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Chromosome Analysis of a Simian Virus 40-transformed Mouse Cell Line and Two Variant Sublines That Are Resistant to Cytochalasin B[1]

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SUMMARY

The chromosomes of an SV40-transformed mouse cell line, SVT2, were analyzed by the acetic-saline-Giemsa banding technique. By contrast to most established mouse lines, SVT2 cells possess a remarkably homogeneous chromosome complement and contain two copies of most chromosomes. However, trisomy for chromosome 3 is a distinct feature of this cell line. Chromosomes 1, 3, 14, and 19 have given rise to biarmed markers.

Two Cytochalasin B-resistant sublines derived from SVT2 are also essentially diploid for all autosomes but contain only one chromosome X; they display an even greater homogeneity than does the SVT2 parental cell line. Each of the Cytochalasin B-resistant cell lines has lost one or several of the biarmed markers from SVT2 and new ones have appeared. Both cell lines have lost one copy of chromosome 3 and one chromosome X.

The results presented illustrate the advantages of cells of defined chromosome constitution, like STV2 cells and derived sublines, to study specific interactions between transforming virus and host chromosomes. The possible role of SV40 in the maintenance of the pseudodiploid karyotype of SVT2 is also discussed.

INTRODUCTION

Most commonly used lines of mouse cells are heteroploid with high numbers of chromosomes, often in the hypotetraploid range (7, 13). That many of the chromosomes show gross structural abnormalities has been recognized for a number of years, but the full extent of the heterogeneity has been recognized only recently, as a consequence of the introduction of fluorescent banding techniques. For example, it is now known that as many as three-quarters of the chromosomes of the A9 line of mouse L-cells show unusual features (1). Two other mouse cell lines, MSWBS, derived from a methylcholanthrene-induced sarcoma, and RAG, derived from a renal adenocarcinoma, are also now known to be much more heterogeneous than had previously been suspected (6).

In this paper, we have used the acetic-saline-Giemsa banding technique (14) to analyze the chromosomes of cells of an SV40[3]-transformed mouse line, SVT2, and 2 CBR sublines derived from SVT2. SVT2 cells have been shown by standard karyotyping to be pseudodiploid (5), unlike most other lines of mouse fibroblasts transformed by SV40, and are, therefore, suited for such study. In addition, SVT2 cells contain viral DNA in an integrated state (12), and a detailed analysis of the viral sequences has been recently reported (2). The 2 CBR cell lines, which were isolated from SVT2 using the ability of Cytochalasin B to kill selectively transformed cells (8), present distinct alterations in the expression of virus-specific functions (9). We show in this paper that all 3 cell lines are pseudodiploid and that most chromosomes have the banding pattern of normal mouse chromosomes. However, we are able to detect specific differences among the karyotypes of these cell lines.

MATERIALS AND METHODS

The SV40-transformed mouse fibroblasts, SVT2, were obtained from Dr. S. Aaronson, NIH, Bethesda, Md. The CBR lines, CBR-1 and CBR-2, were derived from SVT2, as described elsewhere (9). Cells were grown in Dulbecco's modification of Eagle's medium, supplemented with 10% calf serum, on plastic tissue culture dishes (Nunc) at 37° in 5% CO_2.

Chromosome preparations were obtained by use of a standard air-drying procedure, and slides were treated according to the acetic-saline-Giemsa banding technique described by Sumner *et al.* (14).

Chromosomes were counted on photographs of well-spread unbroken metaphase plates and scored as telocentric or biarmed.

Individual chromosomes were identified by their banding pattern using as reference the karyotypes published by Buckland *et al.* (3). Normal chromosomes were numbered as recommended by the committee on standardized genetic nomenclature for mice (4).

RESULTS

The SVT2 Cell Line. Chromosome counts were performed on 100 metaphase spreads. The number of biarmed

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[3] The abbreviations used are: SV40, simian virus 40; CBR, Cytochalasin B-resistant.

chromosomes is plotted in Chart 1 as a function of the total number of chromosomes. The number of chromosomes per cell ranged from 35 to 45, with a mean value of 40. There are from 0 to 4 biarmed chromosomes with a mean value of 3.

Complete karyotypes of 18 cells were prepared, and 1 of these is shown in Fig. 1. In each cell, most elements could be identified as normal mouse chromosomes. Of the 4 biarmed chromosomes observed, 3 were composed of normal elements and they are designated by the number of the chromosomes involved: 1/3, 14/14, and 19/19. The 4th biarmed 19/M1 consists of chromosome 19 and of a marker M1. A small number of chromosomes (from 0 to 3 per cell, with an average of 1.7) could not be identified. This was due in some cases to obvious technical reasons; in others, distinct arrangements of bands could be seen in individual chromosomes. However, the patterns were different from 1 metaphase to the next.

Analysis of the chromosomes in the 18 metaphase spreads examined shows that 2 copies of most autosomes are usually present: very occasionally, the autosomes are mono- or trisomic. Chromosome 1 is also disomic, because there is only one copy in the cells that have the biarmed 1/3 (14 cells) but 2 copies in those that lack it (4 cells). Similarly, the isochromosome 14/14 is present in most cells but may be replaced by 2 single chromosomes. In the same 4 cells, the biarmed 1/3 and 14/14 are replaced by normal chromosomes, an indication that biarmed chromosomes are created by reversible centric fusion of telocentric chromosomes.

Chromosome 3, however, is trisomic in most cells: 2 copies are usually present in addition to a 3rd participating in the biarmed 1/3. Occasionally, 3 copies of chromosome 3 are found, which coincides with the absence of the biarmed 1/3.

Chromosome 19 shows the greatest variability. In most cells, there is one copy of chromosome 19 and either the isochromosome 19/19 or the biarmed 19/M1. The 2 biarmed chromosomes are exclusive of one another. In cells that contain neither of these markers, there are 2 or 3 copies of chromosome 19: cells that have either 19/19 or no biarmed at all have, on the average, 3 equivalents of chromosome 19. Trisomy for this chromosome is thus

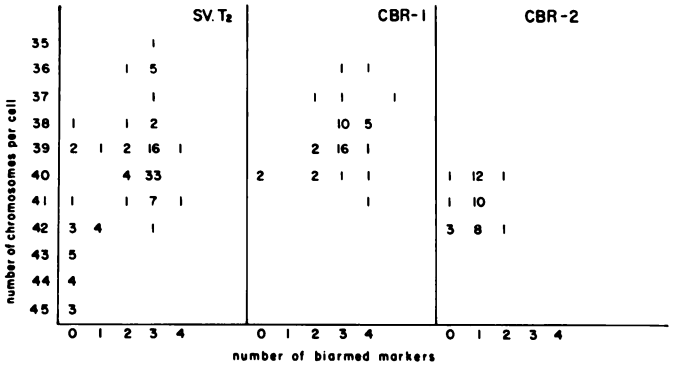

Chart 1. Distribution of the total number of chromosomes and of the number of biarmed markers in SVT2 and in its CBR derivatives. The number of metaphases examined was 100 for SVT2, 46 for DBR-1, and 37 for CBR-2.

frequently encountered, and it seems likely, therefore, that the marker M1 in 19/M1 also contains chromosome 19. This is in agreement with the shape of the proximal segment of M1.

Finally, 2 copies of chromosome X are present in 75% of the cells. No Y chromosome is detectable. These results indicate the probable female origin of SVT2 cells, origin hitherto unknown, since the cell line derives from a mixed embryonic culture.

The CBR Cells, CBR-1 and CBR-2. Chart 1 shows that the 2 CBR cell lines differ from the parental SVT2 cells both in the total number of chromosomes and in the proportion of chromosomes that is biarmed. Also, the chromosome distribution of the 2 cell lines is narrower than that of SVT2 cells. This result is clearly shown in the distribution of chromosome numbers in CBR-2 cells. All metaphase spreads examined contained 40 to 42 chromosomes and from 0 to 2 biarmed chromosomes. In CBR-1 cells, the number of chromosomes per cell ranges from 36 to 41. There are from 0 to 5 biarmed chromosomes, with a mean value of 3.

Chromosome analysis has been carried out on 9 metaphase preparations of CBR-1 cells. All cells contain 3 of the marker chromosomes found in SVT2 cells, 1/3, 14/14, and 19/M1, and 2 new minute chromosomes not found so far in SVT2 cells. The isochromosome 19/19 was never found. In all metaphases, chromosomes 1 and 3 are each present as 1 single chromosome and 1 copy in the biarmed 1/3. Chromosome 19 is present as 1 single copy plus 2 copies in the biarmed 19/M1, and all other autosomes are disomic, except for occasional mono- or trisomy, as in SVT2 cells. Thus, with the exception of chromosome 19, CBR-1 cells appear to be essentially diploid for all autosomes. There is only 1 X chromosome/cell.

Analysis of CBR-2 cells has been carried out on 16 karyotypes, 1 of which is shown in Fig. 2. Only 1 marker chromosome (14/14) found in SVT2 cells is also present in CBR-2 cells. A new marker, M2, appeared and is present in 13 of the 16 cells examined. The CBR-2 cells are also diploid for all autosomes except chromosome 19. The variation in the number of copies of chromosome 19 is reflected in the total number of chromosomes: 8 cells with 40 chromosomes had either 2 or 3 chromosomes 19; 4 cells with 41 chromosomes all had 3 copies and 4 cells with 42 chromosomes had 4 copies. There is only 1 chromosome X/cell.

Comparison of SVT2 and the CBR Cells. Comparison of the average numbers of total chromosomes and biarmed chromosomes/cell suggested that the 2 CBR cell lines each contained 1 fewer chromosome arm than did SVT2 cells. However, after identification of almost every chromosome by Giemsa banding and analysis of the distribution of each species, specific and more complex differences were detected. CBR-1 has only 3 of the 4 biarmed chromosomes of SVT2, 1/3, 14/14, and 19/M1, but 2 additional markers, the minute chromosomes M3. It has lost 1 copy of chromosome 3 and has only 1 chromosome X. CBR-2 has the biarmed 14/14 and another new marker, M2, the origin of which has not yet been identified. Like CBR-1, it is diploid for chromosome 3 and has only 1 chromosome X.

DISCUSSION

By contrast to most lines of established mouse cells, the SV40-transformed mouse fibroblasts, SVT2, appear to possess a remarkably homogeneous chromosome complement. Cells of this line are essentially disomic for most autosomes, although in any 1 cell, monosomy or trisomy for 1 or a small number of chromosomes is often observed. Four autosomes, chromosomes 1, 3, 14, and 19, have given rise to biarmed markers or isochromosomes without further rearrangements, except in the case of chromosome 19/M1, which has an abnormal arm, as yet unidentified with certainty. In addition, most cells contain 3 copies of chromosomes 3 and 19. In 2 cells, disappearance of chromosome 1/3 and of the biarmed markers involving chromosome 19 correlates with the presence of 3 chromosomes 3 and 19. This trisomy, therefore, seems to be a distinct and stable feature of SVT2. Finally, 2 copies of chromosome X are present in most cells.

In view of the well-known chromosomal instability of mouse cells in culture (7, 10), the pseudodiploid karyotype of SVT2 cells is an interesting and unusual property of the cell line. SVT2 cells were generated as a consequence of infecting embryonic cells of the BALB/c strain of mice by SV40 at a stage before the cultures had become permanently established *in vitro* and when most cells were still diploid (S. Aaronson, personal communication); all cell lines established from mouse embryo cells grown in the same conditions, except for the presence of the transforming virus, were tetraploid or hypotetraploid (16). These observations raise the question of the role of SV40 in the maintenance of an almost normal chromosome complement and suggest that the virus may be used to establish pseudodiploid lines of mouse cells. The possible role of viruses in maintaining diploidy has already been emphasized by Tjio and Ostergren (15) in their study of the chromosome constitution of mammary tumors of viral origin in mice. We have recently (F. Kelly, unpublished results) attempted to repeat Aaronson's observation, and indeed we find that quasidiploid clones of transformed cells can be obtained from primary cultures of BALB/c mouse embryos infected with low multiplicities of SV40. Four independent cultures were treated similarly and identical results have been obtained in each instance.

Most studies on transformation of mouse cells by SV40 have been carried out using 3T3 cells that are heteroploid with a median chromosome number of 73 (11, 16). Clearly, SVT2 cells provide a more suitable system if one wants to perform cytogenetic experiments. Chromosome analysis of the 2 CBR cell lines derived from SVT2 cells illustrates this point: we have shown that it is indeed possible to detect specific differences among the 3 cell lines. The most interesting fact is that the 2 cell lines CBR-1 and CBR-2 share the loss of 1 copy of chromosome 3 and 1 copy of chromosome X. As is described elsewhere (9), the 2 cell lines have also lost some viral DNA. Whether the chromosome losses are directly linked to the decreased amount of viral DNA in these cells, thereby indicating specific integration of the virus in chromosomes 3 and/or chromosome X or to some other phenotypic change occurring in the CBR cells, cannot be assessed for the moment, but the type of analysis described (9) can be applied to more cell lines and provides a method to determine if integration of SV40 DNA occurs at a specific site in mouse cells.

SVT2, other near-diploid SV40-transformed cell lines, and variants derived from them provide a favorable system for studying specific interaction between the transforming virus and the host chromosome; but clearly, cell lines with defined chromosome constitution like SVT2 cells are of more general interest.

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REFERENCES

1. Allderdice, P. W., Miller, O. J., Miller, D. A., Warburton, D., Pearson, P. L., Klein, G., and Harris, H. Chromosome Analysis of Two Related Heteroploid Mouse Cell Lines by Quinacrine Fluorescence. J. Cell Sci., *12:* 263-274, 1973.
2. Botchan, M., Ozanne, B., Sugden, B., Sharp, P. A., and Sambrook, J. Viral DNA in Transformed Cells. III. The Amounts of Different Regions of the SV40 Genome Present in a Line of Transformed Mouse Cells. Proc. Natl. Acad. Sci. U. S., *71:* 4183-4187, 1974.
3. Buckland, R. A., Evans, H. J., and Sumner, A. T. Identifying Mouse Chromosomes with the ASG Technique. Exptl. Cell Res., *69:* 231-236, 1971.
4. Committee on Standardized Genetic Nomenclature for Mice: Standard Karyotype of the Mouse, *Mus musculus*. J. Heredity, *63:* 69-71, 1972.
5. Culp, A. L., and Black, P. H. Contact-Inhibited Revertant Cell Lines Isolated from Simian Virus 40-transformed Cells. J. Virol., *9:* 611-620, 1972.
6. Hashmi, S., Allderdice, P. W., Klein, G., and Miller, O. J. Chromosomal Heterogeneity in the RAG and MSWBS Mouse Tumor Cell Lines. Cancer Res., *34:* 79-88, 1974.
7. Hsu, T. C. Chromosomal Evolution in Cell Population. Intern. Rev. Cytol., *12:* 69-145, 1961.
8. Kelly, F., and Sambrook, J. Differential Effect of Cytochalasin B on Normal and Transformed Mouse Cells. Nature, *242:* 217-219, 1973.
9. Kelly, F., and Sambrook, J. Variants of Simian Virus 40-Transformed Mouse Cells That Are Resistant to Cytochalasin B. Cold Spring Harbor Symp. Quant. Biol., *39:* 345-353, 1975.
10. Levan, A., and Biesele, J. J. Role of Chromosomes in Cancerogenesis, as Studied in Serial Culture of Mammalian Cells. Ann. N. Y. Acad. Sci., *71:* 1022-1053, 1958.
11. Pollack, R., Wolman, S., and Vogel, A. Reversion of Virus-Transformed Cell Lines: Hyperploidy Accompanies Retention of Viral Genes. Nature, *228:* 938, 967-970, 1970.
12. Sambrook, J., Westphal, H., Srinivasan, P. R., and Dulbecco, R. The Integrated State of Viral DNA in SV40-Transformed Cells. Proc. Natl. Acad. Sci. U. S., *60:* 1288-1295, 1968.
13. Shannon, J. E. American Type Culture Collection, Registry of Animal

Cell Lines, p. 28. Rockville, Md.: American Type Culture Collection 1972.

14. Sumner, A. T., Evans, H. J., and Buckland, R. A. New Technique for Distinguishing between Human Chromosomes. Nature New Biol., *232:* 31-32, 1971.
15. Tjio, J. H., and Ostergren, G. The Chromosomes of Primary Mammary Carcinomas in Milk Virus Strains of the Mouse. Hereditas, *44:* 451-465, 1958.
16. Todaro, G. J., and Green, H. Quantitative Studies of the Growth of Mouse Embryo Cells in Culture and Their Development into Established Cell Lines. J. Cell Biol., *17:* 299-313, 1963.

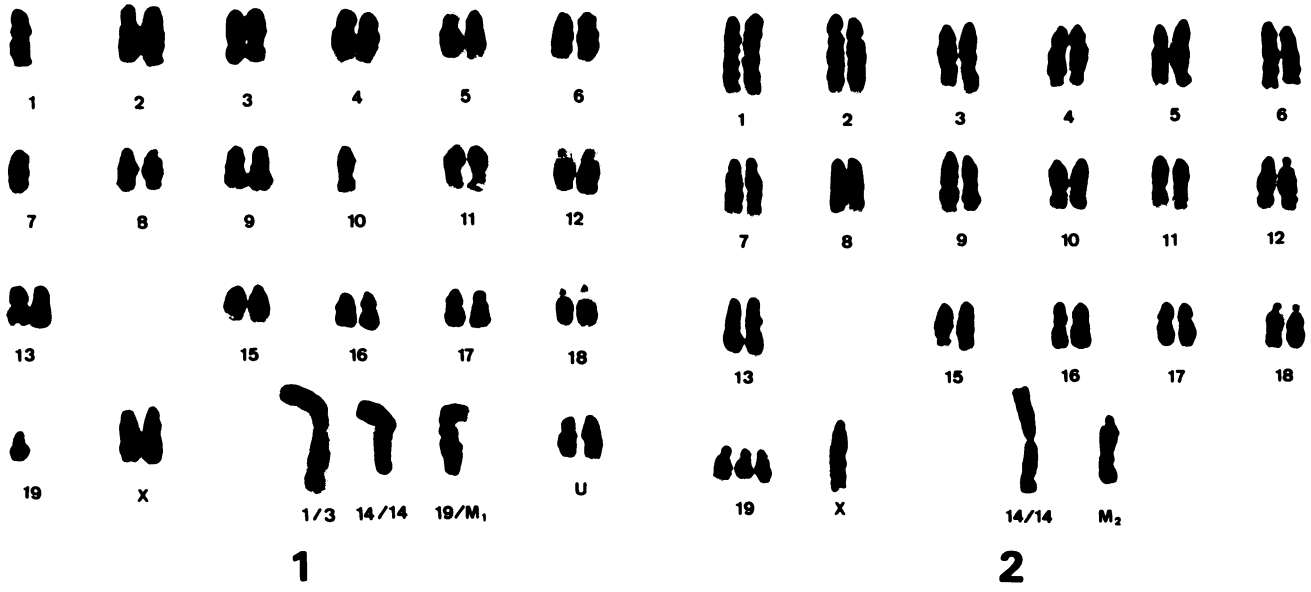

Fig. 1. Acetic-saline-Giemsa karyotype of 1 SVT2 cell. Most chromosomes have the banding pattern of normal mouse chromosomes. Only 2 of them (*U*) remain unidentified. There are 2 copies of most autosomes and 2 chromosomes X. Note 3 of the 4 biarmed markers of SVT2. Also note the trisomy for chromosome 3: there are 2 single copies and a 3rd in the biarmed 1/3.

Fig. 2. Acetic-saline-Giemsa karyotype of 1 CBR-2 cell. This cell is diploid for all autosomes except chromosome 19, which is trisomic. As in SVT2 and CBR-1 cells, the 2 chromosomes 14 are replaced by the biarmed marker 14/14. There is only 1 chromosome X. Note the new marker M2.