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Association of p93^{c-fes} Tyrosine Protein Kinase with Granulocytic/Monocytic Differentiation and Resistance to Differentiating Agents in HL-60 Leukemia Cells

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SUMMARY

A 93-kDa tyrosine protein kinase (p93) identified previously as the gene product of the c-*fes* proto-oncogene, is highly expressed in HL-60 leukemia cells induced to differentiate to the granulocyte or monocyte phenotype. We have now studied the relationship of p93 to the differentiation process by using a dimethyl sulfoxide (DMSO)-resistant subline of HL-60 cells (HL-60/DMSO) or the parental cell line treated with peptide or protein substrates of p93. Treatment of HL-60/DMSO cells with DMSO induced neither differentiation nor the expression of p93; however, cotreatment with IFN-α and DMSO resulted in partial differentiation and the concomitant induction of p93 activity. Treatment of wild-type HL-60 cells by the coaddition of the p93 substrates poly(Glu,Tyr)$_{1:1}$, poly(Glu,Tyr)$_{4:1}$, poly(Glu,Ala,Tyr)$_{6:3:1}$, angiotensin II or vasoactive intestinal peptide with DMSO or IFN-τ partially blocked differentiation and concurrently diminished the induction of p93 activity. The inhibitory concentrations of the p93 substrates were related to their K_m values. These results indicate that there is an obligatory association between the expression of p93 and granulocyte/monocyte differentiation in this cell line.

Promyelocytic leukemia cell line HL-60 can undergo morphologic changes in response to various differentiating agents which result in a phenotype with the characteristics of monocytes/macrophages or myelocytes/neutrophils (1). Although the events initiating such a process are not known, we have recently purified a 93-kDa tyrosine protein kinase which appears to be the c-*fes* gene product (2), and which is highly expressed in differentiated HL-60 cells (3–5). Approximately one-half of the known proto-oncogene products possess tyrosine protein kinase activity (6, 7). These activities have been found to be associated with the membrane receptors for epidermal growth factor (8), platelet-derived growth factor (9), insulin and insulin-like growth factor (10), and M-CSF (11–13). In the latter instance, the tyrosine protein kinase is the gene product of the c-*fms* proto-oncogene. Other proto-oncogene tyrosine protein kinases such as p60^{c-src} (14, 15) and the related *hck* (16) are also elevated following differentiation of HL-60 cells, and generalized increases in uncharacterized tyrosine protein kinase activities have been reported to be associated with the differentiation of HL-60 cells (17–19) as well as murine myelomonocytic cell line WEHI-3B (18). Thus, tyrosine protein kinase activity is a functional parameter between many growth and differentiating factors and the process of differentiation.

The purpose of the present study was to further explore the relationship of p93 to hematopoietic cell differentiation by using a variant of the promyelocytic leukemia cell line HL-60 that was resistant to DMSO (20). In addition, we also examined the use of peptide or protein substrates of p93 for their ability to block the differentiation of HL-60 cells and the induction of p93 activity.

Experimental Procedures

Materials. [τ-^{32}P]ATP (619 Ci/mmol) was obtained from ICN Radiochemicals (Irvine, CA). Tyrosine-agarose, α-napthyl butyrate, NBT, poly(Glu,Tyr)$_{1:1}$, poly(Glu,Tyr)$_{4:1}$, poly(Glu,Ala,Tyr)$_{6:3:1}$ and angiotensin II were purchased from Sigma Chemical Co. (St. Louis, MO). DMSO was obtained from Fisher Scientific (Silver Spring, MD), IFN-τ (10^6 units/mg) was purchased from Cellular Products (Buffalo, NY). Recombinant IFN-α was kindly supplied by Hoffmann-La Roche (Nutley, NJ). Rabbit anti-sheep red blood cell antibodies were obtained from Cooper Biomedical (Malvern, PA).

Tissue culture. HL-60 cells were obtained from the American Type

ABBREVIATIONS: M-CSF, macrophage colony-stimulating factor; DMSO, dimethyl sulfoxide; NBT, nitroblue tetrazolium; IFN-τ, immune interferon; IFN-α, recombinant leukocyte interferon species A; PMSF, phenylmethylsulfonyl fluoride; VIP, vasoactive intestinal peptide; EGTA, ethylene glycol bis-(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid; SDS, sodium dodecyl sulfate; poly(Glu,Tyr)$_{1:1}$, poly(glutamic acid, tyrosine)$_{1:1}$; poly(Glu,Tyr)$_{4:1}$, poly(glutamic acid, tyrosine)$_{4:1}$; poly(Glu,Ala,Tyr)$_{6:3:1}$, poly(glutamic acid, alanine, tyrosine)$_{6:3:1}$; PAGE, polyacrylamide gel electrophoresis; GM-CSF, granulocyte/macrophage colony stimulating factor.

Culture Collection (Rockville, MD). Cells were grown in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum, 40 mM Hepes (pH 7.4), and gentamicin (50 μg/ml) at 37° under an atmosphere containing 5% CO_2. HL-60/DMSO cells were maintained in medium containing 0.8% DMSO. Cell inocula were 1.5×10^7 cells/30 ml of medium in 75-cm^2 flasks, or 5×10^7 cells/100 ml of medium in 175-cm^2 flasks. Cell number was determined with a model ZM Coulter counter. Cells were treated with either 1.25% (v/v) DMSO and/or varying concentrations of IFN-α or 1000 units/ml of IFN-τ for 4 days to induce granulocytic or monocytic differentiation, respectively. Differentiation was measured by the reduction of NBT to formazan (21), the expression of Fc receptors (22), or the phagocytosis of sheep erythrocytes (22).

Cell extracts. DMSO-treated cells were harvested by centrifugation and washed twice with Hanks' balanced salts solution containing 20 mM EDTA without Mg^{2+} and Ca^{2+}. The cell pellet was suspended in 0.5 ml of buffer containing 50 mM Tris-HCl (pH 7.5)/1 mM PMSF/2 mM EGTA/10 mM dithiothreitol/5 μg/ml aprotinin/0.1% Triton X-100, sonicated for 5 sec at 4°, and centrifuged for 5 min at 15,000 × *g* at 4°. The supernatant was removed and the pellet was reextracted with the same buffer containing 1% Triton X-100. Protein concentrations were determined using the Bio-Rad (Richmond, CA) protein reagent with bovine serum albumin as the standard.

Tyrosine protein kinase assays. One per cent Triton X-100 extracts containing 25 μg of protein were dissolved in sample buffer containing 63 mMTris-HCl (pH 6.8)/5 mM dithiothreitol/10% glycerol/0.01% bromphenol blue. Nondenaturing polyacrylamide gel analysis of p93 activity was carried out as described previously (23). Activity was quantitated by cutting out the p93 band located by autoradiography of the dried gel and determining its radioactivity by liquid scintillation counting. Autophosphorylated p93 was characterized in 10% gels by SDS-PAGE (2).

Preparation of antiphosphotyrosine antibodies. Antiphosphotyrosine serum was raised in rabbits as described previously (2).

Partial purification of p93 tyrosine protein kinase. DMSO- and/or IFN-α-treated HL-60 and HL-60/DMSO cells were harvested by centrifugation and extracted with 0.1% and 1% Triton X-100 extraction buffer. One per cent extracts were applied to a tyrosine-agarose column and the eluate was concentrated in a Centricon 30 microconcentrator (Amicon, Danvers, MA). The sample was then incubated for 30 min at 4° with 20 mM Hepes (pH 7.4)/5 mM $MgCl_2$/5 mM $MnCl_2$/50 μM ATP. The autophosphorylated enzyme was then applied to an ultra-affinity high-pressure liquid chromatography column (Beckman Instruments, Fullerton, CA) coupled with antiphosphotyrosine IgG as described previously (2). Fractions containing activity were pooled and concentrated using a Centricon 30 microconcentrator, and the autophosphorylation of p93 was measured as described above.

Results

Induction of differentiation and p93 activity in HL-60 and HL-60/DMSO cells. The differentiation of HL-60 and HL-60/DMSO cells was assessed by their capacity to reduce NBT, express Fc receptors, and phagocytize erythrocytes (Table 1). Following treatment of wild-type HL-60 cells for 4 days with 1.25% DMSO, 77% of the cells were NBT positive, 68% expressed Fc receptors, and 42% were able to phagocytize sheep erythrocytes. In contrast, none of the DMSO-resistant cells were differentiated on the basis of these three criteria. Treatment of either HL-60 or HL-60/DMSO cells with IFN-α alone produced little or no effect on differentiation; however, the combination of DMSO and IFN-α induced a partial response in the DMSO-resistant cell line but did not further enhance the effect of DMSO in the parental cell line. Treatment of HL-60/DMSO cells with DMSO and 1000 units/ml IFN-α induced a response that was 47, 43, and 36% of the response of wild-type cells to the same treatment based on NBT positivity, Fc receptor number, and erythrophagocytosis, respectively.

To ascertain whether the expression of p93 activity was related to differentiation, nondenaturing gel assays of tyrosine protein kinase activity in cell extracts (Fig. 1) and the assess-

TABLE 1

Effect of DMSO and IFN-α on differentiation of HL-60 and HL-60/DMSO cells[a]

Treatment	% Viability	% NBT$^+$ cells	Fc receptors	Phagocytosis
HL-60 cells				
Control	92 ± 5	4 ± 1	8 ± 2	1.5 ± 0.5
DMSO, 1.25%	88 ± 8	77 ± 9	68 ± 6	42 ± 2
IFN-α				
500 units/ml	89 ± 4	6 ± 1	–[b]	–
1000 units/ml	85 ± 6	10 ± 2	–	–
2000 units/ml	73 ± 6	13 ± 5	–	–
DMSO + IFN-α, 1000 units/ml	87 ± 2	83 ± 7	70 ± 5	45 ± 3
HL-60/DMSO cells				
Control	90 ± 3	5 ± 0.2	10 ± 2	1.5 ± 0.5
DMSO, 1.25%	85 ± 6	3 ± 2	9 ± 1	1.0 ± 0.5
IFN-α				
500 units/ml	89 ± 7	10 ± 2	–	–
1000 units/ml	83 ± 4	8 ± 1	15 ± 2	3.5 ± 1.5
2000 units/ml	69 ± 6	7 ± 1	–	–
DMSO + IFN-α, 500 units/ml	83 ± 8	26 ± 4	–	–
DMSO + IFN-α, 1000 units/ml	81 ± 2	39 ± 6	30 ± 3	16 ± 3
DMSO + IFN-α, 2000 units/ml	56 ± 8	58 ± 5	–	–

[a] Each value is the mean ± standard deviation of three to six experiments.
[b] –, not determined.

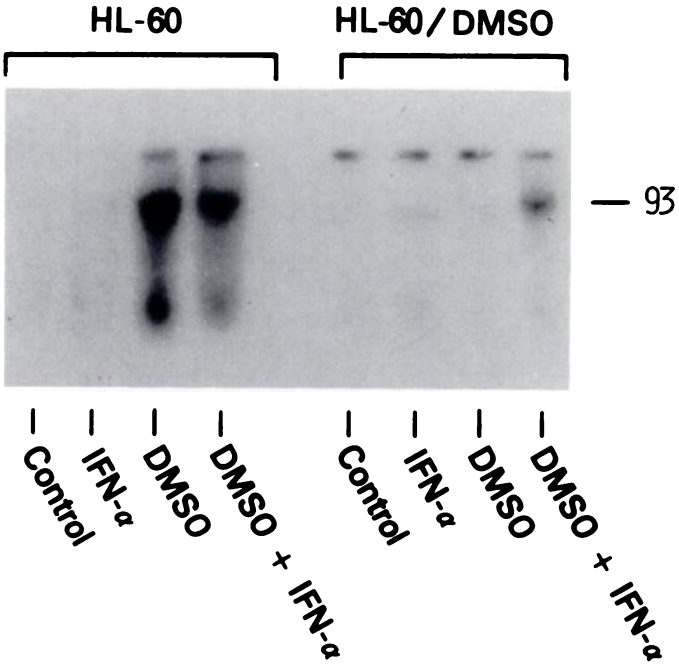

Fig. 1. p93 Tyrosine protein kinase activity in HL-60 and DMSO-resistant HL-60 cells after treatment with DMSO and/or IFN-α. Cells were treated and tyrosine protein kinase activity was measured in nondenaturing gels as described under Experimental Procedures. The radioactivity (net dpm) present in the respective lanes, *DMSO* and *DMSO + IFN-α*, was 2800 and 2200 for HL-60 cells, and 150 and 1100 for HL-60/DMSO cells. The other lanes were at background level (100 dpm).

ment of autophosphorylation in partially purified preparations of p93 were performed (Fig. 2). DMSO induced p93 activity in the differentiated HL-60 cells, but barely detectable levels of p93 could be measured in similarly treated HL-60/DMSO cells (Fig. 1). When HL-60/DMSO cells were induced to differentiate by cotreatment with IFN-α and DMSO, p93 activity appeared in proportion to their differentiative response (Fig. 1). The autophosphorylation of partially purified p93 also correlated with the percentage of differentiated cells in both cell lines (Fig. 2).

Inhibition of differentiation by p93 substrates. Previous studies examining the substrate specificity of p93 isolated from DMSO-differentiated HL-60 cells indicated that selective peptides and synthetic heteropolymers such as VIP and poly(Glu,Tyr)$_{4:1}$ were effective substrates but possessed markedly different K_m values (2). To see whether the addition of these substrates to the medium could modulate the response of HL-60 cells to the monocytic or granulocytic differentiating agents, IFN-τ or DMSO, respectively, cells were cotreated with IFN-τ and three synthetic heteropolymers or with DMSO and two peptides (Table 2). Treatment with 20 μM poly(Glu,Tyr)$_{4:1}$, 25 μM poly(Glu,Tyr)$_{1:1}$, or 20 μM poly(Glu,Ala,Tyr)$_{6:3:1}$ reduced the percentage of NBT-positive cells following treatment with IFN-τ by 43, 48, and 21%, respectively. A 20–25 μM concentration of these heteropolymers was optimal and higher concentrations did not further block differentiation. None of the polymers induced differentiation, were cytotoxic, inhibited cell growth, or affected the pH of the medium. However, highly charged polymers such as poly(Glu,Tyr)$_{1:1}$ could possibly produce nonspecific surface effects, and, thus, smaller peptide substrates such as angiotensin II and VIP, which do not present

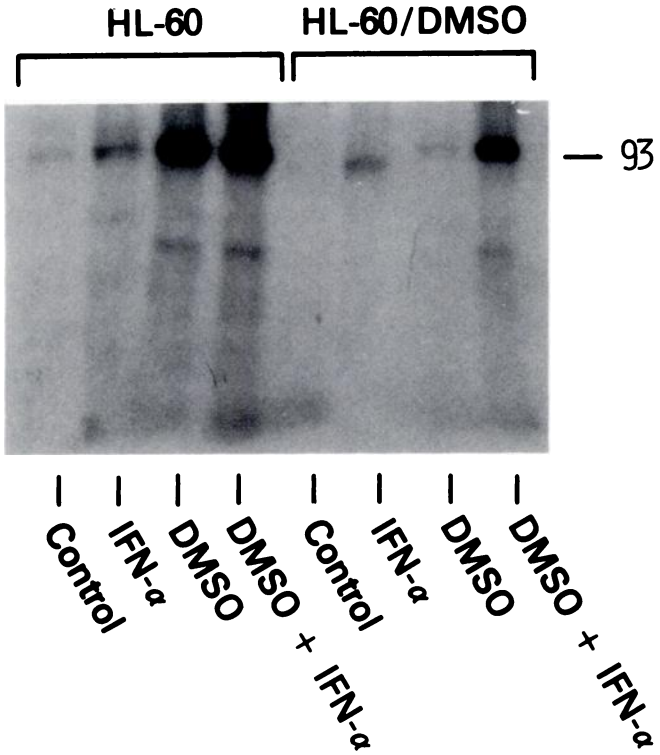

Fig. 2. Autophosphorylation *in vitro* of p93 tyrosine protein kinase in HL-60 and HL-60/DMSO cells after treatment with DMSO and/or IFN-α. Cells were treated and autophosphorylation of p93 was measured after SDS-PAGE as described under Experimental Procedures.

TABLE 2

Inhibition of differentiation of HL-60 cells by substrates of p93 tyrosine protein kinase*

Treatment	% Viability	% NBT$^+$
Control	91 ± 5	5 ± 1
IFN-τ, 1000 units/ml	90 ± 5	61 ± 3
poly(Glu,Tyr)$_{4:1}$		
10 μM	91	4
20 μM	91 ± 6	6 ± 2
40 μM	93	3
poly(Glu,Tyr)$_{1:1}$		
12.5 μM	93	4
25 μM	87 ± 6	9 ± 1
poly(Glu,Ala,Tyr)$_{6:3:1}$		
10 μM	87 ± 5	5 ± 3
20 μM	91	4
IFN-τ + poly(Glu,Tyr)$_{4:1}$		
10 μM	91	64
20 μM	91 ± 5	35 ± 3
40 μM	88	41
IFN-τ + poly(Glu,Tyr)$_{1:1}$		
12.5 μM	87	43
25 μM	88 ± 1	32 ± 10
IFN-τ + poly(Glu,Ala,Tyr)$_{6:3:1}$		
10 μM	87 ± 2	48 ± 8
20 μM	88	41
Control	92 ± 5	5 ± 1
DMSO, 1.6%	94 ± 1	79 ± 9
VIP, 1 μM	89 ± 4	3 ± 1
DMSO + VIP		
1 μM	92 ± 3	30 ± 6
0.1 μM	86 ± 3	73 ± 9
Control	90 ± 5	6 ± 1
DMSO, 1.6%	86 ± 3	83 ± 7
Angiotensin II		
50 μM	89 ± 2	8 ± 3
500 μM	80 ± 3	4 ± 2
DMSO + Angiotensin		
50 μM	84 ± 2	58 ± 10
500 μM	75 ± 6	34 ± 7

* Each value is the mean ± standard error of three to five experiments or the mean of two experiments.

these limitations, were examined. Treatment with either VIP or angiotensin II alone did not produce cytotoxicity or induce differentiation (Table 2); however, coaddition of these substrates with DMSO blocked the appearance of NBT-positive cells by 66–70% at the highest concentrations tested.

Assessment of p93 activity in nondenaturing gels indicated that treatment of either IFN-τ or DMSO-treated cells with poly(Glu,Tyr)$_{1:1}$ (Fig. 3A) or VIP (Fig. 3B), respectively, significantly reduced p93 activity and correlated with the inhibition of differentiation by these proteins (Table 2).

Discussion

Previous studies from our laboratory have focused on the 93-kDa c-*fes* tyrosine protein kinase which is highly expressed at the onset of and during granulocytic and monocytic differentiation of HL-60 cells (2–5). Although p93 is highly expressed in the differentiated phenotype, it was not known whether it was expressed in cells resistant to differentiation or whether substrates of p93 could modulate differentiation. The present study has demonstrated that a variant of HL-60 cells resistant to DMSO did not express p93 activity. However, when DMSO-resistant HL-60 cells were induced to differentiate by the combination of IFN-α and DMSO as shown previously (20), p93 activity was concomitantly enhanced. Although the mech-

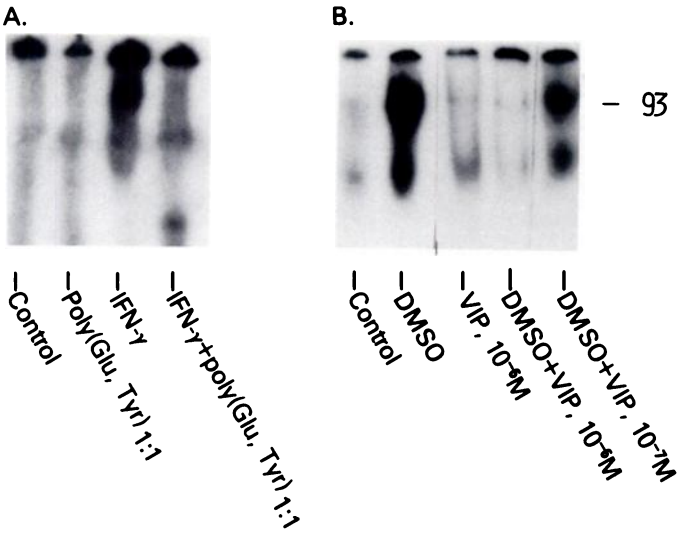

Fig. 3. Effect of poly(Glu,Tyr)$_{1:1}$ and VIP on the expression of p93 tyrosine protein kinase activity. Cells were treated with the indicated agents and tyrosine protein kinase activity was measured in nondenaturing gels as described under Experimental Procedures.

anism for the effect of IFN-α is unknown, it has been hypothesized that it may sensitize the variant cells through a generalized alteration of the cell membrane (20). In any event, the data indicate that IFN-α does not act through a cytotoxic effect or directly through changes in p93 activity. These results indicate that there is a close, if not absolute, association between hematopoietic cell differentiation and p93 activity.

Previous studies have reported that the proto-oncogene c-*fes* (24, 25) and the related avian proto-oncogene c-*fps* (26) tyrosine protein kinases were expressed in particularly high levels in bone marrow stem cells and in hematopoietic cell lines of the monocyte/macrophage and granulocyte lineages. Since there does not appear to be a transmembrane domain for the c-*fes*/*fps* gene product (27), it is unlikely that this protein serves as a receptor for proliferative or differentiating factors as in the case of the c-*fms* proto-oncogene tyrosine protein kinase, which is the cellular receptor for M-CSF (11–13). In our study, p93 activity was related positively to monocytic/granulocytic differentiation as assessed by its absence in DMSO-resistant cells, its appearance in DMSO-resistant cells rendered partially responsive to differentiation, and by its reduction in cells inhibited from undergoing either monocytic or granulocytic differentiation by peptides which are substrates for p93. Thus, p93 expression may be more involved with the global response of myeloid cells to differentiation rather than directly with a specific receptor. In this regard, Gasson and colleagues (28, 29) have reported an increase in GM-CSF receptors in DMSO-differentiated HL-60 cells of a magnitude similar to that which we observe for the elevation in p93 activity following a comparable treatment regimen. Thus, it may be plausible that p93 is involved in mediating signal transduction by various growth and differentiation factors in myeloid cells.

References

1. Hemmi, H., and T. R. Breitman. Induction of differentiation in the myelocytic cell line HL-60 and primary cultures of human leukemia cells: a model for clinical treatment, in *Developments in Cancer Chemotherapy* (R. I. Glazer, ed.). CRC Press, Boca Raton, FL, 247–280 (1984).
2. Yu, G., and R. I. Glazer. Purification and characterization of p93^{fms}- and p60^{src}-related tyrosine protein kinase activities in differentiated HL-60 leukemia cells. *J. Biol. Chem.* **262**:17543–17548 (1987).
3. Glazer, R. I., M. S. Chapekar, and M. C. Knode. Appearance of membrane-bound tyrosine kinase activity during differentiation of HL-60 leukemia cells by immune interferon and tumor necrosis factor. *Biochem. Biophys. Res. Commun.* **140**:908–915 (1986).
4. Glazer, R. I., M. S. Chapekar, K. D. Hartman, M. C. Knode, and G. Yu. Induction of membrane-bound tyrosine kinase activity in human promyelocytic leukemia cells by differentiating agents, in *Membrane Proteins: Proceedings of the Membrane Protein Symposium* (S. S. Goheen, ed.). Bio-Rad Laboratories, Richmond, CA 715–728 (1987).
5. Chapekar, M. S., K. D. Hartman, M. C. Knode, and R. I. Glazer. Synergistic effect of retinoic acid and calcium ionophore A23187 on differentiation, c-*myc* expression, and membrane tyrosine kinase activity in human promyelocytic leukemia cell line HL-60. *Mol. Pharmacol.* **31**:140–145 (1986).
6. Hunter, T. A thousand and one protein kinases. *Cell* **50**:823–829 (1987).
7. Bishop, J. M. Viral oncogenes. *Cell* **42**:23–38 (1986).
8. Cohen, S., G. Carpenter, and I. King, Jr. Epidermal growth factor-receptor protein kinase interactions. Co-purification of receptor and epidermal growth factor-enhanced phosphorylation. *J. Biol. Chem.* **255**:4834–4842 (1980).
9. Frackelton, A. R., P. Tremble, Jr., and L. T. Williams. Evidence for the platelet-derived growth factor-stimulated tyrosine phosphorylation of the platelet-derived growth factor receptor *in vivo*. *J. Biol. Chem.* **259**:7909–7915 (1984).
10. Kasuga, M., Y. F. Yamaguchi, D. L. Blithe, and C. R. Kahn. Tyrosine-specific protein kinase activity is associated with the purified insulin receptor. *Proc. Natl. Acad. Sci. USA* **80**:2137–2141 (1983).
11. Sacca, R., E. R. Stanley, C. J. Sherr, and C. W. Rettenmier. Specific binding of the mononuclear phagocyte colony-stimulating factor CSF-1 to the product of the v-*fms* oncogene. *Proc. Natl. Acad. Sci. USA* **83**:3331–3335 (1986).
12 Sherr, C. J., C. W. Rettenmier, R. Sacca, M. F. Roussel, A. T. Look, and E. R. Stanley. The c-fms proto-oncogene product is related to the receptor for the mononuclear phagocyte growth factor, CSF-1. *Cell* **41**:665–676 (1985).
13. Rettenmier, C. W., R. Saca, W. L. Furman, M. F. Roussel, J. T. Holt, A. W. Nienhuis, E. R. Stanley, and C. J. Sherr. Expression of the human c-*fms* proto-oncogene (colony-stimulating factor-1 receptor) on peripheral blood mononuclear cell and choriocarcinoma cell lines. *J. Clin. Invest.* **77**:1740–1746 (1986).
14. Barnekow, A., and M. Gessler. Activation of the pp60^{c-src} kinase during differentiation of monomyelocytic cells *in vitro*. *EMBO J.* **4**:701–705 (1986).
15. Gee, C. E., J. Griffin, L. Sastre, L. J. Miller, T. A. Springer, H. Piwnica-Worms, and T. M. Roberts. Differentiation of myeloid cells is accompanied by increased levels of pp60^{c-src} protein and kinase activity. *Proc. Natl. Acad. Sci. USA* **83**:5131–5135 (1986).
16. Quintrell, N., R. Lebo, H. Varmus, J. M. Bishop, M. J. Pettenati, M. M. Le Beau, M. O. Diaz, and J. D. Rowlet. Identification of a human gene (*HCK*) that encodes a protein-tyrosine kinase and is expressed in hematopoietic cells. *Mol. Cell. Biol.* **7**:2267–2275 (1987).
17. Frank, D. A., and A. C. Sartorelli. Regulation of protein phosphotyrosine content by changes in tyrosine kinase and protein phosphotyrosine phosphatase activities during induced granulocytic and monocytic differentiation of HL-60 leukemia cells. *Biochem. Biophys. Res. Commun.* **140**:440–447 (1987).
18. Frank, D. A., and A. C. Sartorelli. Alterations in tyrosine phosphorylation during granulocytic maturation of HL-60 leukemia cells. *Cancer Res.* **48**:52–58 (1988).
19. Kraft, A. S., and R. L. Berkow. Tyrosine kinase and phosphotyrosine phosphatase activity in human promyelocytic leukemia cells and human polymorphonuclear leukocytes. *Blood* **70**:356–362 (1987).
20. Grant, S., K. Bhalla, B. Weinstein, S. Pestka, M. D. Mileno, and P. B. Fisher. Recombinant human interferon sensitizes resistant myeloid leukemic cells to induction of terminal differentiation. *Biochem. Biophys. Res. Commun.* **130**:379–388 (1985).
21. Breitman, T. R., S. E. Selonick, and S. J. Collins. Induction of differentiation of the human promyelocytic leukemia cell line (HL-60) by retinoic acid. *Proc. Natl. Acad. Sci. USA* **77**:2936–2940 (1980).
22. Breitman, T. R., B. R. Keene, and H. Hemmi. Studies of growth and differentiation of human myelomonocytic leukemia cells in serum-free medium, in *Methods for Serum-Free Culture of Neuronal and Lymphoid Cells*. Alan R. Liss, Inc., New York, 215–236 (1984).
23. Glazer, R. I., G. Yu, and M. C. Knode. Analysis of tyrosine kinase activity in cell extracts using nondenaturing polyacrylamide gel electrophoresis. *Anal. Biochem.* **164**:214–220 (1987).
24. Feldman, R. A., J. L. Gabrilove, J. P. Tam, M. S. Moore, and H. Hanafusa. Specific expression of the human cellular *fps/fes* encoded protein NCP92 in normal and leukemic myeloid cells. *Proc. Natl. Acad. Sci. USA* **82**:2379–2383 (1985).
25. MacDonald, I., J. Levy, and T. Pawson. Expression of the mammalian c-*fes* protein in hematopoietic cells and identification of a distinct *fes*-related protein. *Mol. Cell. Biol.* **5**:2543–2551 (1985).
26. Mathey-Prevot, B., H. Hanafusa, and S. Kawai. A cellular protein is immunologically crossreactive with and functionally homologous to the Fujinami sarcoma virus transforming protein. *Cell* **28**:897–906 (1982).
27. Roebroek, A. J. M., J. A. Schalken, J. S. Verbeek, A. M. W. Van den

Ouweland, C. Onnekink, H. P. J. Bloemers, and W. J. M. Van den Ven. The structure of the human c-*fes/fps* proto-oncogene. *EMBO J.* **4**:2897–2903 (1985).
28. Gasson, C. J., S. E. Kaufman, R. H. Weisbart, M. Tomonaga, and D. W. Golde. High-affinity binding of granulocyte-macrophage colony-stimulating factor to normal and leukemic human myeloid cells. *Proc. Natl. Acad. Sci. USA* **83**:669–673 (1986).
29. Tomonaga, M., D. W. Golde, and J. C. Gasson. Biosynthetic (recombinant) human granulocyte-macrophage colony-stimulating factor: effect on normal bone marrow and leukemia cell lines. *Blood* **67**:31–36 (1986).

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