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COMPARATIVE CYTOGENETIC STUDY OF A CONTINUOUS HUMAN CELL LINE AND ITS SUBLINE SUSCEPTIBLE AND RESISTANT, RESPECTIVELY, TO COXSACKIE B3 VIRUS

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Summary. — Cytogenetic characteristics of the J-96 human cell line and its J-41 subline, highly susceptible and resistant, respectively, to coxsackie B3 virus, were compared. The J-41 subline, as compared to the original J-96 line, had fewer chromosomes in the modal class cells (54—57 and 58—62, respectively) mostly at the expense of normal chromosomes. In most J-41 cells chromosome 21 was eliminated and the number of homologues of chromosomes 2, 9, 11, and 12 was reduced. The percentage of marker chromosomes in the J-41 subline (31.3) was slightly higher than in the J-96 line (24.3). The relationship between differences in the karyotypes and properties of the cultures such as resistance to coxsackie B3 virus, capacity to produce virus-induced interferon and to acquire an antiviral state after treatment with interferon were discussed.

Key words: antiviral resistance; karyotypes; chromosomes; coxsackie virus

Introduction

The J-96 continuous cell line was isolated by Osgood and Brooke (1955) from the blood of a patient with monocyte leukaemia. A subline, J-41, was derived from it by three treatments of J-96 cells with large doses of coxsackie B3 virus and found to be highly and specifically resistant to this virus (Gulevich, 1968). The resistance to coxsackie B3 virus acquired by the J-41 subline proved to be a stable heritable property persisting for over 15 years of alternating periods of cultivation and freezing.

J-41 cells were shown to excrete no coxsackie B3 virus, to be no virus carriers, and to produce no substances of the type of antibody or interferon (Soloviev and Gulevich, 1966). Morphological and cytochemical studies revealed in the resistant cells some decrease in the intensity of reactions to RNA, total protein, protein SH groups, and a marked reduction in the activity

of enzymes acting at alkaline pH, particularly alkaline phosphatase, as compared with the original cells (Amchenkova and Gulevich, 1968).

Routine karyological analysis of both cell lines revealed a stable decrease in the chromosome numbers in cells of the modal class (Varshaver and Gulevich, 1964). The decrease in the modal number is mainly due to the loss of group G chromosomes (Khesin *et al.*, 1974). By the R-method of differential chromosome staining we established that chromosome 21 was lost most frequently (Khesin *et al.*, 1978).

A certain role in changes of the cell karyotypes and selection of the resistant cells probably played the fact that the process of formation of the specific antiviral resistance of the J-41 subline was accompanied by a prolonged (about 4 months) period of a virus-carrier state (Gulevich, 1968; Soloviev and Khesin, 1970).

We analysed the results obtained from the point of view of cytogenetic control of susceptibility or resistance of cells to certain virus groups. At present, this problem is in the stage of accumulation of facts (Miller *et al.*, 1974; Carrit and Goldfarb, 1976; and others).

Detailed cytogenetic studies similar to the present one offer valuable information on karyotype-phenotype relationships at the cell population level. Heneen (1976) described karyotypes of the original continuous human cell line Lu-106 and of a measles virus-carrying line derived from it. We are not aware of any report on the cytogenetic characteristics of a continuous human cell line which, as a result of specially arranged experiments, has become specifically resistant to a virus to which the original line had been highly susceptible. The present study deals with a comparative cytogenetic analysis of such a system.

Materials and Methods

Cell cultures. The continuous human cell line J-96 and its subline J-41 (see above) were used.

Karyological analysis. Preparations were made on the 2nd-3rd day of cultivation. To determine the modal class, chromosomes were counted in 100—200 metaphase plates. Karyotypes were determined by the G-method (Seabright, 1971), R-method (Dutrillaux and Lejeune, 1971) and C-method (Sumner, 1972). From 10 to 30 metaphase plates of either culture were examined by each method. Identification and description of the chromosomes was based on the recommendations of the Paris Conference (1972). The results were evaluated statistically by the Fisher-Student method.

Results

Both cultures are heteroploid. Modal class cells contain 58—62 chromosomes in the J-96 line and 54—57 chromosomes in the J-41 subline (Khesin *et al.*, 1972). The chromosome sets of cells of both lines consist of normal chromosomes typical of normal human karyotype and marker chromosomes resulting from structural rearrangements (Figs 1—8). The proportion of normal chromosomes in J-96 cells is 75.7% on the average; this value is markedly lower in the J-41 subline (68.7%; $P < 0.0001$). The number of homologues of normal chromosomes varied in individual cells of both lines. The rate of

Table 1. Arithmetic mean counts of normal and marker chromosomes in J-96 and J-41 cells

Chromosome	J-96	J-41	t	P <
1	1.5 ± 0.17	1.5 ± 0.11		
2	2.5 ± 0.17	1.3 ± 0.10	6.5	0.001
3	1.5 ± 0.16	1.3 ± 0.12		
4	1.7 ± 0.10	1.5 ± 0.11		
5	1.9 ± 0.13	1.8 ± 0.12		
6	1.8 ± 0.14	1.6 ± 0.10		
7	2.1 ± 0.16	2.2 ± 0.12		
8	1.7 ± 0.18	2.1 ± 0.12		
9	2.6 ± 0.15	1.7 ± 0.10	2.56	0.01
10	2.6 ± 0.11	2.2 ± 0.11		
11	2.3 ± 0.15	1.6 ± 0.13	3.43	0.001
12	2.3 ± 0.13	1.8 ± 0.15	2.8	0,01
X	1.2 ± 0.15	1.2 ± 0.15		
13	0.7 ± 0.10	1.0 ± 0.11		
14	1.0 ± 0.11	1.3 ± 0.10		
15	1.5 ± 0.11	1.1 ± 0.10		
16	2.8 ± 0.16	2.8 ± 0.11		
17	2.3 ± 0.21	2.0 ± 0.13		
18	2.2 ± 0.14	1.9 ± 0.10		
19	2.3 ± 0.16	2.3 ± 0.13		
20	2.1 ± 0.17	1.8 ± 0.11		
21	1.5 ± 0.17	0.3 ± 0.10	6.27	0.001
22	1.4 ± 0.17	1.1 ± 0.13		
Y	0.4 ± 0.12	0.2 ± 0.07		
Total normal chromosomes	45.7 ± 0.72	37.6 ± 0.41	9.73	0.0001
%	75.7	68.7		
Marker chromosomes J—96	14.7 ± 0.58	12.9 ± 0.47		
%	24.3	23.6		
New markers	—	4.2 ± 0.13		
%	—	7.7		
Total chromosomes	60 4 ± 0.45	54.7 ± 0.17	11.8	0.0001

Data obtained by the three methods on 32 (J-96) and 38 (J-41) metaphase plates. In columns "t" and "P <" only statistically significant differences are presented. In all the other cases $t \geqq 2.5$ and $P > 0.5$.

occurrence of normal and marker chromosomes in cells of the cultures studied is summarized in Table 1.

The greatest differences in cell karyotypes concerned the average number of individual normal autosomes per cell. In the J-96 line we found all autosomes of the normal human karyotype, whereas most cells of the J-41 subline lacked the normal chromosome 21 (0.3 ± 0.1 per cell). The number of homologues of chromosome 2 per cell was significantly higher (2.5 ± 0.17) in the J-96 line than in the J-41 subline (1.3 ± 0.1; $P < 0.001$); the same was true for chromosomes 9, 11, and 12, although, unlike chromosome 21, all these normal chromosomes were present practically in all cells of both lines. As for the other autosomes, cells of both lines exhibited similar trends in variations of chromosome numbers around disomy. In both lines, the number of homologues of chromosomes 16 and 19 was greater than disomic

(on the average 2.3—2.8 per cell); the number of homologues of chromosomes 1, 3, 13, 14, 15, and 22 was less than disomic (from 1.5 to 0.7). As concerns sex chromosomes, both lines were nearly identical both in the number of X-chromosomes per cell (1.2 ± 0.15 in both cultures) and in the marked decrease of the number of "Y" chromosomes per cell (0.4 ± 0.12 in J-96 and 0.2 ± 0.07 in subline J-41). The quotation marks ("Y") mean that this chromosome did not meet all the criteria of the Paris Conference for a normal human Y-chromosome. It was identical with the normal Y-chromosome in size, centromeric index and localization of the heterochromatin block in the distal portion of the long arm demonstrable by the C-method of staining (insets in Figs 3 and 6), but exhibited no specific bright fluorescence of the distal portion upon staining with acridine dyes.

The amount of marker chromosomes in karyotypes of J-96 and J-41 cells was 24.3 and 31.3% respectively ($P < 0.001$). Nearly 85% of marker chromosomes of the J-96 line were present at different rates in the resistant J-41 subline. At the same time approximately 25% of markers of the J-41 subline represented new chromosome variants absent from cells of the original line. Data concerning which chromosomes of the normal karyotype are included into the marker chromosome set will be reported elsewhere.

Discussion

Studies in which cell cultures resistant to various viruses were obtained by inoculation of susceptible cultures with the homologous virus have been numerous (Walker, 1964). Karyological examinations in some of these experiments have thus far revealed only a decrease in the number of chromosomes in cells of the modal class at the expense of certain chromosome groups (Gulevich *et al.*, 1970; Kusano *et al.*, 1970; and others). Current methods of identification of individual chromosomes allow a detailed comparison of the properties of the cultures studied with changes in their karyological characteristics.

Our results showed that the decrease in the modal number of chromosomes in cells of the resistant J-41 subline as compared with the J-96 cell line occurred mostly at the expense of normal chromosomes. Some of the latter were eliminated from the cells and others were involved in structural rearrangements and transferred to marker chromosomes.

Previously we reported about an association between the susceptibility of human cells to coxsackie B3 virus and the presence of normal chromosome 21, and about the disappearance of this chromosome from most cells of the resistant J-41 subline (Khesin *et al.*, 1978). We found another manifestation of a decrease in the number of normal chromosome 21 and changes in the function of those parts of this chromosome which were incorporated into the marker set characteristic exclusively of the J-41 subline. The interferon-induced antiviral state of human cells is determined by a gene localized in chromosome 21 (Tan, 1975; Epstein and Epstein, 1976; and others). Soloviev *et al.*, 1978*a* showed that even by treatment with 1600 units/ml [recalculated to international standard B for human interferon (69/19)] of human leukocyte

interferon no antiviral state of the J-41 cells could be achieved. This indicates either a lack of the appropriate locus in chromosome 21 or, less likely, a loss by the gene of this locus of the capacity to become activated by interferon.

Another difference between J-96 and J-41 cell cultures was manifested by their capacity to produce interferon in response to virus inducers: the titre of interferon induced under similar conditions by Newcastle disease and Sendai viruses in the resistant J-41 subline was 8 times lower than in the original J-96 line (Soloviev *et al.*, 1978*b*).

According to the data obtained in experiments on somatic cell hybrids, the structural and regulatory genes responsible for the production of leukocyte and fibroblast interferons in human cells could be localized in chromosomes 1, 2, 5 and 9 (Tan *et al.*, 1974; Creagan *et al.*, 1975; Meager *et al.*, 1979). The numbers of chromosomes 2 and 9 in the J-96 line were significantly higher (2.5 ± 0.17 and 2.6 ± 0.15, respectively) than in the J-41 subline (1.3 ± 0.1 and 1.7 ± 0.1, respectively; $P < 0.01$). The two cultures practically do not differ in the number of chromosomes 1 and 5. These results obtained on a new model are in good accordance with the reports on the role of the genes of human chromosomes 2 and 9 in regulation of the production of virus-induced interferon in human cell cultures.

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Explanation of Figures (Plates I-IV):

Figs 1—6. Metaphase plates of J-96 (*1, 3, 5*) and J-41 (*2, 4, 6*) cell cultures stained by the G — (*1, 2*), R — (*3, 4*) and C — (*5, 6*) method. Insets: *3* — chromosomes 21 and 22; 6 — chromosome "Y".

Figs 7 and 8. Karyotypes of J-96 (7) and J-41 (8) cells. Staining by the R-method. Upper 4 rows — chromosomes of normal human karyotype; lower 2 rows — marker chromosomes: M — metacentric, Sm — submetacentric, St — subtelocentric, A — acrocentric, m — microchromosomes. Figures under marker chromosomes indicate their numbers.