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ONCOGENE EXPRESSION IN HUMAN HEPATOMA CELLS PLC/PRF/5

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SUMMARY: The expression of 7 cellular oncogenes in a human hepatoma cell line PLC/PRF/5 was studied using Northern blot analyses. Among the oncogenes tested, c-abl, c-fes, c-fms, c-myc, c-Ha-ras and c-sis were expressed. The oncogene c-Ki-ras was not expressed. The length of the mRNAs expressed was almost consistent with published data. Compared to the oncogene expression in Daudi lymphoma cells, the same kind of oncogenes were expressed in PLC/PRF/5 cells, but the intensity of the signal in each oncogene expression was stronger in Daudi cells than in PLC/PRF/5 cells. Considering the cellular localization and the function of each oncogene, the oncogene survey in hepatoma cells broadens the knowledge of hepatocarcinogenesis and the character of human hepatoma cells.

The activation of oncogenes has been reported to be associated with carcinogenesis (1). The expression of cellular oncogenes has been studied in many types of tumors (2, 3, 4, 5, 6). As for hepatoma (or primary carcinoma of the liver), mainly myc and ras oncogenes have been investigated using rat hepatomas (7, 8, 9, 10, 11) and human hepatoma tissues (12).

However, some other oncogenes might be expressed in hepatoma cells, and such oncogenes could play important roles in hepatocarcinogenesis. For this reason, oncogene surveys are needed. Moreover, there is no report concerning oncogene expression in human hepatoma cell lines. In this study, we examined the expression of 7 different oncogenes in a human hepatoma cell line PLC/PRF/5.

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MATERIALS AND METHODS

Cells: A human hepatoma cell line PLC/PRF/5 was established by Alexander et al. (13). The cells were grown as monolayers in Eagle's minimal essential medium (MEM) supplemented with 10% fetal bovine serum (FBS). Daudi lymphoblastoid cells were grown in suspension cultures in RPMI 1640 medium with 10% FBS. Both cell lines were obtained from the American Type Culture Collection (Rockville, MD). Human peripheral blood leukocytes were obtained from healthy volunteers.

Preparation of total RNA and Northern blotting: Total RNA from the cells was isolated by extraction with guanidine thiocyanate followed by centrifugation through a cesium chloride cushion as previously described (14, 15). 40 μg of total RNA from each cell line was denatured with glyoxal and electrophoresed on a horizontal 1.5% agarose gel at 4°C overnight. Transfer, baking and hybridization were carried out as previously described (16, 17). The oncogene DNA probes were nick-translated with α-[^{32}P]dCTP. The hybridization was done under a stringent condition (in 50% formamide at 42°C). After washing the filters four times for 5 min each at room temperature in 2X SSC, 0.1% SDS followed by two washes of 15 min each at 50°C in 0.1X SSC, 0.1% SDS, they were autoradiographed using intensifying screens at -80°C.

Probes: The oncogene probes used in this study are shown in Table 1. As Hunter (25) described, oncogenes can be classified into 4 or 5 classes as in Table 1 according to the cellular

Table 1. Oncogene DNA probes used in this study

Cellular oncogene	Restriction fragment	Size	Cellular location	Function	Class	Reference
abl	BamHI-HindIII	0.65 kb	Plasma membrane	Tyrosine specific protein kinase	Class 1	(18)
fes	BamHI	0.8 kb	Cytoplasm			(19)
fms	EcoRI-HindIII	0.9 kb	Plasma and cytoplasmic membranes	Cytoplasmic domain of a growth-factor receptor	Class 1-related	(20)
sis	BamHI	1.0 kb	Secreted	Platelet-derived growth factor like	Class 2	(21)
Ha-ras	BamHI	6.6 kb	Plasma membrane	GTP binding	Class 3	(22)
Ki-ras	EcoRI-HindIII	2.0 kb				(23)
myc	EcoRI-HindIII	9.0 kb	Nucleus	DNA binding	Class 4	(24)

Oncogenes were classified according to the cellular location and the function of each oncogene as described by Hunter (25).

localization and the function. The probes of c-myc and c-Ha-ras were obtained from the American Type Culture Collection (Rockville, MD). The rest were purchased from the Oncogene Science, Inc. (Mineola, NY).

RESULTS

Total RNA isolated from human hepatoma cells PLC/PRF/5 as well as Daudi cells (a positive control) and normal human leukocytes (a negative control) were denatured with glyoxal, electrophoresed, transferred to nitrocellulose paper and hybridized with 7 different oncogene probes as described in Materials and Methods.

To assess the quality of total RNA, the gel was stained with methylene blue. The characteristic 18S and 28S rRNA bands were observed with no apparent degradation (Fig. 1).

The results of Northern blot analyses are shown in Fig. 2. All of the oncogenes tested, with the exception of c-Ki-ras, were expressed in PLC/PRF/5 cells as well as in Daudi cells. The intensity of each signal observed in PLC/PRF/5 cells was lower than that in Daudi cells. Smears or very faint signals were found in leukocytes, but no specific band for any oncogene transcript was detected.

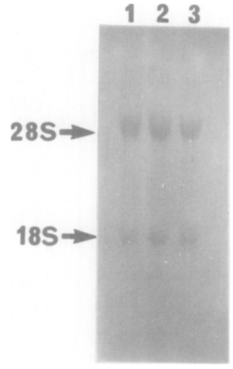

Fig. 1 Total RNA from PLC/PRF/5 cells (lane 1), Daudi cells (lane 2) and normal human leukocytes (lane 3). 8 μg of each RNA sample was glyoxalated and run on a 1.5% agarose gel and stained with 0.2% methylene blue.

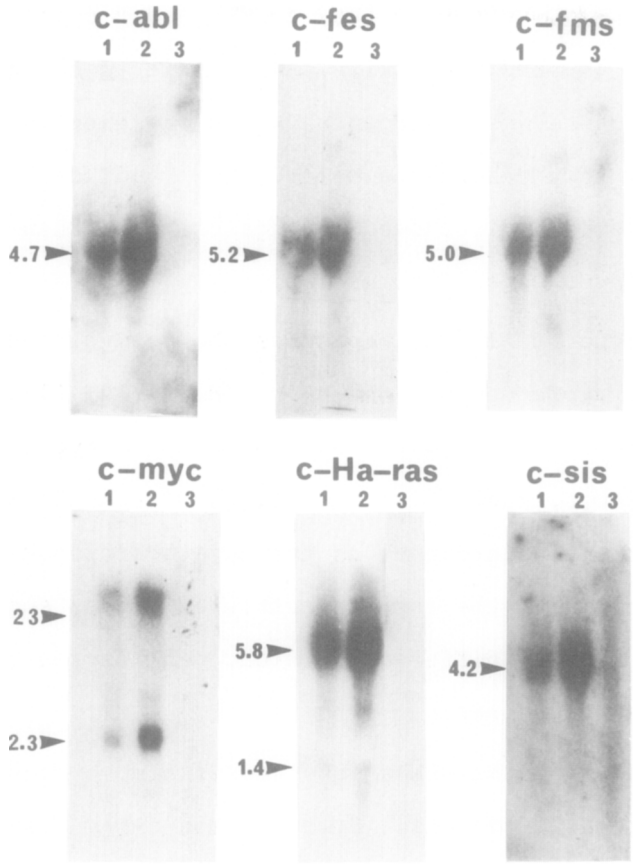

Fig. 2 Northern blot analyses of total RNA from PLC/PRF/5 cells compared to Daudi cells (a positive control) and leukocytes (a negative control) using nick-translated oncogene probes. 40 μg of each RNA sample was denatured with glyoxal, electrophoresed, transferred to nitrocellulose paper and hybridized with the oncogene probes. λ DNA digested with EcoRI and HindIII was used as a marker.
Lane 1; PLC/PRF/5, Lane 2; Daudi and Lane 3; leukocyte RNA.

In addition to a 2.3 kilobase (kb) transcript of c-myc, 2 larger bands (>23 kb) were found in PLC/PRF/5 and Daudi cells but not in leukocytes. There were 2 different sizes (1.4 and 5.8 kb) of transcripts of c-Ha-ras in PLC/PRF/5 and Daudi cells. The size of the mRNA of c-abl, c-fes, c-fms and c-sis was 4.7, 5.2, 5.0 and 4.2 kb, respectively.

DISCUSSION

An oncogene survey was done in human hepatoma cells PLC/PRF/5 using 7 different oncogene probes. Our data show

that c-abl, c-fes, c-fms, c-myc, c-Ha-ras and c-sis were expressed in the hepatoma cells. The c-Ki-ras oncogene was not expressed. There was no oncogene which was expressed specifically in the hepatoma cells. The Daudi cell line, used as a positive control, showed stronger signals than PLC/PRF/5 cells in every oncogene expression.

It is reported in rat hepatomas that c-myc, c-Ha-ras and c-Ki-ras are expressed but not c-abl (8, 9, 10, 11). Su et al. (12) has reported the reduced expression of c-myc in human hepatoma tissues, suggesting that c-myc expression might not be required for the maintenance of the hepatocarcinogenesis. Our data, together with the reports mentioned above, suggest that there might be some differences in oncogene expression between rat and human hepatomas.

The length of the mRNAs observed is almost consistent with previously reported values. However, the c-fms transcript of 5.0 kb is different in size from the 3.7 kb transcript reported by Slamon et al. (4). The nature of the difference is unclear. In addition to the 2.3 kb transcript of c-myc, we found the larger bands (>23 kb) in PLC/PRF/5 and Daudi cells but not in normal leukocytes. These bands might be detected due to the existence of an RNA species with homology to c-myc or a precursor of the c-myc mRNA.

There is a strong association between human hepatoma and chronic infection with hepatitis B virus, HBV, (26). HBV DNA is integrated in the genome of the human hepatoma cell line PLC/PRF/5 (27). Since HBV does not carry its own oncogenes (28), it might activate the expression of a neighboring cellular oncogene or might disrupt a cellular regulator gene (29). Our data show that there was no overexpressed oncogenes tested in PLC/PRF/5 cells. Thus far, there is no evidence for

integration of HBV DNA adjacent to any of the known cellular oncogenes (29).

Fox and Watanabe (30) have recently reported an active cellular oncogene in liver tumors arising spontaneously in 24 month old B6C3F1 mice. Such a new transforming gene might contribute to hepatocarcinogenesis.

In conclusion, among the known cellular oncogenes, c-abl, c-fes, c-fms, c-myc, c-Ha-ras and c-sis were expressed in human hepatoma cells PLC/PRF/5, but none of them were hepatoma-specific, compared to the expression of the oncogenes in Daudi lymphoma cells. Further studies are needed to delineate the role of oncogenes in hepatocarcinogenesis.

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REFERENCES

1. Cooper, G.M. (1982) Science 218, 801-806.
2. Eva, A., Robbins, K.C., Andersen, P.R., Srinivasan, A., Tronick, S.R., Reddy, E.P., Ellmore, N.W., Galen, A.T., Lautenberger, J.A., Papas, T.S., Westin, E.H., Wong-Staal, F., Gallo, R.C. and Aaronson, S.A. (1982) Nature 295, 116-119.
3. Westin, E.H., Wong-Staal, F., Gelmann, E.P., Dalla-Favera, R., Papas, T.S., Lautenberger, J.A., Eva, A., Reddy, E.P., Tronick, S.R., Aaronson, S.A. and Gallo, R.C. (1982) Proc. Natl. Acad. Sci. U.S.A. 79, 2490-2494.
4. Slamon, D.J., deKernion, J.B., Verma, I.M. and Cline, M.J. (1984) Science 224, 256-262.
5. Tatosyan, A.G., Galetzki, S.A., Kisseljova, N.P., Asanova, A.A., Zborovskaya, I.B., Spitkovsky, D.D., Revasova, E.S., Martin, P. and Kisseljov, F.L. (1985) Int. J. Cancer 35, 731-736.
6. Rijnders, A.W.M., van der Korput, J.A.G.M., van Steenbrugge, G.J., Romijn, J.C. and Trapman, J. (1985) Biochem. Biophys. Res. Commun. 132, 548-554.
7. Fausto, N. and Shank, P. (1983) Hepatology 3, 1016-1023.
8. Makino, R., Hayashi, K., Sato, S. and Sugimura, T. (1984) Biochem. Biophys. Res. Commun. 119, 1096-1102.
9. Hayashi, K., Makino, R. and Sugimura, T. (1984) Gann 75, 475-478.

10. Yaswen, P., Goyette, M., Shank, P.R. and Fausto, N. (1985) Mol. Cell. Biol. 5, 780-786.
11. Cote, G.J., Lastra, B.A., Cook, J.R., Huang, D.P. and Chiu, J.F. (1985) Cancer Lett. 26, 121-127.
12. Su, T.S., Lin, L.H., Lui, W.Y., Chang, C., Chou, C.K., Ting, L.P., Hu, C.P., Han, S.H. and P'eng, F.K. (1985) Biochem. Biophys. Res. Commun. 132, 264-268.
13. Alexander, J.J., Bey, E.M., Geddes, E.W. and Lecatsas, G. (1976) S. Afr. Med. J. 50, 2124-2128.
14. Glišin, V., Crkvenjakov, R. and Byus, C. (1974) Biochemistry 13, 2633-2637.
15. Chirgwin, J.M., Przybyla, A.E., MacDonald, R.J. and Rutter, W.J. (1979) Biochemistry 18, 5294-5299.
16. Thomas, P.S. (1983) Methods Enzymol. 100, 255-266.
17. Berent, S.L., Mahmoudi, M., Torczynski, R.M., Bragg, P.W. and Bollon, A.P. (1985) BioTechniques 3, 208-220.
18. Heisterkamp, N., Groffen, J. and Stephenson, J.R. (1983) J. Mol. Appl. Genet. 2, 57-68.
19. Groffen, J., Heisterkamp, N., Grosveld, F., Van de Ven, W.J.M. and Stephenson, J.R. (1982) Science 216, 1136-1138.
20. Heisterkamp, N., Groffen, J. and Stephenson, J.R. (1983) Virology 126, 248-258.
21. Groffen, J., Heisterkamp, N., Stephenson, J.R., van Kessel, A.G., de Klein, A., Grosveld, G. and Bootsma, D. (1983) J. Exp. Med. 158, 9-15.
22. Santos, E., Tronick, S.R., Aaronson, S.A., Pulciani, S. and Barbacid, M. (1982) Nature 298, 343-347.
23. Shimizu, K., Goldfarb, M., Suard, Y., Perucho, M., Li, Y., Kamata, T., Feramisco, J., Stavnezer, E., Fogh, J. and Wigler, M.H. (1983) Proc. Natl. Acad. Sci. U.S.A. 80, 2112-2116.
24. Alitalo, K., Bishop, J.M., Smith, D.H., Chen, E.Y., Colby, W.W. and Levinson, A.D. (1983) Proc. Natl. Acad. Sci. U.S.A. 80, 100-104.
25. Hunter, T. (1984) Sci. Am. 251, 70-79.
26. Beasley, R.P., Hwang, L.Y., Lin, C.C. and Chien, C.S. (1981) Lancet 2, 1129-1133.
27. Marion, P.L., Salazar, F.H., Alexander, J.J. and Robinson, W.S. (1980) J. Virol. 33, 795-806.
28. Varmus, H.E. (1984) Viral hepatitis and liver disease, pp 411-414, Grune & Stratton, Orlando.
29. Tiollais, P., Pourcel, C. and Dejean, A. (1985) Nature 317, 489-495.
30. Fox, T.R. and Watanabe, P.G. (1985) Science 228, 596-597.