Vol. 157, No. 1, 1988 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 30, 1988 Pages 395-401

CSF-1 AND C-FMS GENE EXPRESSION IN HUMAN CARCINOMA CELL LINES

Junko Horiguchi[1], Matthew L. Sherman[1], Adam Sampson-Johannes[2], Barbara L. Weber[1] and Donald W. Kufe[1]

[1]Laboratory of Clinical Pharmacology, Dana-Farber Cancer Institue and Harvard Medical School, Boston, MA 02115

[2]Department of Cell Biology, Cetus Corporation, Emeryville, CA 94608

Received October 10, 1988

The macrophage-specific colony stimulating factor CSF-1 is required for the growth and differentiation of monocytes. The cell surface receptor for CSF-1 is identical to the product of the c-fms proto-oncogene. The present studies have monitored CSF-1 and c-fms expression in human carcinoma cell lines. Two of three human ovarian carcinoma cell lines expressed multiple species of CSF-1 mRNA. Furthermore, detection of CSF-1 transcripts was associated with secretion of CSF-1 protein that was increased after phorbol ester treatment. CSF-1 mRNA was also detectable in 4 breast and 2 lung carcinoma cell lines. In contrast, c-fms expression was found only in SK-Br-3 breast carcinoma cells. Similar studies in 2 human choriocarcinoma cell lines demonstrated the presence of c-fms, but not CSF-1, transcripts. While phorbol ester treatment was associated with increased c-fms mRNA levels in choriocarcinoma cells, this agent had no effect on CSF-1 expression. These findings indicate that: 1) CSF-1 expression is frequent in human ovarian, breast and lung carcinoma cells; and 2) coexpression of the CSF-1 and c-fms genes, as found in monocytes is infrequent in malignant epithelial and choriocarcinoma cell lines.

The growth factor CSF-1 is required for the survival, proliferation and differentiation of cells in the mononuclear phagocyte series (1). CSF-1 also appears to play a role in the development of the mouse placenta (2). Mouse cells which produce CSF-1 include uterine epithelium (2), fibroblasts (3) and bone marrow stroma (4). Moreover, human monocytes (5-7) and certain pancreatic carcinoma cell lines (8,9) have been shown to produce this factor.

The effects of CSF-1 are mediated by binding to a single class of high affinity CSF-1 receptor (1). The CSF-1 receptor and the product of the c-fms proto-oncogene have been shown to be identical molecules

Abbreviations used: CSF-1, colony-stimulating factor 1; TPA, 12-0-tetradecanoylphorbol-13-acetate.

0006-291X/88 $1.50

(10). In this regard, c-fms transcripts have been detected in placental tissues (11,12) and in monocytes (13,14). c-fms mRNA and protein have also been detected in human choriocarcinoma cell lines derived from malignant trophoblasts (15-17). Other studies have demonstrated the presence of both c-fms and CSF-1 mRNA in cells differentiated along the monocytic lineage (18), thus suggesting that this factor may act by an autocrine mechanism in mononuclear phagocytes.

We previously demonstrated that the CSF-1 gene is expressed at the RNA and protein levels in two human pancreatic carcinoma cell lines (8,9). In contrast, the present work has examined both CSF-1 and c-fms expression in a variety of other types of human malignant epithelial cell lines.

MATERIALS AND METHODS

Cell Culture

The human epithelial cell lines used in this study included: two derived from choriocarcinomas (BeWo, JEG); four derived from ovarian carcinomas (OV-S, OV-D, OV-M, SK-OV-3); four derived from breast carcinomas (BT-20, T-47D, ZR-75-1, SK-BR-3); and two derived from lung carcinomas (A549, Calu-3). The ovarian cell lines were provided by Dr. R. Knapp, Brigham and Womens' Hospital, Boston, MA (19); all other epithelial cells as well as the HL-60 human promyelocytic cell line were obtained from the American Type Culture Collection, (Rockville, MD). The BeWo, JEG, OV-S, OV-D, OV-M and HL-60 cells were treated with 32 nM TPA for varying intervals.

RNA analysis

Total cellular RNA (20 ug) was prepared from each cell line and analyzed by Northern hybridization as described previously (13). The ^{32}P-labeled DNA probes included the 0.6 kb AccI/EcoRI fragment of a human CSF-1 cDNA purified from the pcCSF-12 plasmid (8) and the 4 kb EcoRI fragment of the human c-fms gene in the pc-fms 102 plasmid (20).

CSF-1 determinations

The concentration of CSF-1 in tissue culture supernatants was determined by the CSF-1-specific radioimmunoassay (1).

RESULTS

RNA prepared from 3 human ovarian carcinoma cell lines was analyzed for the presence of CSF-1 and c-fms related transcripts (Fig. 1). CSF-1 mRNA was detected in two of these cell lines, OV-S and OV-D, while none was found in the OV-M cells (Fig. 1). There were two transcripts of approximately 4.6 and 4.0 kb in the OV-S and OV-D cells. The larger CSF-1 transcript was similar to that in TPA-treated HL-60 cells (Fig. 1) and monocytes (12,13), while the smaller

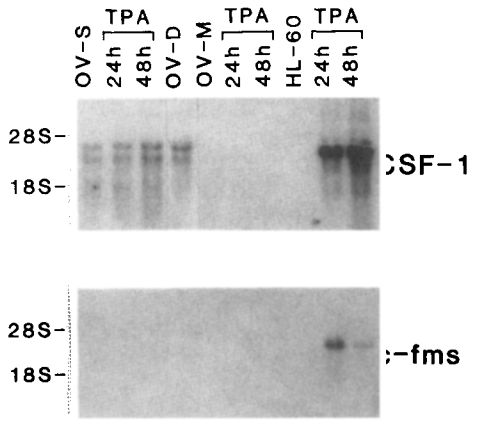

Figure 1. Expression of CSF-1 and c-fms mRNA in human ovarian carcinoma cells. Total cellular RNA (20 ug) was analyzed for CSF-1 and c-fms transcripts following Northern transfer and hybridization to the ^{32}P-labeled probes. The OV-S and OV-M cells were treated with 32 nM TPA for the indicated times before mRNA isolation. Similar amounts of RNA from HL-60 cells induced by TPA were included as a relative measure of CSF-1 and c-fms expression. CSF-1 protein in OV-S, OV-D and OV-M cell supernatants was: <83, 168 and <83 U/ml, respectively (control medium <83 U/ml). Supernatant from OV-S and OV-D cells treated with TPA for 48 h had 105 and 261 U CSF-1/ml, respectively.

transcript has not been reported for hematopoietic cells. Furthermore, the level of CSF-1 mRNA in these cells was detectably less than that in HL-60 cells treated with TPA for 24 h and 48 h (Fig. 1). In contrast to these findings, there was no detectable c-fms mRNA in these three cell lines (Fig. 1).

The three ovarian cell lines were treated with TPA for 24 and 48 h to determine if, as in HL-60 cells, there was an associated induction of CSF-1 and c-fms transcripts. TPA increased CSF-1 mRNA levels in OV-S cells (Fig. 1). In contrast, this agent had no detectable effect on levels of CSF-1 transcripts in the OV-D (data not shown) or the OV-M cells (Fig. 1). Moreover, there was no detectable induction of c-fms mRNA in these cells by TPA (Fig. 1). The untreated and TPA-treated ovarian carcinoma cells were also monitored for secretion of CSF-1 protein. There was no detectable CSF-1 in the OV-S and OV-M cell supernatants. However, this protein was present in the supernatant of TPA-treated OV-S cells. Furthermore, CSF-1 protein was present in OV-D supernatant and increased after treatment of these cells with TPA (legend to Fig. 1).

A variety of other human carcinoma cell lines were monitored for CSF-1 and c-fms transcripts. Both breast and lung carcinoma cell lines had detectable CSF-1 mRNA (Fig. 2A). Several of these cell lines expressed two transcripts that were similar in size to those found in the OV-S and OV-D cells (Fig. 2A). However, the level of

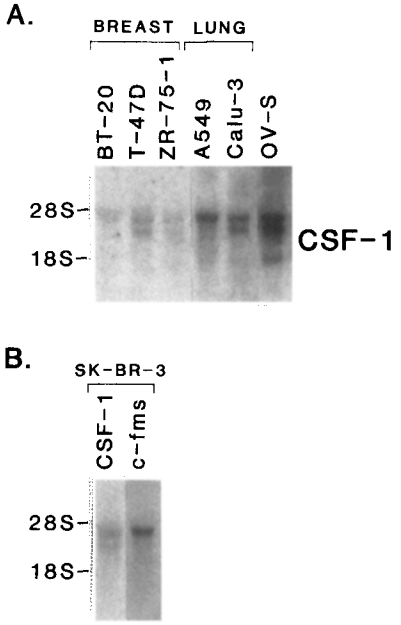

Figure 2. Expression of CSF-1 and c-fms mRNA in human breast and lung carcinoma cells. A. Total cellular RNA (20 ug) was isolated from the indicated carcinoma cells and hybridized to the ^{32}P-labeled CSF-1 probe. RNA from the OV-S cells was included for comparison. B. The ^{32}P-labeled CSF-1 and c-fms probes were hybridized to RNA isolated from the SK-BR-3 breast carcinoma cells.

CSF-1 mRNA in the breast and lung cancer cell lines was relatively lower than that in the ovarian cells (Fig. 2A). Furthermore, there was no detectable CSF-1 protein in the supernatants of these cell lines. In contrast to the presence of CSF-1 transcripts, none of these breast or lung carcinoma cell lines had detectable c-fms mRNA, even after prolonged autoradiographic exposure of the filters (data not shown). Indeed, only one additional malignant breast carcinoma cell line (SK-BR-3) expressed c-fms transcripts. Moreover, the SK-BR-3 cells also had detectable levels of CSF-1 mRNA (Fig. 2B). These findings suggested that CSF-1, but not c-fms expression, is relatively frequent among ovarian, breast and lung carcinoma cell lines.

Previous work has demonstrated that c-fms mRNA is expressed in cell lines derived from human choriocarcinomas (15). However, other studies have not examined whether these cells also express the CSF-1 gene. The BeWo and JEG choriocarcinoma cell lines had levels of c-fms mRNA that were comparable to that in TPA-treated HL-60 cells (Fig. 3). Moreover, treatment of these cells with TPA resulted in increased c-fms mRNA levels that were maximal at 24 to 48 h of drug exposure (Fig. 3). In contrast to these findings, there was no

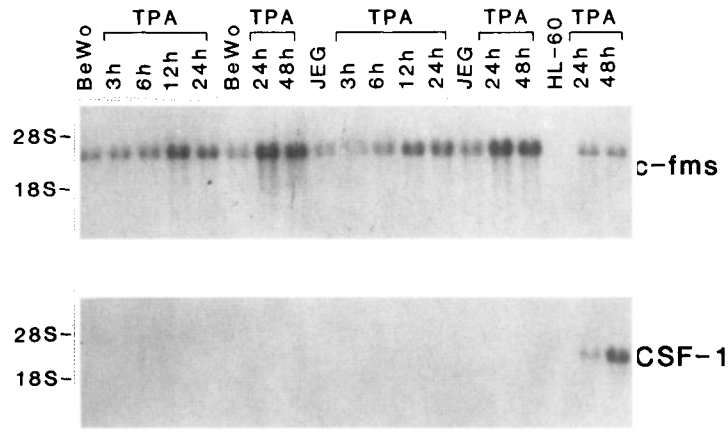

Figure 3. Expression of c-fms and CSF-1 mRNA in human choriocarcinoma cells. Total cellular RNA (20 ug) isolated from untreated and TPA-treated choriocarcinoma cells was hybridized to the ^{32}P-labeled probes. RNA from HL-60 cells was included for comparison.

detectable CSF-1 expression in these cells, even after TPA treatment (Fig. 3). Taken together, these findings indicated that the choriocarcinoma cell lines have contrasting patterns of c-fms and CSF-1 expression to that in the ovarian, breast and lung carcinoma cell lines.

DISCUSSION

Studies of human cells that produce CSF-1 have been limited, although recent work has demonstrated that this growth factor is produced by cells of the monocytic lineage (5-7). Multiple species of CSF-1 mRNA have also been detected in certain human pancreatic carcinoma cell lines (8,9), while expression of this gene in human monocytes (5-7) and myeloid leukemia cells (21) is predominantly as the larger 4.6 kb transcript. The present studies demonstrate that the CSF-1 gene is widely expressed in a variety of human ovarian, breast and lung carcinoma cell lines. Moreover, the presence of multiple species of CSF-1 mRNA in these cells is consistent with the alternative splicing of a large primary transcript (22). However, the basis for the expression of different CSF-1 mRNA species in malignant epithelial cells and not in monocytes remains unclear.

We have previously demonstrated that monocytes express both the CSF-1 and c-fms (CSF-1 receptor) genes (18). These findings have suggested that cells differentiated along the monocytic lineage are capable of regulating their own growth through the production of CSF-1. In this regard, we also monitored ovarian, breast and lung

carcinoma cell lines for the presence of c-fms transcripts. With the exception of the SK-BR-3 cell line, c-fms mRNA was undetectable in these cells. These findings suggested that CSF-1 is not capable of stimulating autocrine growth of the majority of human malignant epithelial cell lines. The SK-BR-3 cell line, however, may be an exception and additional work on these cells is underway. Furthermore, while we were unable to detect c-fms transcripts in the other ovarian, breast and lung cell lines, these findings do not prelude the possibility that this gene is expressed in these tumors in vivo.

In contrast, to the findings with malignant epithelial cells, lines derived from human choriocarcinomas constitutively express c-fms transcripts (15). However, we were unable to detect CSF-1 mRNA in these cells. Previous studies have demonstrated that TPA induces c-fms and CSF-1 expression in myeloid leukemic cell lines (5,7,13). In the present work, TPA also increased c-fms mRNA levels in the choriocarcinoma cells and CSF-1 mRNA levels in the OV-S cells. Moreover, TPA increased production of CSF-1 protein from the OV-D cells, a finding similar to that for myeloid leukemia cells (21). However, there was no detectable CSF-1 expression in TPA-treated choriocarcinoma cells or c-fms expression in TPA-treated ovarian cells. In this regard, although both transcripts can be induced by TPA, the regulation of CSF-1 and c-fms expression is controlled by distinct mechanisms (7 and unpublished data).

We have previously demonstrated that human malignant epithelial cell lines constitutively express the tumor necrosis factor gene at the RNA and protein levels (23). Recent findings from our laboratory have also demonstrated that the platelet-derived growth factor (PDGF)-1 and PDGF-2 genes are expressed by ovarian, breast and lung carcinoma cell lines (24). Transcriptional and posttranscriptional mechanisms regulate tumor necrosis factor gene expression in human monocytes (25). Similar mechanisms appear to regulate PDGF gene expression in myeloid cell lines and monocytes (26). Moreover, the TNF, PGDF and CSF-1 genes appear to share common control mechanisms at the post-transcriptional level. However, it is presently not clear whether these mechanisms contribute to the expression of these genes in malignant epithelial cells.

ACKNOWLEDGMENTS

This investigation was supported by PHS Grants CA-34183, CA-1092 and by a Burroughs Wellcome Clinical Pharmacology Scholar Award (DWK).

REFERENCES

1. Stanley, E.R., Guilbert, L.J., Tushinski, R.J. and Bartelmez, S.H. (1983) J. Cell. Biochem. 21, 151-159.
2 Pollard, J.W., Bartocci, A., Arceci, R., Orlofsky, A., Ladner, M.B. and Stanley, E.R. (1987) Nature 330, 484-486.
3. Stanley, E.R. and Heard, P.M. (1977) J. Biol. Chem. 252, 4305-4312.
4. Lanotte, M., Metcalf, D. and Dexter, T.M. (1982) J. Cell. Phys. 112, 123-127.
5. Horiguchi, J., Warren, M.K., Ralph, P. and Kufe, D. (1986) Biochem. Biophys. Res. Commun. 141, 924-930.
6. Horiguchi, J., Warren, M.K. and Kufe, D. (1987) Blood 69, 1259-1261.
7. Horiguchi, J., Sariban, E. and Kufe, D. (1988) Mol. Cell. Biol. 8, 3951-3954.
8. Kawasaki, E.S., Ladner, M.B., Wang, A.M., Van Arsdell, J., Warren, M.K., Coyne, M.Y., Schweickart, V.L., Lee, M-T, Wilson, K.J., Boosman, A., Stanley, E.R., Ralph, P. and Mark, D.F. (1985) Science 230, 291-296.
9. Ralph, P., Warren, M.K., Lee, M., Csejtey, J., Weaver, J., Broxmeyer, H., Williams, D., Stanley, E. and Kawasaki, E. (1986) Blood 68, 633-639.
10. Sherr, C.J., Rettenmier, C.W., Sacca, R., Roussel, M.F., Look, A.T. and Stanley, E.R. (1985) Cell 41, 665-676.
11. Muller, R., Slamon, D.J., Adamson, E.D., Tremblay, J.M., Muller, D., Cline, M.J. and Verma, I.M. (1983) Mol. Cell. Biol. 3, 1062-1069.
12. Hoshina, M., Hishio, A., Bo, M., Boime, I. and Mochizuki, M. (1985) Acta Obstet. Gynec. Jpn. 37, 2791-2798.
13. Sariban, E., Mitchell, T. and Kufe, D. (1985) Nature 316, 64-66.
14. Nienhuis, A.W., Bunn, H.F., Turner, P.H., Gopal Venkat, T., Nash, W.G., O'Brien, S.J. and Sherr, C.J. (1985) Cell 42, 421-428.
15. Muller, R., Tremblay, J., Adamson, E. and Verma, I. (1983) Nature 304, 4543-4546.
16. Woolford, J., Rothwell, V. and Rohrschneider, L. (1985) Mol. Cell. Biol. 5, 3458-3466.
17. Rettenmier, C., Sacca, R., Furman, W., Roussel, M., Holt, J., Nienhuis, A., Stanley, E. and Sherr, C. (1986) J. Clin. Invest. 77, 1740-1746.
18. Wakamiya, N., Horiguchi, J and Kufe, D. (1987) Leukemia 1, 518-520.
19. Masuho, Y., Zalutsky, M., Knapp, R.C. and Bast, R.C. (1984) Cancer Res. 44, 2813-2819.
20. Coussens, L., Van Beveren, C., Smith, D., Chen, E., Mitchell, R.L., Isacke, C.M., Merma, I.M. and Ullrich, A. (1986) Nature 320, 277-280.
21. Rambaldi, A., Wakamiya, N., Vellenga, E., Horiguchi, J., Warren, M.K., Kufe, D. and Griffin, J.D. (1988) J. Clin. Invest. 81, 1030-1035.
22. Ladner, M.B., Martin, G.A., Noble, J.A., Nikoloff, D.M., Tal, R., Kawasaki, E.S. and White, T.J. (1987) EMBO. J. 6, 2693-2698.
23. Spriggs, D., Imamura, K., Rodriguez, C., Sariban, E. and Kufe, D. (1988) J. Clin. Invest. 81, 455-460.
24. Sariban, E., Sitaras, N.M., Antoniades, H.N., Kufe, D.W. and Pantazis, P. (1988) J. Clin. Invest. (in press).
25. Sariban, E., Imamura, K., Luebbers, R. and Kufe, D. (1988) J. Clin. Invest. 81, 1506-1510.
26. Sariban, E. and Kufe, D. (1988) Cancer Res. 48, 4498-4502.