EXPRESSION OF CELLULAR ONCOGENES IN HUMAN TUMORS TRANSPLANTABLE INTO NUDE MICE

D. D. Spitkovskii and E. S. Revazova

UDC 616-006-092:616-006-008.949.5:615.277.4]-033-092.9

KEY WORDS: cellular oncogenes; human tumors; expression.

It is an undoubted fact that cellular oncogenes are implicated in the process of carcinogenesis, and that not one, but several oncogenes participate in the transformation of normal cells [2]. However, the direct role of these genes in the multistage conversions of normal cells leading to tumor formation is not yet clear. Most of the data on expression of cellular oncogenes have been obtained by the study of transplantable cell lines. Meanwhile it has now become clear that the molecular processes and, in particular, oncogene expression, accompanying transplantation in vivo and in vitro, may be not completely identical [3, 6, 15].

The aim of this investigation was to study oncogene expression in strains of human tumors transplantable into nude mice. This a topic of great interest because these strains are distinguished by stable characteristics.

EXPERIMENTAL METHOD

Experiments were carried out on 20 strains of human tumors transplantable into nude mice, obtained in the Laboratory of Experimental Models, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR: carcinoma of the colon 7, carcinoma of the lung 3, melanoma 3, Burkitt's lymphoma 2, carcinoma of the body of the uterus (CBU) 2, carcinoma of the liver (CL) 1, fibrosarcoma (FS) 1, carcinoma of the bladder (CB) [1, 4, 5]. Total RNA preparations were isolated from the tumors with the aid of guanidine thiocyanate [6], fractionated in 1% agarose under denaturing conditions, using formaldehyde, and transferred

TABLE 1. Oncogene Expression in Human Tumors Transplantable into Nude Mice

Tumor	Oncogene				
	c-myc	c-fos	c-ras	c-sis	c-myb
Carcinoma of the colon:					
CC-1	+	+	+	—	N.d.
CC-7	+	+	+	—	N.d.
CC-9	+	+	+	—	—
CC-10	+	—	+	—	—
CC-11o	—	+	+	—	—
CC-11m	+	+	+	—	—
CC-12	—	+	+	—	—
Carcinoma of the lung:					
CL-1	+	+	+	—	—
CL-3	+	+	N.d.	—	—
CL-4	+	+	+	—	—
Melanoma:					
Mel-2	+	+	+	—	—
Mel-3	—	N.d	+	—	N.d.
Mel-5	+	+	+	—	—
Burkitt's lymphoma:					
BL-1P	+	+	+	—	—
BL-2N	+	+	+	—	—
CH	+	—	+	—	—
FS	+	+	—	—	—
CB	+	+	+	—	—

Legend. +) Oncogene expression present; —) oncogene expression absent; N.d.) not determined.

Institute of Carcinogenesis, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Eksperimental'noi i Meditsiny, Vol. 105, No. 3, pp. 326-329, March, 1988. Original article submitted April 9, 1987.

0007-4888/88/0003-0385$12.50

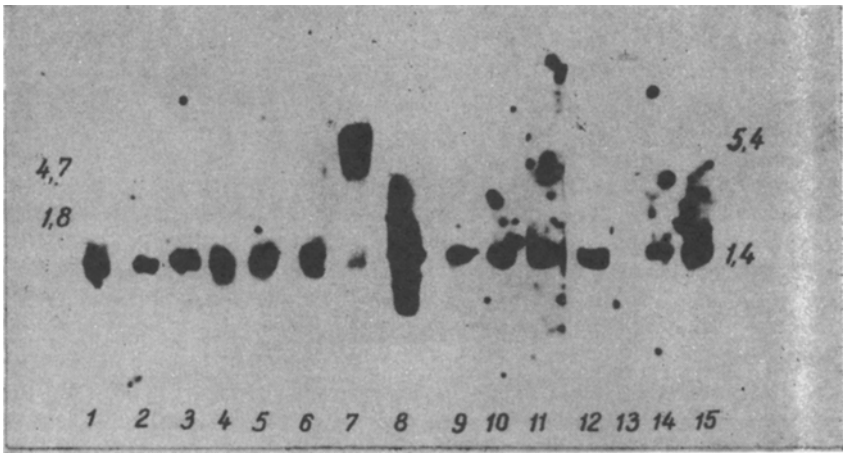

Fig. 1. Blot-hybridization of ^{32}P-c-ras with RNA from tumors transplanted into nude mice. 1) CC-1; 2) CC-7; 3) CC-9; 4) CC-11m; 5) CC-11o; 6) CC-12; 7) CH; 8) Mel-5; 9) CL-4; 10) BL-1P; 11) BL-2N; 12) CB; 13) normal spleen; 14) CBU 77; 15) CBU 77A.

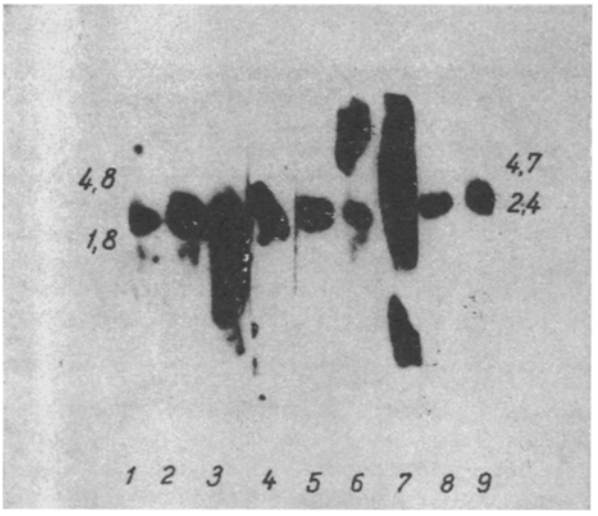

Fig. 2. Blot-hybridization of ^{32}P-c-fos with RNA from tumors transplanted into nude mice. 1) Mel-2; 2) FS; 3) CH; 4) CB; 5) CL-3; 6) CC-9; 7) CC-11m; 8) CC-11o.

to nitrocellulose by the standard method [15]. These filters were hybridized with ^{32}P-labeled probes for various oncogenes in myc-translation reactions: c-myc [10], v-fos [8], c-Ha-ras [13], v-mos [7], c-myb [12], v-sis [11], B-lym [9]. Ribosomal RNA was used as molecular weight marker. The conditions of hybridization, washing the filters, and autoradiography were similar to those described previously [3].

EXPERIMENTAL RESULTS

Data on expression of five of the seven cellular oncogenes studied are given in Table 1.

Strains of Carcinoma of the Colon. Expression of the c-ras oncogene was discovered in all tumors (Table 1, Fig. 1). The transcripts measured 1.4 kbp. The c-fos oncogene was found in six of the seven tumors tested. Strain CC-10, in which c-fos production could not be found, was an undifferentiated carcinoma with high production of mucus. However, high production of mucus also was a feature of strains CC-9, CC-11m, CC-11o, and CC-12. In addition, strain CC-12 was an undifferentiated adenocarcinoma. The c-fos transcripts discovered in the tumors were represented by bands in the 2.2 kbp zone (Fig. 2).

It will be clear from Fig. 2 that the level of expression of c-fos in CC-11m, obtained from a metastasis, was higher than in CC-11o, obtained from the original tumor in the same

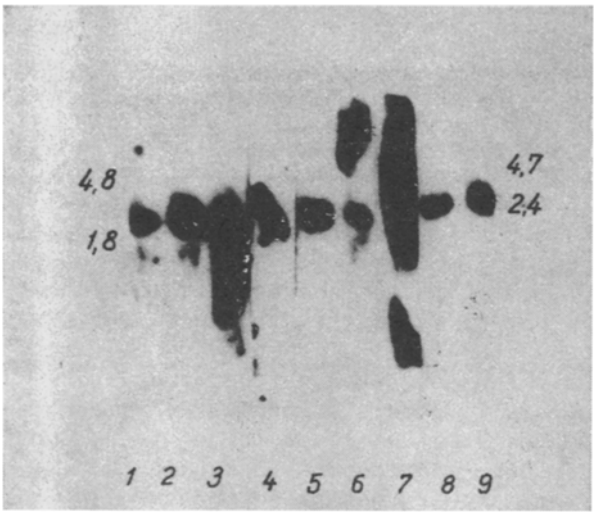

Fig. 3. Blot-hybridization of ^{32}P-c-myc with RNA from tumors transplanted into nude mice. 1) CC-1; 2) CC-7; 3) CC-9; 4) CL-1; 5) CL-4; 6) Mel-2; 7) BL-1P; 8) BL-2N; 9) CB.

patient. The c-myc oncogene was expressed in five of seven strains of carcinoma of the colon. It is an interesting fact that expression of c-myc was not found in CC-11o, whereas it was readily detected in CC-11m. This is the only oncogene for which expression differed greatly in strains of a carcinoma and its metastasis. Tumors CC-11o and CC-12, in which expression of c-myc could not be found by the method used, have a similar histological structure (adenocarcinomas with high mucus production). However, CC-9 and CC-11m have the same histological structure. The c-myc transcripts discovered measured 2.4 kbp in all cases (Fig. 3). Expression of the four other oncogenes studied in strains of carcinoma of the colon could not be found.

Strains of Melanomas. In all three specimens of melanoma expression of the c-ras oncogene was found, although it differed: in Mel-2 and Mel-3 transcripts measuring 5.4 and 1.4 kbp were detected, and the 5.4 kbp fragment was the major version. Superexpression of this oncogene was observed in Mel-5, and the transcripts in this case were observed in the form of a diffuse band (Fig. 1). The c-myc oncogene, represented by a standard 2.4 kbp transcript, was discovered in two of the three strains. In strain Mel-3, which, like Mel-2, had an alveolar-lobular RNA structure, no c-myc was discovered. In both tumors, c-fos transcripts measuring 2.2 kbp were found: Mel-2 (Fig. 2) and Mel-5. Among the other four oncogenes studied in strains of melanomas, expression of the c-myb oncogene was discovered in Mel-2. The transcripts found measured 4.5 and 3.5 kbp.

Strains of Lung Carcinoma. Expression of c-myc oncogenes, with transcripts measuring 2.4 kbp (Fig. 3), and of c-fos oncogenes, with transcripts measuring 2.2 kbp (Fig. 2), was discovered in all strains of lung carcinoma used in the investigation. All three strains had a different histological structure: CL-1 was a papillary cystadenocarcinoma, CL-3 a squamous-cell carcinoma, and CL-4 a glandular-solid carcinoma with high mucus production. Expression of c-ras was analyzed in lung carcinomas CL-1 and CL-4. In both cases transcripts measuring 1.4 kbp were found. Expression of four other oncogenes could not be found.

Strains of Burkitt's Lymphomas. Oncogenes c-myc, c-fos, and c-ras were expressed in both tumors. The c-ras (Fig. 1) and c-fos transcripts discovered were of standard size. Differences were observed in the expression of the c-myc oncogene: a single c-myc transcript measuring 2.4 kbp was found in BL-2N, whereas besides this transcript, an additional transcript measuring 4.7 kbp was present in BL-1P (Fig. 3). Data on hybridization of RNA from BL-1P with a specific probe for c-myc are evidence of superexpression of this oncogene. Expression of c-mos, B-lym, c-sis, and c-myb oncogenes was not observed in the Burkitt's lymphomas.

Other Tumor Strains. On analysis of expression of oncogenes in different tumor strains (CH, FS, CB, CBU) samples of expression of oncogenes similar to those obtained for other tumors transplanted into nude mice were observed. Expression of c-mos, B-lym, c-sis, and c-myb oncogenes could not be discovered. Expression of c-ras was observed in four of five

tumors, and it could not be discovered only in the fibrosarcoma. The c-ras transcripts measured 1.4 kbp, and an additional transcript measuring 5.4 kbp also was found in CH (Fig. 1). Among the strains investigated, expression of the c-fos oncogene was absent in CH only. The c-fos transcripts measured 2.2 kbp. In all five cases c-myc transcripts were found, and they measured 2.4 kbp.

No significant differences were thus found in oncogene expression among different types of tumors transplanted into nude mice, or among the same types of transplanted tumors, differing in histological structure. Analysis of transcription of cellular oncogenes was carried out using total RNA preparations, so that only increased expression of these genes (more than 15-20 copies per cell), which is not characteristic of healthy tissues [6], could be detected. No difference in principle was found between expression of oncogenes in primary tumors [6, 14] and in tumors transplanted into nude mice. In both kinds of tumors expression of three oncogenes was mainly found: c-myc, c-fos, and c-ras. The differences are that expression of the above oncogenes in tumors transplanted into nude mice is found in a higher percentage of cases: c-myc in 17 of 20, c-fos in 16 of 18, and in c-ras in 18 of 19 cases. Levels of expression of the above-mentioned oncogenes, detected by the intensity of hybridization with labeled probes, were higher than those observed in primary tumors. In some cases, besides c-myc and c-ras transcripts, with standard size (2.4 kbp for c-myc and 1.4 kbp for c-ras), additional transcripts differing from them in molecular weight were discovered. In the melanoma strains and in one CH, c-ras transcripts measuring 5.4 kbp were observed, whereas in a strain of Burkitt's lymphoma, an additional c-myc transcript measuring 4.7 kbp was found. Probably, of the heterogeneous cell population in the primary tumors in nude mice, those cells in which expression of c-myc, c-fos, and c-ras oncogenes was enhanced possessed a selective advantage of growth. It is also quite probable that the increase in expression of these cellular oncogenes takes place during passage of transformed cells through nude mice.

LITERATURE CITED

1. N. G. Blokhina, Yu. N. Solov'ev, A. V. Ozherel'ev and E. S. Revazova, Byull. Éksp. Biol. Med., No. 3, 330 (1985).
2. F. L. Kiselev and D. D. Spitkovskii, Zh. Vses. Khim. Obshch. D. I. Mendeleeva, No. 6, 272 (1986).
3. E. S. Revazova and Yu. N. Solov'ev Byull. Éksp. Biol. Med., No. 2, 189 (1985).
4. E. S. Revazova, Yu. N. Solov'ev, and T. V. Yudicheva, Byull. Éksp. Biol. Med., No. 7, 71 (1985).
5. D. D. Spitkovskii, I. B. Zborovskaya, and F. L. Kiselev, Mol. Biol., No. 5, 1409 (1986).
6. A. G. Tamosyan, S. A. Galetskii, I. B. Zborovskaya, et al., Dokl. Akad. Nauk SSSR, 272, No. 3, 726 (1983).
7. I. M. Chumakow, E. R. Zabarovsky, V. S. Prasolov, et al., Gene, 17, 19 (1982).
8. T. Curran, G. Peters, C. Van Veberen, et al., J. Virol., 44, 674 (1982).
9. A. Diamond, J. M. Devine, and G. M. Cooper, Science, 225, 516 (1982).
10. C. Gazin, S. Dinachin, A. Hampe, et al., EMBO J., 3, 383 (1984).
11. E. R. Gellman, F. Wong Staal, R. A. Kramer, et al., Proc. Natl. Acad. Sci. USA, 78, 3373 (1981)
12. D. Leprince, S. Saule, C. Tausue, et al., EMBO J., 12, 1075 (1983).
13. C. Shin and R. A. Weinberg, Cell, 29, 161 (1982).
14. D. J. Slamon, J. B. Kernion, I. M. Verma, et al., Science, 224, 256 (1984).
15. A. G. Tatosyan, S. A. Galetskii (S. A. Galetzki), N. P. Kiseleva (N. P. Kisseljova), et al., Int. J. Cancer, 35, 731 (1985).