Karyotypes of Vasoformative Sarcomas Arising from BALB/3T3 Cells Attached to Polycarbonate Plates

Michihiro C. Yoshida, Motomichi Sasaki, Noritoshi Takeichi, and Charles W. Boone

Chromosome Research Unit, Faculty of Science, Hokkaido University, Sapporo, Japan [M. C. Y., M. S.], and Cell Biology Section, Laboratory of Carcinogenesis, National Cancer Institute, NIH, Bethesda, Maryland 20014 [N. T., C. W. B.]

SUMMARY

Four vasoformative sarcoma in vivo tumor lines arising from the s.c. implantation of 3 x 10⁴ BALB/3T3 cells attached to a 1- x 5- x 10-mm polycarbonate platelet were shown to be quite similar in chromosome constitution to the parental 3T3 line. All tumors contained the same marker chromosomes that were present in the parent BALB/3T3 cells. The findings conclusively showed that the tumors had derived from BALB/3T3 cells and not from host cells or from the in vivo fusion of the inoculated 3T3 cells with host cells. Each tumor line had a unique karyotype that was more homogeneous than that of the parental BALB/3T3 cells, strongly suggesting that each tumor represented a separate clone. An M1 marker chromosome was consistently present in each of the four tumor lines but was present in only about 50% of the parent BALB/3T3 cells; it therefore appeared to be a distinct and stable feature of the tumors.

INTRODUCTION

The BALB/3T3 line, which exhibits the properties of marked postconfluence inhibition of cell division (8, 17), anchorage dependence (16), and high serum requirement (19), has been widely used in many laboratories as a representative nontumorigenic line that can be compared with tumorogenic sublines transformed with various agents (1, 13, 14, 18). Earlier studies (4, 5) demonstrated that the s.c. implantation of less than 3 x 10⁴ BALB/3T3 cells attached to 3-mm glass beads or 1- x 5- x 10-mm polycarbonate platelets into syngeneic mice produced tumors, presumably because their anchorage dependence was accommodated in vivo during the initial period of tumor development. In this paper, we have used the quinacrine banding technique to analyze the chromosomes of 4 vasoformative sarcomas arising from s.c. implants of BALB/3T3 cells attached to polycarbonate platelets. The results provide conclusive evidence that the tumors did indeed arise from the BALB/3T3 cells, and they support the previous conclusion (5) that each tumor is monoclonal.

MATERIALS AND METHODS

Cell Line. Cline A31 BALB/3T3 cells were obtained from the American Type Culture Collection, Rockville, Md., and maintained in Eagle’s minimal essential medium supplemented with 10% calf serum.

Tumors. As described in detail previously (5), 4 in vivo tumor lines, P3/1, P3/2, P3/3, and P3/4, were derived from 4 tumors that arose from the s.c. implantation of the A31 BALB/3T3 cells attached to 1- x 5- x 10-mm polycarbonate platelets into BALB/c mice.

Karyotypes. Solid tumors removed from animals were minced with scissors in a 0.075 M KCl solution containing colchicine, 0.5 μg/ml, and dissociated by pipeting. The resulting single-cell suspension was processed for air-drying preparations as described previously (22). Slides were stained with Giemsa or quinacrine mustard combined with 33258 Hoechst by the method of Yoshida et al. (21). Karyotypes were arranged according to the standard mouse karyotype (7, 12). The BALB/3T3 cells were processed with essentially the same methods as above.

RESULTS

Karyotype of Parental A31 BALB/3T3 Line. Chromosome numbers of the parental A31 BALB/3T3 line in 50 cells examined varied from 68 to 78 with a mode of 75. Ten karyotypes banded after quinacrine fluorescence staining, such as the one shown in Fig. 1, are summarized in Table 1. As seen in Table 1, the numbers of each normal chromosome appeared to be inconsistent from one cell to another except chromosomes 2 and 16, of which 3 copies per cell were present. The X chromosome was usually present in duplicate or triplicate. No Y chromosome was detectable. Nine different chromosomes normally not present in mouse metaphases were identified. The origin of these marker chromosomes is illustrated in Fig. 2. The number of markers ranged from 68 to 78. As shown in Table 1, the BALB/3T3 cells generally lacked 1 or 2 of each marker except chromosomes 5 and 16, of which 3 copies per cell were present. As shown in Table 2, each line was characterized by its own distinctive chromosome constitution. Marker chromosomes present in tumors were identical with those found in the parental cells (Fig. 3). The number of marker chromosomes

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ranged from 9 to 18 in each tumor line. Each line had a distinctive set of markers (Table 2). In addition, several marker chromosomes were consistently present in all lines: M1, M2, M3, M7, and M8. Line P3/4 acquired a new large biarmed chromosome which resulted from centric fusion of No. 1. Since there was no such biarmed chromosome in the parental 3T3 cells, the 1/1 isochromosome must have occurred during tumor progression in vivo. The chromosome constitution in tumor lines appeared to be definitely more homogeneous than that of the parental 3T3 cells. The distribution of the number of copies per cell of each chromosome appeared to be more frequently in the triploid range than that of the parental 3T3 cells.

DISCUSSION

The similarity in karyotype pattern and marker chromosomes between cells of the A31 BALB/3T3 line and cells of the tumors that arose from implants of BALB/3T3 cells attached to plastic substrates proves conclusively that the tumors did indeed originate from the BALB/3T3 cells and not from host cells or from in vivo fusion between the implanted cells and host cells. Every cell of the 4 tumor lines contained the M1 marker chromosome, in contrast to its presence in only about 50% of the parent cells; the M1 chromosome therefore appeared to be a distinct and stable feature of the tumor lines.

The cells from each tumor had a unique chromosomal complement with a relatively high degree of homogeneity, in contrast to the cells of the parent BALB/3T3 line, which had a relatively heterogeneous chromosome complement similar to most commonly used heteroploid lines (the 2 mouse lines MSWBS and RAG, for example, have a remarkably high degree of heterogeneity associated with the continuous and relatively rapid structural rearrangements (9)). The fact that each of the 4 tumors possessed a unique karyotype of relatively high homogeneity supports the conclusion reported previously (5) that BALB/3T3 cells may be considered highly preneoplastic and that, when a monolayer is implanted in vivo, one of the cells in the population eventually undergoes spontaneous transformation to the condition of anchorage independence and forms a monoclonal tumor.

In studies on malignant cell transformation, malignancy or nonmalignancy has been considered to be related to the gene balance associated with particular chromosomes (3, 6, 10, 11). This balance hypothesis would precisely predict an altered ratio between 2 chromosomes. We tabulated the total number of copies per cell of both normal and marker chromosomes in the nontumorigenic parental BALB/3T3 cells and the derived tumor cells. Chromosomes in excess of the 4 expected in a tetraploid cell were Nos. 1, 3, 4, 6, 9, 15, 17, 18, and 19 in the BALB/3T3 cells. The tumor cells also contained these excess chromosomes except for No. 9. The following chromosomes were consistently deficient in both the parental and tumor cells: Nos. 2, 16, and X. Thus, no evidence of a chromosome imbalance in the tumor cells relative to the parental 3T3 cells was seen, although the imbalance hypothesis may not be applied to such heterogeneous, already imbalanced karyotypes as those in preneoplastic 3T3 cells and sarcomas originating from them.

One tumor line, P3/4, acquired an isochromosome of the 1/1 centric fusion. Since there was no such chromosome in the parental 3T3 cells, this homology association must have been formed during in vivo progression. The P3/4 was a line that was positive only for endogenous oncornaviruses and reverse transcriptase (5). Whether the formation of the 1/1 centric fusion is directly related to the presence of endogenous viruses cannot be assessed for the moment, because homology association in chromosomes is frequently observed in mouse cell lines (2, 15, 20).

REFERENCES


Table 1  

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<th>Marker chromosome</th>
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<td>1     2     3     4     5     6     7     8     m</td>
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<td>4     2     7</td>
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Table 2  

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<tr>
<td>P3/4</td>
<td>76</td>
<td>3     3     3     3     3     3     4     4     4     4     3     3     3     4     4     4     4     2     1     1     1     1     1     1     1     1     1</td>
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</table>

* Isochromosome of No. 1.


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