COMPARATIVE KARYOLOGIC STUDY OF TRANSPLANTABLE MIO AND MIO-r CELLS SENSITIVE AND RESISTANT TO POLIOVIRUS

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The MIO continuous cell line, maintained in culture for over 15 years, was first obtained by Gulevich from tonsil tissue of a healthy monkey (Macaca rhesus) [3]. The line consists of polygonal cells of reticular type, with a large round nucleus. MIO cells are highly sensitive to type I poliovirus, and yield a high concentration of virus in the culture fluid. By treatment of cells of the MIO line with massive doses of poliovirus (type I, Brunanders strain), followed by culture of cells which remain viable, a resistant subline of cells, spontaneously freeing itself of virus, but secreting virus for a short time, then becoming specifically highly resistant to infection with homologous virus, was obtained. This subline was called MIO-r [1, 2].

The change in the karyotype of cells becoming specifically resistant to the action of poliovirus is particularly interesting in connection with recently obtained data on the role of genes located in particular chromosomes in sensitivity of cells to one or other virus. Sensitivity of human cells to poliovirus is linked with chromosome 19 [6]. There is also evidence of the role of genes of chromosome 3 in sensitivity to herpesvirus [5] and of chromosome 21 in sensitivity to Coxsackie B virus [4].

EXPERIMENTAL METHOD

Cells were cultured by the usual method. Medium 199 with 10% bovine serum, inactivated by heating to 56°C for 30 min, with the addition of antibiotics (penicillin 100 units/ml, streptomycin 10 mg/ml), was used as growth medium.

Chromosome preparations were obtained by the method in [7] and stained with azure-eosin by the Romanovsky–Giemsa method to count the number of chromosomes and to determine the modal class and also by G and C differential staining methods for karyotyping and identification [9, 10].

Chromosomes were counted in 100-200 metaphases for each culture.

Karyotyping was carried out in accordance with the Denver nomenclature and Paris classification of human chromosomes [8], for most chromosomes of this line are indistinguishable from normal human chromosomes with respect to G bands. Besides these chromosomes, the karyotype of MIO and MIO-r cells contains marker chromosomes, which differ in their morphology both from normal human chromosomes and from chromosomes of Macaca rhesus.

EXPERIMENTAL RESULTS

The two lines are heteroploids. Karyologic analysis of MIO cells compared with the resistant variant – cells of the MIO-r subline – revealed certain differences between them.

A tendency toward a reduction in the number of chromosomes was found in the MIO-r subline. The modal class of MIO cells corresponded to 60-61 chromosomes (40% of cells contained that number of chromosomes) whereas for MIO-r cells it was 58-60 chromosomes (58% of cells contained that number of chromosomes); the number of chromosomes in subline MIO-r, moreover, varied from 53 to 65 and in line MIO from 52 to 62 chromosomes (Fig. 1).

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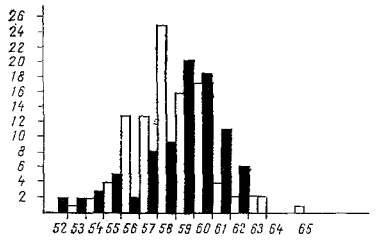

Fig. 1. Histogram of distribution of number of chromosomes in cultures of continuous cell lines MIO and MIO-r. Abscissa, number of chromosomes; ordinate, number of cells (in %). Unshaded columns – MIO-r cells; shaded columns – MIO cells.

TABLE 1. Mean Number of Copies of Individual Intact Chromosomes per Cell

Chromosome	MIO	MIO-r
1	1,75±0,3	1,32±0,22*
2	2,05±0,45	2,5±0,52*
3	2,14±0,47	1,95±0,7
4	1,90±0,19	1,82±0,42
5	1,95±0,35	1,68±0,67
6	1,95±0,25	1,87±0,28
7	1,85±0,23	2,04±0,26
8	1,55±0,25	1,91±0,45
9	1,80±1,60	1,68±0,68
10	1,90±0,39	2,14±0,68
11	1,65±0,33	2,09±0,37*
12	1,55±0,25	2,77±0,86*
13	1,55±0,25	1,27±0,3
14	1,70±0,31	1,36±0,42
15	1,75±0,68	1,00±0,27*
16	2,50±0,65	2,23±0,63
17	2,00±0,70	2,50±0,45
18	1,75±0,68	2,45±0,45*
19	1,65±0,33	1,00±0*
20	1,70±0,41	1,86±0,31
21	1,75±0,68	1,27±0,48*
22	1,55±0,65	1,36±0,79
"X"	0,85±0,43	1,05±0,42
Total number of normal chromosomes, %	66,5	67,8
Number of markers, %	33,5	32,2

*$\overline{P < 0.01}$.

Normal (intact) and marker (with structural changes) chromosomes were clearly distinguished in the karyotypes of both lines of cells. The percentage of marker chromosomes per cell was 33.5 in the MIO culture and 32.2 in the MIO-r subline, i.e., there was virtually no difference in the frequency of marker chromosomes in the two cultures.

The number of normal chromosomes per cell varied around disomy about equally in the two cultures. For instance, in cells of the MIO line chromosomes 2, 3, 4, 5, 6, 7, 10, and 17, and in cells of subline MIO-r chromosomes 3, 6, 7, 8, 10, 11, 12, and 20 were near-disomic (from 1.85 to 2.2 ± 0.25) (Table 1).

Definite differences in the mean number of copies of intact chromosomes per cell were found in the karyotypes. A statistically significant reduction in the mean number of chromosomes 1, 15, 19, and 21 and an increase in the number of copies of chromosomes, 2, 11, 12, and 18 were observed in subline MIO-r. The decrease in the number of chromosomes 13 and 14 and the increase in the number of copies of chromosome 17 in cells of subline MIO-r compared with the karyotype of the MIO cells was less significant.

During analysis of the marker chromosomes they were subdivided depending on size and location of the centromere into the following groups: 1) metacentric chromosomes; 2) submetacentric; 3) subtelocentric; 4) acrocentric, and 5) microchromosome.

1. A large metacentric chromosome, larger than human chromosome 1, was found in all MIO cells tested. It probably consists of chromosome 3 and a region of an unidentified chromosome translocated to it. This chromosome was absent in all cells of the MIO-r subline. The remaining marker chromosomes of this subgroup were found with equal frequency in the cells of both lines.

2. In the subgroup of submetacentric chromosomes, a large submetacentric chromosome, larger than chromosome 1, was found in each cell of subline MIO-r examined. The short arm of this chromosome most probably originates from the long arm of chromosome 1, and its long arm is a derivative of the long arm of chromosome 2. This chromosome was not found in any of the cells of line MIO studied. The other marker chromosomes of this subgroup were found in cells of both lines, as also were the chromosomes of subgroup 3 (subtelocentric).

In the subgroup of acrocentric chromosomes two large acrocentric chromosomes were found. One of them (the larger) was found in all cells of the MIO-r subline. This chromosome was close in size to chromosomes of the B group, it was not found in the MIO cells, and it most probably originates from chromosome 13 with a region of an unidentified chromosome translocated to the terminal region of its long arm. The second large acrocentric chromosome was larger than the D group chromosomes and in all probability is a derivative of chromosome 14.

The facts described above thus point to genetic differences between the two cell lines compared. However, these differences do not include absence of chromosome 19 from the subline of cells specifically resistant to poliovirus, as would be expected on the basis of data in [6]. The explanation of this discrepancy must be sought by a further study, for it could lie in fine structural or mutational changes in chromosome 19 in the MIO-r line or in the fact that the cell lines studied were developed from monkey cells through their possible contamination and hybridization with human cells.

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