes} (35). Consistent with this hypothesis is the recent finding that treatment of HL-60 cells with MepSO results in large increases in both p93\textsuperscript{c-fes} tyrosine kinase activity and in the level of autophosphorylated p93 (34).

**Further Characterization of Tyrosine Kinases by Tyrosine-Agarose and ABP-Agarose Chromatography**—To further characterize the low mobility tyrosine kinase activities detected in the various cell lines by nondenaturing gel electrophoresis, the chromatographic properties of the enzymes were investigated by hydrophobic-interaction chromatography on tyrosine-agarose, followed by affinity chromatography on ABP-agarose. Membrane proteins solubilized in buffer containing 1% Triton X-100 were applied to tyrosine-agarose columns in high salt buffer, eluted in low salt buffer, and analyzed for tyrosine kinase activity using the nondenaturing gel assay. In each cell line, all of the kinase activity was present in the elution fraction, as was observed for the Western blots. Based on protein recovery, this step resulted in approximately 5-fold enrichment of p93\textsuperscript{c-fes} in each case. A similar result was observed at this step of the purification of p93\textsuperscript{c-fes} from MepSO-treated HL-60 cells (12). The elution fractions from tyrosine-agarose were then adsorbed to ABP-agarose, and greater than 80% of the tyrosine kinase activity applied was retained for each cell line. Quantitative recovery of tyrosine kinase activity was achieved following elution with 40 mM phenyl phosphate, suggesting that the ABP moiety may interact with p93\textsuperscript{c-fes} at its active site. This step resulted in an additional 2–3-fold purification of p93\textsuperscript{c-fes} protein. Nondenaturing gel assays of the fractions eluted from tyrosine-agarose and ABP-agarose are shown in Fig. 6.

In summary, the differentiation-associated p93\textsuperscript{c-fes} tyrosine
is identical to that of the deduced amino acid sequence (15), and its reactivity with a family of antisera raised against cloned c-fes peptides spanning almost the entire cDNA sequence. Expression of immunoreactive p93c-fes is especially high in mature peripheral monocytes and granulocytes and in leukemia cell lines capable of myelomonocytic differentiation. A strong correlation exists between the levels of p93c-fes observed on Western blots and the activity of the low mobility tyrosine kinases observed on non-denaturing gels. These kinases showed electrophoretic mobility and chromatographic behavior identical to the p93c-fes tyrosine kinase of Me6SO-differentiated HL-60 cells, establishing their identity as p93c-fes tyrosine kinases.

The finding that p93c-fes expression and its associated tyrosine kinase activity appear to be linked to myelomonocytic differentiation suggests that this kinase may play a role in the acquisition and maintenance of the mature phenotype, in a manner analogous to the c-fms/macrophage colony-stimulating factor receptor tyrosine kinase (see Introduction). Although the amino acid sequence of c-fes does not appear to contain a transmembrane domain (15), its association with the membrane in differentiated HL-60 cells suggests that it may be involved in mediating responsiveness to other hematopoietic growth factors. In this regard Dipersio et al. (23) have shown that mature granulocytes and monocytes as well as the cell lines KG-1 and HL-60 express cell-surface receptors for GM-CSF, whereas no receptors could be detected on KG-1a cells. In addition, treatment of HL-60 cells with Me6SO, which induces p93c-fes tyrosine kinase activity in concert with the granulocytic phenotype, led to parallel increases in GM-CSF binding (Fig. 7); HL-60 cells resistant to Me6SO did not express p93c-fes or GM-CSF receptors. The possible connection between the mechanism of GM-CSF action and p93c-fes tyrosine kinase activity is currently under investigation.
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