CYTOGENETIC STUDY OF ROUS SARCOMAS INDUCED BY CARR – ZIL'BER VIRUS IN THE ADULT *Macaca mulatta*

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Cells of two tumors induced by a highly oncogenic variant of Rous sarcoma virus in adult monkeys (Macaca mulatta) have the characteristic karyotype (2n = 42) of this species and possess a comparatively high level of structural mutations of the chromosomes. Metaphases with deletions, pulverization, and pycnosis of the chromosomes are found.

The oncogenic activity of Rous sarcoma virus (RSV) in newborn monkeys has been described [2, 6]. In a recent report, Obukh and collaborators have described the isolation of a variant of RSV, highly oncogenic toward the adult Macaca mulatta, from the Carr–Zil'ber strain [7].

This paper describes a cytogenetic investigation of tumors induced in adult monkeys (Macaca mulatta) by this strain of RSV.

EXPERIMENTAL METHOD

The test objects were Rous sarcomas in two monkeys of the species Macaca mulatta aged 2.5-3 years: 1) male No. 10,827, with a tumor in the region of the spine (age of tumor 42 days); 2) female No. 10,821 with a tumor in the region of the right thigh (age of tumor 31 days). Colchicine (0.6 mg/kg body weight) was injected intravenously into the animals and also into the region of the tumor 3 h before biopsy. The tumor material was trypsinized, treated with hypotonic solutions, and fixed by the usual methods [4]. As the control, together with tumor material, a sample of bone marrow from the same monkeys was taken for investigation. Chromosomal preparations were stained by the Romanowsky–Giemsa method. Metaphase plates were distinguished by the criteria described previously [1]. Chromosomal aberrations were analyzed in metaphases containing 40-43 chromosomes. Approximately equal numbers of metaphases were analyzed in the tumor and bone marrow from each monkey. In tumors from each of the two monkeys no statistically significant differences in the numbers of cells with chromosomal aberrations were found. The results obtained from the two monkeys are therefore combined in the tables.

EXPERIMENTAL RESULTS

The results of cytogenetic analysis, as reflected in the number of abnormal cells, are given in Table 1.

As Table 1 shows, the number of aberrant metaphases in tumors induced by Rous virus was significantly higher than in the bone marrow. Besides aberrant cells, metaphases with numerous gaps and a small number of cells with pulverization of the chromosomes were observed. It can be postulated that these changes are due to the presence of a virus in the tumors, as was shown previously [3].

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TABLE 1. Incidence of Abnormal Cells in Tumors and in Bone Marrow of Adult Monkeys Induced by Rous Virus

Object	No. of metaphases	No. of metaphases with chromosomal aberrations		No. of metaphases with gaps and pulverization of chromosomes	
		abs.	%	abs.	%
Tumor	244	37	15.12±2.29	40	16.39±2.36
Bone marrow	513	2	0.4 ± 0.08	0	0

TABLE 2. Incidence of Different Types of Chromosomal Aberrations in Tumors and Bone Marrow

Type of aberration	Tumor		Bone marrow	
	abs.	per 100 cells	abs.	per 100 cells
Single fragments	33	13.5	2	0.4
Paired fragments	11	4.5	0	0
Total number of aberrations	44	18.0	2	0.4
Ring chromosomes	14	5.7	0	0

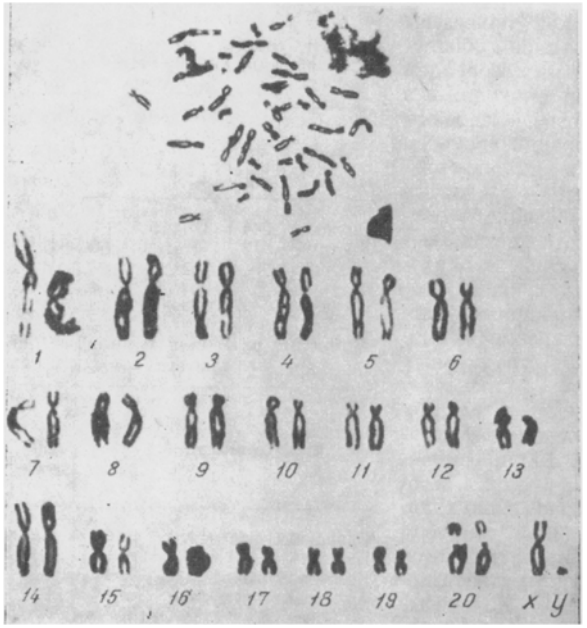

Fig. 1. Metaphase plate and karyotype of Rous sarcoma cell of adult monkey. Gaps in 1st, 3rd, and 5th pairs of chromosomes; ring chromosome in 16th pair; satellite chromosomes in 20th pair.

Metaphases with gaps and pulverization of the chromosomes were not included in the category of aberrant cells and were considered separately. No such abnormalities were found in the bone marrow.

Results for the types of chromosomal aberrations discovered in the tumors and bone marrow are given in Table 2. Single fragments are the chief type of chromosomal aberrations in both the tumor and bone marrow, but their frequency in the tumors was higher than in the marrow. In addition, paired fragments and ring chromosomes were observed in the tumor cells. The number of structural lesions of the chromosomes discovered in the tumor cells was 18 aberrations per 100 cells analyzed. Consequently, cells of a tumor induced by Rous virus are characterized by increased mutability of their chromosomes. Ring chromosomes were not accompanied by fragments. It is possible that there was a clone of cells with a ring chromosome. Another possible suggestion is that the rings are formed by adhesion of telomers. The solution of this problem requires analysis of a large number of metaphases and karyotyping of high-quality plates with ring chromosomes.

The tumor cells had a normal karyotype (2n=42) characteristic of monkeys of this species (Fig. 1). The karyotype of Macaca mulatta has been described by Chiarelli [5]. Figures for the frequency of aneuploidy in the region of diploid numbers and polyploidy in the tumor and bone marrow are given in Table 3.

It will be seen that both in the bone marrow and in the tumor about 90-95% of the cells were euploid (2n = 42). Aneuploid cells were mainly of the hypodiploid type, evidently on account of loss of chromosomes during hypotonic treatment. The degree of hypodiploidy of "artefact" type was higher for the bone marrow than for the tumor. Polyploids were rarely seen.

TABLE 3. Frequency of Aneuploidy and Polyploidy in Tumor and Bone Marrow

Object	Cells with number of chromosomes				Number of aneuploids		% of polyploids
	40	41	42	43	abs.	%	
Tumor	5	7	230	2	14	5.74±1.03	2.47±0.99
Bone marrow	10	42	460	1	53	10.52±1.35	0.92±0.42

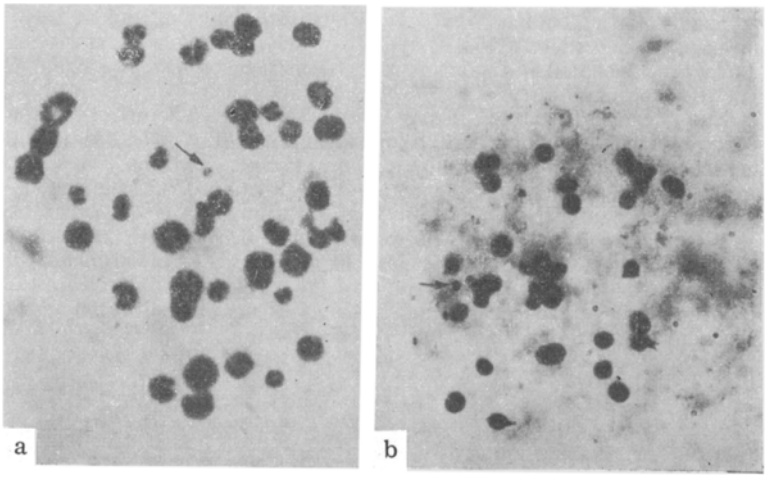

Fig. 2. Abortive metaphases: a) number of pycnotic chromosomes 42; b) number of chromosomes less than 42. Arrows indicate figures corresponding to smallest Y-chromosome.

A special type of karyorrhexis, due to passage of the interphase nucleus into mitosis (abortive mitoses), was found in the tumor material. In this condition the chromosomes remain in the metaphase stage and become pycnotic and spherical in shape. Rarely is the number of these structures equal to the diploid number of chromosomes (Fig. 2a). More commonly their number is less than 42, for some of them fuse to form conglomerates (Fig. 2b). The number of "abortive" mitoses in the tumor was about 40% of the total number of dividing cells. Their true frequency was evidently different. "Nonabortive" metaphases (60%) were accumulated in the course of 3 h by means of colchicine, but the time of accumulation of the "abortive" metaphases is unknown. It is possible that growth of the tumor depends on the ratio between the numbers of "abortive" and "nonabortive" mitoses.

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