CONCORDANT SEGREGATION OF THE EXPRESSION OF SV40 T ANTIGEN AND HUMAN CHROMOSOME 7 IN MOUSE-HUMAN HYBRID SUBCLONES

By CARLO M. CROCE and HILARY KOPROWSKI
(From The Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania 19104)
(Received for publication 30 January 1974)

When Simian virus 40 (SV40)-transformed human cells were hybridized with mouse cells, 82% of the hybrid clones expressed SV40 T antigen and contained the human chromosome 7 (1). 18% of the hybrid clones were SV40 T antigen-negative (1). Those data indicated positive correlation between the expression of SV40 T antigen and human chromosome 7 (1). Assignment of the gene(s) for the expression of SV40 tumor-specific transplantation antigen(s) (TSTA) to human chromosome 7 has also been established (A. J. Girardi and C. M. Croce, in preparation). Preferential retention of the human chromosome 7 in the hybrids formed between SV40-transformed human cells deficient in hypoxanthine-guanine-phosphoribosyltransferase (HGPRT$^-$) and mouse cells deficient in thymidine kinase (TK$^-$) was observed (2). Due to the highly preferential retention of human chromosome 7 in this type of hybrid, the possibility arises that the correlation between human chromosome 7 and SV40 T antigen might be specious. It is possible, in fact, that in the mouse-human hybrids the SV40 genome is transferred to a mouse chromosome, and, since the majority of the mouse-human hybrid clones retain the human chromosome 7, that the apparent correlation between SV40 T antigen and human chromosome 7 is only coincidental.

In order to exclude this possibility and to confirm the assignment of the SV40 genome to human chromosome 7, we subcloned SV40 T-antigen-positive clones and analyzed the subclones for expression of SV40 T antigen and for the presence or absence of human chromosome 7 as identified by Giemsa-banding staining. Clones of T antigen-positive human-mouse hybrids again segregate into T antigen-positive and T antigen-negative subclones, while retaining all mouse chromosomes. Thus, if a T antigen-positive clone segregates into T antigen-negative subclones which have lost the human chromosome 7, while retaining all the mouse chromosomes, this excludes the possibility of SV40 T antigen expression due to transfer of the SV40 genome to a mouse chromosome.

Materials and Methods

Hybrid Cells.—The three cell clones used in this study were derived from hybridization of LN-SV SV40-transformed human cells deficient in HGPRT with CI-1D mouse cells deficient in TK (1, 2).

* This work was supported in part by U.S. Public Health Service Research Grants CA 10815 from the National Cancer Institute, RR 05540 from the Division of Research Resources, and funds from the Commonwealth of Pennsylvania.
Mass cultures of the three different clones were subcloned by plating single hybrid cells in microtest plates in either hypoxanthine-aminopterin-thymidine (HAT) medium or in Eagle's minimal essential medium (MEM) containing 30 μg/ml of bromodeoxyuridine (BrdU).

At the time of subcloning, one of the clones (52–58 Cl 19) grown in HAT medium contained human chromosomes C-7 (25% of the metaphase plates), C-11 (45% of the metaphase plates), E-17 (100% of the metaphase plates), and about 25% of cells of this clone showed the presence of SV40 T antigen.

In contrast, more than 90% of the cells of the second and third clones (52–62 (1) Cl 5 BrdU, and 52–62 (1) Cl 16 BrdU) which were backselected by growing in medium containing BrdU, showed the presence of SV40 T antigen. More than 90% of the cells of the clone 52–62 (1) Cl 5 BrdU contained only one human chromosome, the C-7. More than 90% of the cells of the clone 52–62 (1) Cl 16 BrdU contained the human chromosome 7 and approximately 50% contained the human chromosome 12.

15 subclones of the clone 52–58 Cl 19, nine subclones of the clone 52–62 (1) Cl 5 BrdU, and five subclones of the clone 52–62 (1) 16 BrdU were available for analysis. In addition, cells of the 52–62 (1) Cl 5 BrdU clone were hybridized with IR (HGPRT−) mouse cells (3). 20 triple hybrid clones selected in HAT medium were analyzed for the presence of SV40 T antigen and of the human chromosome 7.

**Karyological Analysis.**—At least 25 metaphases of each subclone population were analyzed by Giemsa-banding staining as previously described (4).

**SV40 T Antigen.**—Presence of the antigen was determined by indirect immunofluorescent staining of the subclone population cells by the technique described before (5).

**RESULTS AND DISCUSSION**

As it is shown in Table I, only three of the 15 subclones of the clone 52–58 Cl 19 were T antigen-positive. The remaining 12 subclones were T antigen-negative. The karyological analysis of the three T-antigen-positive subclones following Giemsa-banding staining confirmed the presence of the human chromosome 7 and 17 and in one of them the additional presence of chromosome 11. All the T antigen-negative subclones contained the human chromosome 17 and five of them contained the human chromosome 11.

Seven out of nine subclones of the clone 52–62 (1) Cl 5 BrdU showed the presence of SV40 T antigen in at least 90% of the cells (Table I). All these seven subclones contained human chromosome 7 (Fig. 1). Contrary to this, the two T antigen-negative subclones did not contain human chromosome 7 or any other human chromosome.

Four out of five subclones of the clone 52–62 (1) Cl 16 BrdU were T antigen-positive.

**TABLE I**

| Hybrid clones       | No. of subclones or triple hybrids | No. of T antigen-positive subclones or triple hybrids |
|---------------------|------------------------------------|------------------------------------------------------|
| 52-58 Cl 19         | 15                                 | 3                                                    |
| 52-62 (1) Cl 5 BrdU | 9                                  | 7                                                    |
| 52-62 (1) Cl 16 BrdU| 5                                  | 4                                                    |
| 52-62 (1) Cl 5 BrdU × IR | 20                        | 12                                                   |
Fig. 1. Karyotype of the T antigen-positive subclone 27, derived from the clone 52–62 (1)
Cl 5 BrdU. Only the human chromosome 7 is present. All the other chromosomes are of
mouse origin.

positive (Table I) and contained human chromosome 7. Two of them contained,
in addition, the human chromosome 12. The T antigen-negative subclone of
the clone 52–62 (1) Cl 16 BrdU contained only the human chromosome 12.

12 out of 20 triple hybrid clones between 52–62 (1) Cl 5 BrdU and IR cells
were T antigen-positive and contained human chromosome 7. The remaining
eight clones were SV40 T antigen-negative and did not contain any human
cromosomes.

As is shown in the ideogram (Fig. 2) there is positive correlation between the
expression of the SV40 T antigen and the presence of the human chromosome
7 in all the subclones or triple hybrid clones examined. These results confirm
the assignment of the SV40 T antigen gene to human chromosome 7 and rule
All Hybrid Subclones and Triple Hybrids

Human Chromosome 7

| SV40 T Antigen | +  | 26 | 0 |
|----------------|----|----|---|
|                | -  | 0  | 23|

Fig. 2. Positive correlation between the presence of SV40 T antigen and human chromosome 7 in 29 subclones of three T antigen-positive mouse-human hybrid clones and in 20 triple hybrid clones derived from the fusion of 52-62 (1) C15 BrdU with mouse IR cells.

out the possibility that the expression of the SV40 T antigen in SV40 T antigen-positive hybrid clones was caused by a transfer of the viral genome to a mouse chromosome, since all the hybrid subclones which have lost the human chromosome 7, but retained all the mouse Cl 1D chromosomes, were SV40 T antigen-negative.

SUMMARY

Subcloning of Simian virus 40 (SV40) T antigen-positive mouse-human hybrids, derived from the fusion of mouse cells deficient in thymidine kinase with SV40-transformed Lesch Nyhan fibroblasts, resulted in their segregation into T antigen-positive and negative subclones. Positive correlation between the presence of human chromosome 7 and the expression of SV40 T antigen was established in the subclones examined. These results negate the possibility of a transfer of the SV40 genome to a mouse chromosome.

REFERENCES

1. Croce, C. M., A. J. Girardi, and H. Koprowski. 1973. Assignment of the T antigen gene of Simian Virus 40 to human chromosome C-7. *Proc. Natl. Acad. Sci. U.S.A.* 70:3617.
2. Croce, C. M., B. B. Knowles, and H. Koprowski. 1973. Preferential retention of the human chromosome C-7 in human-(thymidine kinase deficient) mouse hybrids. *Exp. Cell Res.* 82:457.
3. Nabholz, M., V. Miggiano, and W. Bodmer. 1969. Genetic analysis using human-mouse somatic hybrids. *Nature (Lond.)* 223:358.
4. Croce, C. M., G. Litwack, and H. Koprowski. 1973. Human regulatory gene for inducible tyrosine aminotransferase in rat-human hybrids. *Proc. Natl. Acad. Sci. U.S.A.* 70:1268.
5. Riggs, J. L., N. Takemori, and E. H. Lennette. 1965. Detection of Adenovirus type 17 neoantigen(s) in continuous human amnion cell line (FL) by immuno-fluorescence. *Proc. Soc. Exp. Biol. Med.* 120:832.