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Somatic Cell Hybrids Between Mouse Peritoneal Macrophages and Simian-Virus-40-Transformed Human Cells: II. Presence of Human Chromosome 7 Carrying Simian Virus 40 Genome in Cells of Tumors Induced by Hybrid Cells*

[tumor (T) antigen/transformed phenotype/"nude" mice]

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ABSTRACT **Cells derived from tumors induced in "nude" mice after injection of cells that were hybrids between mouse peritoneal macrophages and simian virus 40 (SV40)-transformed human cells were found to retain the human chromosome 7 carrying the SV40 genome, and to express SV40-induced tumor (T) antigen. These results indicate that the presence of human chromosome 7 carrying the SV40 genome is responsible for the expression of the tumorigenic phenotype in the hybrid cells.**

Through somatic cell hybridization techniques, we have previously assigned the gene for simian virus 40 (SV40) tumor (T) antigen and the SV40 genome to human chromosome 7 in SV40-transformed cells of various origins (1–3). Furthermore, we have shown that hybridization of mouse peritoneal macrophages (which are nondividing cells) with SV40-transformed human cells resulted in the formation of *transformed* somatic cell hybrids in which the human chromosome 7 carrying the SV40 genome was always present (4). Those results indicate that the human chromosome 7 that carries the SV40 genome contains gene(s) coding for transforming gene product(s) (4). In addition, we have recently shown (Koprowski, Aden, and Croce, manuscript in preparation) that somatic cell hybrids between mouse peritoneal macrophages and SV40-transformed human cells are able to induce tumors in "nude" mice, in which heterotransplantation of human tumors can be successfully achieved (5–7).

We undertook these studies to determine if the human chromosome 7 carrying the SV40 genome and coding for SV40 T antigen is present in cells derived from tumors induced in "nude" mice by the injection of hybrid cells that contain many different human chromosomes, including chromosome 7. If the tumorigenic phenotype is determined by the presence of a specific human chromosome, this chromosome should always be present in tumor cells recovered from "nude" mice. This communication reports the results of these studies.

MATERIALS AND METHODS

Hybrid Cells. The production of somatic cell hybrids between C57BL/6 mouse peritoneal macrophages and the human cells LN-SV (SV40-transformed Lesch–Nyhan fibroblasts) is described elsewhere (4). A heterogeneous population of hybrid cells containing 14 different human chromosomes (Table 1) and a quasi-tetraploid number of mouse chromosomes was injected under the abdominal skin of 20 Balb/c *nu/nu* ("nude") mice at a concentration of 2 to 4 $\times$ 10^7 cells per mouse.

Tumor Cells. The inoculated mice were observed daily for tumor growth, and when a palpable mass was detected, the tumor was removed aseptically and fragments were immediately frozen, fixed in acetone and stained for SV40 T antigen (8). The remaining portion was cut in small fragments and seeded either in 60 mm petri dishes or 75 cm^2 Falcon flasks in Eagle's minimal essential medium (MEM) containing 10% fetal calf serum. The cells derived from the tumors obtained from five "nude" mice were transferred five to eight times in culture and stained for the presence of SV40 T antigen. LN-SV SV40-transformed human cells were found to be tumorigenic in "nude" mice. On the contrary, no tumors were induced by the inoculation of C57BL/6 mouse peritoneal macrophages.

Karyotypical Analysis. A modification of the Giemsa banding technique of Seabright (9) was used in this study (10, 11). Fifty-one metaphases of the inoculated hybrid cells were analyzed (Table 1). A minimum of 26 metaphases of each "tumor" culture derived from a different "nude" mouse was also analyzed following Giemsa banding staining. This permitted identification of each human and mouse chromosome present in the cells (10, 11).

Reinoculation of Tumor Cells. Cells derived from the tumor of "nude" mouse 8 were transferred for eight passages in culture and reinjected (2 $\times$ 10^7 cells per mouse) in 10 "nude" mice. All these animals developed tumors. Three of these tumors were transferred in tissue culture and the tumor cells were subjected to karyological analysis. Cells derived from a tumor produced in "nude" mouse 1 (Table 2), 17.8% of which contained human chromosome 5, 77.8% of which contained chromosome 6, and 100% of which contained chromosome 7, were inoculated into five "nude" mice at a concentration of 4 $\times$ 10^7 cells per mouse. A tumor obtained from one of these mice was processed for culture as above except that a portion of the tumor was cut into small fragments (2–3 mm in diameter) and the cell fragments were implanted subcutaneously into five other "nude" mice. In addition the cultured cells derived from this tumor were injected in nine "nude" mice. All these mice developed very large masses (9–17 g in weight). Three tumors were transferred in culture and the tumor cells were subjected to karyological analysis.

Abbreviations: SV40, simian virus 40; T antigen, tumor antigen.

* Paper I of this series is ref. 4.

TABLE 1. *Presence of different human chromosomes in hybrid cells between C57BL/6 peritoneal macrophages and LN-SV human cells*

Human chromosome	Frequency in hybrids, %*	Human chromosome	Frequency in hybrids, %*
4	5.9	13	5.9
5	60.8	14	7.8
6	70.6	15	17.6
7	100†	16	2.0
9	5.9	17	60.8
11	54.9	18	3.9
12	23.5	20	5.9

* A total of 51 metaphases was analyzed.

† On the average the hybrid cells contained 2.3 chromosomes 7 per cell.

RESULTS

Karyological Analysis of the Hybrid Cells Used as Inoculum. As shown in Table 1, 14 out of the 24 different human chromosomes were present in the hybrid cells. The human chromosome 7 was the only human chromosome present in 100% of the metaphases. This frequency was followed by that of human chromosome 6 in 70.6%, human chromosomes 5 and 17 in 60.8%, and human chromosome 11 in 54.9% of metaphases. The remaining human chromosomes were encountered in less than 24% of the cells analyzed.

SV40 T Antigen in the Hybrid Cells Used as Inoculum. The hybrid cells injected in the "nude" mice were stained for the presence of T antigen and all were found to show the presence of this antigen. A minimum of 10^3 cells was analyzed. Since the gene for SV40 T antigen has been assigned to human chromosome 7 in SV40-transformed human cells (1, 2), these data confirm the results of the karyological analysis indicating the presence of human chromosome 7 in all cells of the hybrid population.

Characterization of Cells Obtained from Tumors of "Nude" Mice Injected with Hybrid Cells. The tumors that developed in five mice after the inoculation of hybrid cells were removed at the times indicated in Table 2. Examination by SV40 T antigen staining of frozen sections revealed SV40 T antigen in cells of all the tumors examined. Cells derived from each tumor grew in culture and, after five to eight transfers, they were seeded on coverslips and stained for SV40 T antigen. A minimum of 10^3 cells of each culture was analyzed for the presence of SV40 T antigen. One-hundred percent of the cells of the cultures derived from each tumor showed the presence of SV40 T antigen.

Karyological Analysis of Tumor Cells Grown in Culture. As can be seen in Table 2, chromosome 7 was found in 100% of the metaphases of the cells of the cultures obtained from tumors of the "nude" mice. In addition, the cells derived from "nude" mice 7 and 8 tumors contained human chromosome 6 in less than 39% of the metaphases (Table 2). "Nude" mouse 4 tumor cells contained the human chromosome 6 in 64.1% of the cells, the human chromosome 17 in 38.4% of the cells and the human chromosome 11 in only one cell out of 39 cells examined (Fig. 1). "Nude" mouse 2 tumor cells contained human chromosome 6 in 21 out of 30 metaphases. "Nude" 1 tumor cells contained human chromosome 6 in 77.8% of metaphases and human chromosome 5 in 17.8% of the metaphases. On the average, cells derived from the tumors of the five "nude" mice contained three human chromosomes 7 per cell.

Inoculation of "Nude" Mice with Cells Derived from the "Nude" 8, 1, and 9 Tumors. Tumor cells derived from "nude" 8 were transferred for eight passages in culture and inoculated into 10 "nude" mice, all of which developed tumors. Cells of three of the 10 tumors were analyzed for the presence of SV40 T antigen and human chromosomes. The results of the analysis are reported in Table 3. The tumor cells derived from these tumors contained only one human chromosome, the 7. On the average, 2.6 chromosomes 7 per cell were retained by the hybrids. The entire complement of mouse chromosomes was present in all the cells examined. Tumor cells derived from "nude" 1 tumor were transferred for eight passages in culture and inoculated into five "nude" mice. Sixty-seven days after inoculation, a tumor mass (3.1 g) was removed from one of these "nude" mice ("nude" 9). Presence of SV40 T antigen was detected in the frozen sections of the tumors following indirect immunofluorescence staining for SV40 T antigen and in the cultured cells derived from this tumor. Karyological analysis of 41 metaphases (Table 3) of the cultured cells revealed the presence of chromosome 7 in all metaphases. On the average 3.0 chromosomes 7 per cell were retained by the hybrids. Twenty-nine out of 41 metaphases showed also the presence of one human chromosome 6. All the cells retained the entire complement of mouse parental chromosomes. The tumor cells derived from the "nude" 9 tumor were inoculated in nine "nude" mice. Very large tumor masses developed

TABLE 2. *Karyological analysis of cells recovered from tumors induced in "nude" mice by inoculation of hybrid cells*

Source of tumors	Removal, days after inoculation	Percent of cells showing SV40 T antigen	Human chromosomes*				
			5	6	7	11	17
"Nude" 1	22	100	8/45	35/45	45/45	0/45	0/45
"Nude" 2	28	100	0/30	21/30	30/30	0/30	0/30
"Nude" 4	42	100	0/39	25/39	39/39	1/39	15/39
"Nude" 7	70	100	0/26	10/26	26/26	0/26	1/26
"Nude" 8	76	100	0/33	3/33	33/33	0/33	0/33

* Numbers of metaphases showing presence of this chromosome over total analyzed.

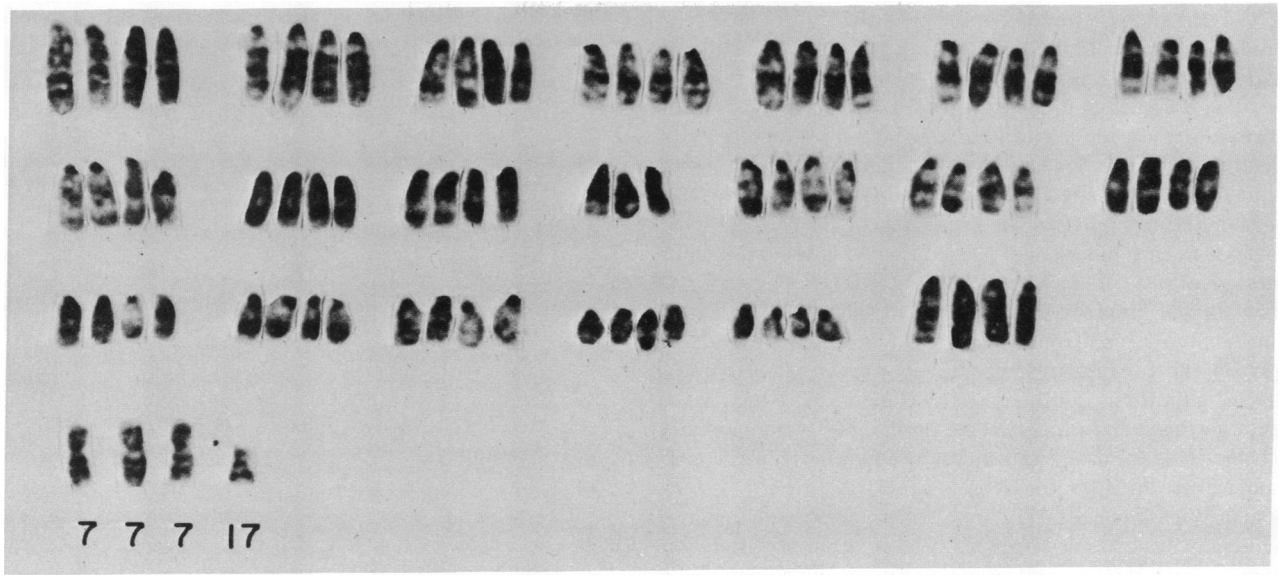

FIG. 1. Karyotype of a hybrid cell derived from "nude" 4 tumor. The hybrid cell contains a quasi-tetraploid number of mouse chromosomes and four human chromosomes (three chromosomes 7 and one chromosome 17).

(9–17 g) in all of these mice. Three of the nine tumors were transferred into culture and subjected to karyological analysis (Table 3).

Implantation of Fragments of "Nude" 9 Tumor in "Nude" Mice. Five mice were implanted with fragments of the tumor derived from "nude" 9. All animals showed the presence of rapidly growing tumor masses. Tumors were removed from two mice on the 22nd day after implantation, from one mouse on the 26th day, and from the two remaining mice on the 37th day after implantation. SV40 T antigen was found in the cells of all tumors examined. Cultures of cells derived from these tumors, which have been transferred in culture for at least eight passages, again showed the presence of T antigen in 100% of the cells. Karyological analysis of the cells grown out of these five tumors in tissue culture indicated (Table 4) the presence of human chromosome 7 in all metaphases examined, human chromosome 6 in less than 70% of the metaphases, and in no cell could we detect human chromosome 5. Again, the entire complement of mouse chromosomes was present in all cells examined.

TABLE 3. *Karyological analysis of cells of tumors derived from the inoculation of "nude" 8 and "nude" 9 tumor cells in "nude" mice*

Tumors	Removal, days after inoculation	Percent of cells showing SV40 T antigen	Human chromosomes* 5	6	7
"Nude" 8-1	40	100	0/32	0/32	32/32
"Nude" 8-2	40	100	0/37	0/37	37/37
"Nude" 8-3	56	100	0/37	0/37	37/37
"Nude" 9†	67	100	0/41	29/41	41/41
"Nude" 9-1	24	100	0/31	23/31	31/31
"Nude" 9-2	38	100	0/26	20/26	26/26
"Nude" 9-3	39	100	0/30	21/30	30/30

* Number of metaphases showing presence of this human chromosome over total analyzed. No other human chromosomes were present in the hybrid cells.

† Hybrid cells derived from "nude" 1 were inoculated into "nude" 9.

DISCUSSION

We have shown that somatic cell hybrids between normal diploid human cells and SV40-transformed cells behave as transformed cells *in vitro* (12). This indicates that, in hybrids between normal and SV40-transformed human cells, the transformed phenotype is dominant. We have also observed that hybrid cells between normal mouse cells (peritoneal macrophages) and SV40-transformed human cells, which retained without exception the human chromosome 7, behaved as transformed cells *in vitro* (4). This indicates that the human chromosome 7 that carries the SV40 genome contains genes coding for "transforming factors" (4).

The fact that the hybrid cells between mouse peritoneal macrophages and SV40-transformed human cells were found to produce tumors in "nude" mice made it possible to characterize the tumorigenic hybrid cells. The results of these investigations indicate that the human chromosome 7 carrying

TABLE 4. *Karyological analysis of cells derived from tumors induced in five "nude" mice by the implantation of fragments of "nude" 9 tumor*

Tumors	Removal, days after inoculation	Percent of cells showing SV40 T antigen	Human chromosomes* 5	6	7
"Nude" 9-1 F†	22	100	0/31	20/31	31/31
"Nude" 9-2 F	22	100	0/33	23/33	33/33
"Nude" 9-3 F	26	100	0/33	21/33	33/33
"Nude" 9-4 F	37	100	0/26	17/26	26/26
"Nude" 9-5 F	37	100	0/29	19/29	29/29

* Number of metaphases showing presence of this chromosome over total analyzed.

† Implanted with fragments of "nude" 9 tumor.

the SV40 genome is retained by 100% of the hybrid cells recovered in culture from the "nude" tumors, whereas the other human chromosomes present in the inoculated cells are eliminated. The presence of multiple copies of human chromosome 7 in the cells recovered from the tumors may have represented a selective advantage for their growth. Thus, the presence of the human chromosome 7 carrying the SV40 genome seems to be essential not only for the expression of the transformed phenotype *in vitro* but also for the expression of the oncogenic phenotype *in vivo*.

Since in our hybrid cells the full complement of mouse chromosomes derived from the normal mouse parent was retained by the tumorigenic hybrid cells, our results contrast with the hypothesis formulated by Harris and Klein, who postulated that a hybrid between a normal and a tumorigenic cell behaves as a normal cell unless the chromosomes of the normal cell are lost from the hybrid (13–15).

Somatic cell hybridization with mouse peritoneal macrophages may be extended to cells derived from a variety of human cancers. If this results in the production of transformed somatic cell hybrids that retain specific human chromosomes, it may soon be possible to identify the human chromosomes responsible for the expression of the tumor phenotype in human malignancies.

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