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Cytological alteration of cultured rat liver cells by 3'-methyl-4-dimethylaminoazobenzene with special reference to chromosome changes, changes of growth patterns at a colony level and alpha-fetoprotein production.

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Abstract

A near diploid clone derived from a rat liver cell line was continuously treated with various concentrations of 3'-methyl-4-dimethylaminoazobenzene (3'-Me-DAB) in culture. By treatment with 2.8 micrograms/ml, cells with 41 chromosomes formed a mode and which then shifted to 39. The chromosome numbers of cells treated with 5.4 micrograms/ml were widely distributed at early stages, but later the mode shifted to hypotetraploid region. Untreated control cells were confirmed as near diploid. Increased plating efficiency by 3'-Me-DAB as well as the appearance of large sized colonies was obtained. The production of alpha-fetoprotein (AFP) by the cells was slightly enhanced by treatment with 3'-Me-DAB. The cells treated with and without 3'-Me-DAB did not produce any tumor in rats 6 months after their intraperitoneal injection.

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CYTOLOGICAL ALTERATION OF CULTURED RAT LIVER CELLS BY 3′-METHYL-4-DIMETHYLAMINOAZOBENZENE WITH SPECIAL REFERENCE TO CHROMOSOME CHANGES, CHANGES OF GROWTH PATTERNS AT A COLONY LEVEL AND α-FETOPROTEIN PRODUCTION

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Abstract. A near diploid clone derived from a rat liver cell line was continuously treated with various concentrations of 3′-methyl-4-dimethylaminoazobenzene (3′-Me-DAB) in culture. By treatment with 2.8 μg/ml, cells with 41 chromosomes formed a mode and which then shifted to 39. The chromosome numbers of cells treated with 5.4 μg/ml were widely distributed at early stages, but later the mode shifted to hypotetraploid region. Untreated control cells were confirmed as near diploid. Increased plating efficiency by 3′-Me-DAB as well as the appearance of large sized colonies was obtained. The production of α-fetoprotein (AFP) by the cells was slightly enhanced by treatment with 3′-Me-DAB. The cells treated with and without 3′-Me-DAB did not produce any tumor in rats 6 months after their intraperitoneal injection.

Key words: 3′-Me-DAB, liver cells, chromosome numbers, plating efficiency, α-fetoprotein.

A number of studies on malignant transformation of rat liver epithelial cells by aminoazo dyes have been attempted and various properties of the transformed cells have already been reported (1–5). However, few communications have been concerned with chromosome changes, changes of growth patterns at a colony level or AFP production in rat liver epithelial cells during the course of aminoazo dye-treatment. In the present study, we investigated these properties with the aim of surveying reliable *in vitro* markers of tumorigenicity in near diploid rat liver cells during the course of 3′-Me-DAB-treatment.

MATERIALS AND METHODS

Cells. A liver cell line, J-5-2 (6) derived from a normal rat (7-day-old) was colonialy cloned and the CL-1 cells thus obtained were used. The culture medium was Eagle's minimal essential medium (MEM, Chiba Pref. Serum Inst.,

Ichikawa, Japan) supplemented with 20% inactivated bovine serum. Subcultures were conducted with 0.2% trypsin solution (Difco, Detroit) every 10 days and 1×10^5 cells/ml were inoculated into TD15 flasks.

Treatment of cells with 3'-Me-DAB. 3'-Me-DAB (Tokyo Kasei Co., Tokyo) was dissolved in dimethylsulfoxide (Sigma, Saint Louis, Missouri), diluted with culture medium and added to the cells at a final concentration of 2.8 μg/ml, 5.4 μg/ml or 11.4 μg/ml.

Chromosome analysis. Chromosome preparation was made and stained with a procedure described by Rothfels and Siminovitch (7). G-banding was accomplished by incubation in 0.1% trypsin from 5–15 sec at 20°C.

Plating efficiency. The plating efficiency was estimated by counting the colonies formed 10 days after inoculation of 300 or 30 cells per dish.

Production of AFP. When the cells treated with 3'-Me-DAB reached confluence after cultivation in the medium without carcinogen, the culture medium was replaced by that without bovine serum. The cells were cultured for another 2 days and then submitted for assay. The concentration of AFP was determined by competitive binding radioimmunoassay with ^{125}I-labelled AFP (8, 9).

RESULTS

The cells treated with 11.4 μg/ml of 3'-Me-DAB for 20 days did not show growth in culture because of its toxic effect. Cells continuously treated with 5.4 μg/ml of 3'-Me-DAB suffered slightly from the toxic effect of 3'-Me-DAB but continued to grow. On the other hand, 2.8 μg/ml 3'-Me-DAB-treated cells showed more active growth than untreated control cells after the 60th day of the continuous treatment (Fig. 1). Chromosome analysis of the cells was carried out at 20, 40 and 100 days after the initiation of treatment with 3'-Me-DAB. The control cells were confirmed with chromosome number 40 (near diploid). When the cells were treated with 2.8 μg/ml of 3'-Me-DAB, the cells with 41 chromosomes became dominant and formed a mode. Forty days after treatment by 3'-Me-DAB, the mode of chromosome number shifted to 39. Thereafter, the mode did not change even at 100 days after treatment. When the cells were treated with 5.4 μg/ml of 3'-Me-DAB, the chromosome numbers were widely distributed in the 20th day of treatment and the mode shifted to hypotetraploid region 100 days after the initiation of treatment (Fig. 2). The same marker chromosome as that in the cells with 41 chromosomes was found in the cells with 40 chromosomes by G-banding analysis, suggesting the same origin of the cells with 41 and 40 chromosomes (Figs. 3, 4).

During 123 to 161 days after the treatment, the plating efficiency of the cells treated with 2.8 μg/ml and 5.4 μg/ml of 3'-Me-DAB was much higher than in the control cells (Table 1). Large sized colonies were formed in 5.4 μg/ml of 3'-Me-DAB-treated cultures as compared with those in the cells treated with 2.8 μg/ml.

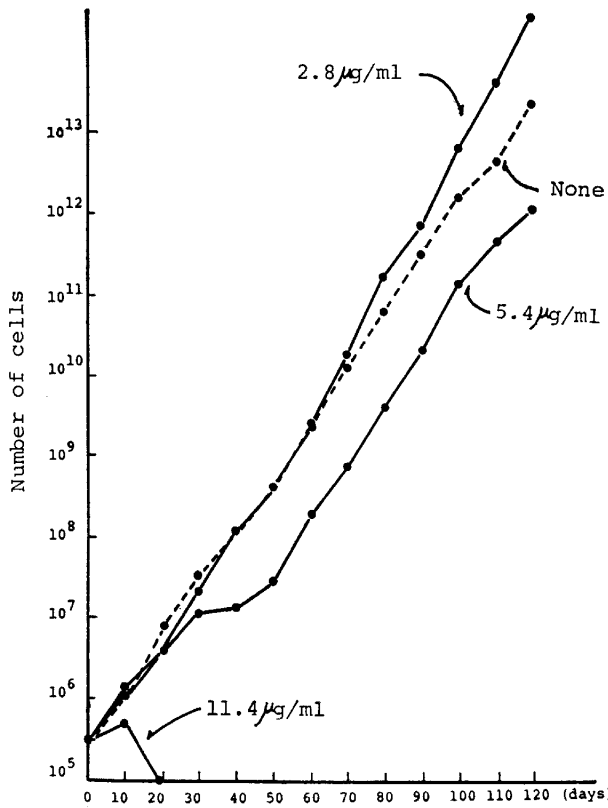

Fig. 1. Cumulative growth curves of the cells treated with or without 3′-Me-DAB.

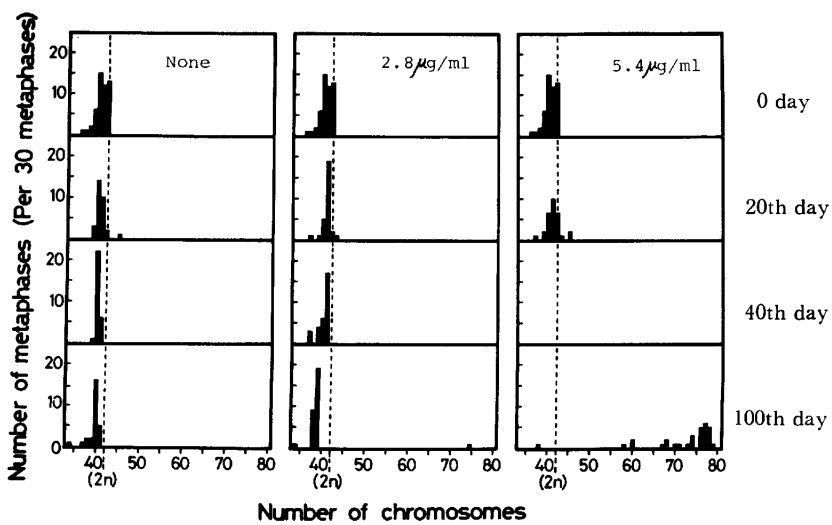

Fig. 2. The distribution of chromosome numbers of the cells treated with or without 3′-Me-DAB.

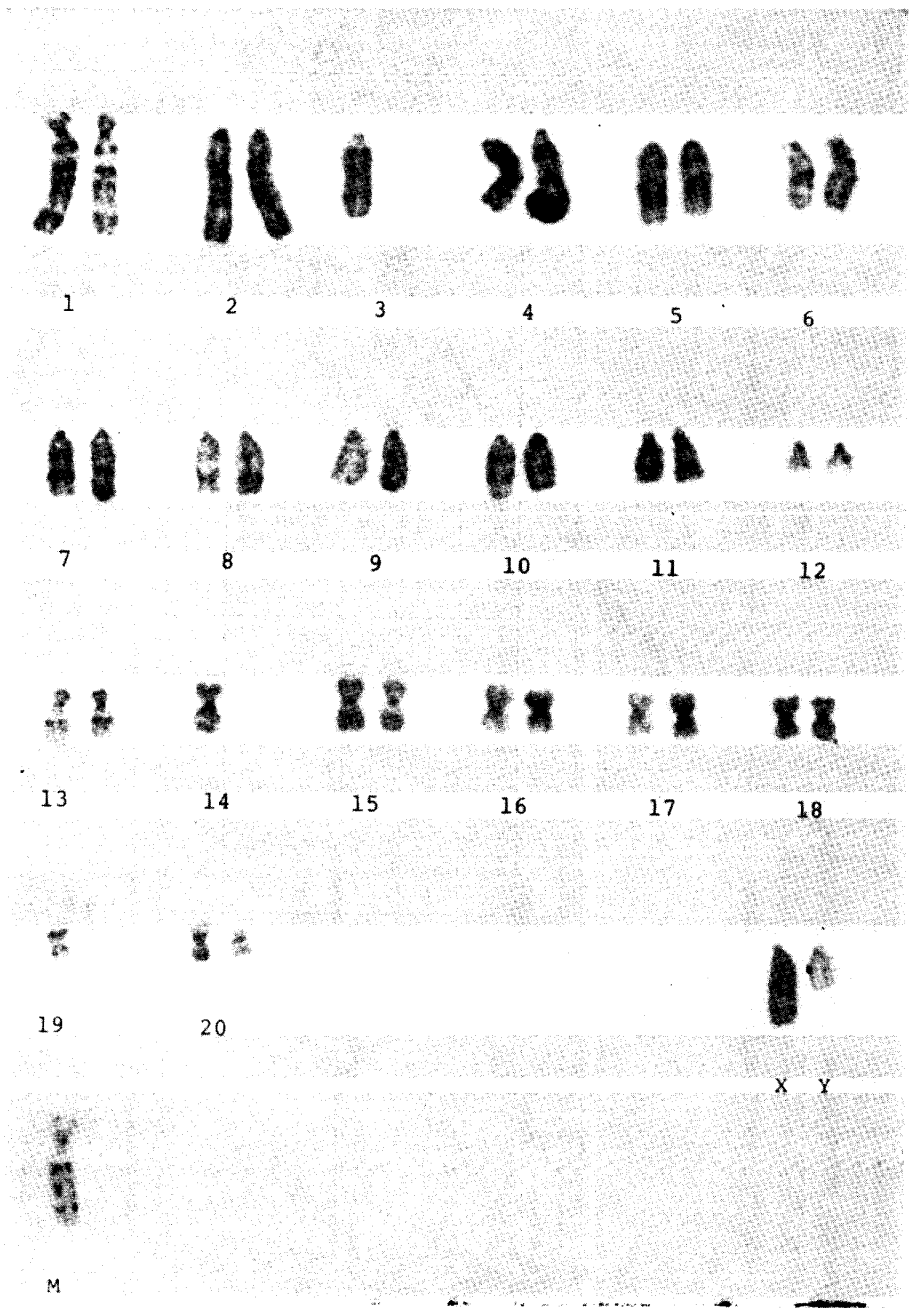

Fig. 3. G-band karyotype. 40, XY, −3, −14, −19, +t (3 ; 14q).

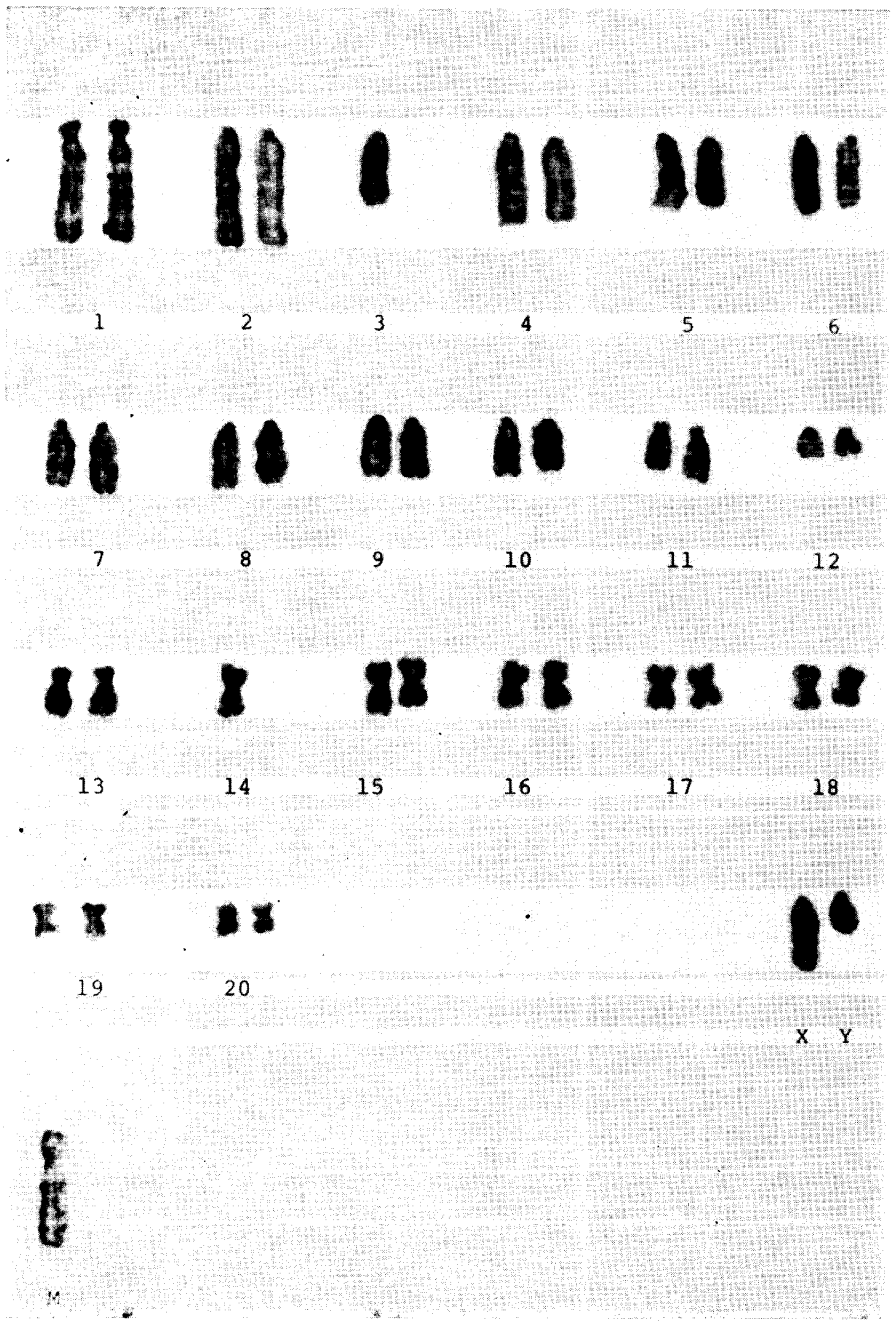

Fig. 4. G-band karyotype. 41, XY, −3, −14, +t (3; 14q).

TABLE 1. PLATING EFFICIENCY OF CELLS TREATED WITH OR WITHOUT 3'-ME-DAB

Treatment μg/ml	Culture[a] days	PE[b] (%)[c] on inoculum size					
		30 cells/dish			300 cells/dish		
		L[d]	S[e]	Total	L[d]	S[e]	Total
None	27	0	13.3	13.3	1.8	21.0	22.8
None	161	4.3	29.0	33.3	1.8	26.6	28.4
2.8	161	11.0	26.7	37.7	13.7	35.4	49.1
5.4	123	34.3	20.0	54.3	25.1	19.0	44.1

a, Day after the initiation of study; *b*, Plating efficiency; *c*, Each value represents the average from three dishes; *d*, Large colony with more than 1 mm in diameter; *e*, Small colony with less than 1 mm in diameter.

TABLE 2. PRODUCTION OF AFP IN CULTURE BY THE CELLS TREATED WITH OR WITHOUT 3'-ME-DAB

Treatment μg/ml	AFP (ng/ml)	
	Day[a]	
	6	20
None	37.5	36.6
2.8	n.d.[b]	43.2
5.4	19.6	51.0

a, Details are described in the text. Each value represents the average from two TD15 flasks. *b*, Not determined.

AFP levels in the culture medium were examined by radioimmunoassay. As shown in Table 2, production of AFP by the cells was slightly enhanced by treatment with 3'-Me-DAB for 20 days.

Cells of 1×10^6 numbers obtained in the 81st and 90th day after treatment with and without 3'-Me-DAB were inoculated intraperitoneally into rats. No tumors were produced at least within 6 months after the inoculation.

DISCUSSION

A limited number of studies deal with chromosome changes of cells after treatment with 3'-Me-DAB or DAB. Different cytological responses were shown by treatment with 2.8 μg/ml and 5.4 μg/ml of 3'-Me-DAB in the present experiment. It would be reasonable to say that cells with 41 chromosomes were selected by 2.8 μg/ml of 3'-Me-DAB and underwent chromosomal mutation in further cultivation. On the other hand, continuous treatment with 5.4 μg/ml of 3'-Me-DAB induced chromosomal mutation of cells at an early stage of treatment since the chromosome numbers were widely distributed 20 days after treatment with 5.4 μg/ml.

Plating efficiency was chosen as a marker for growth analysis during chromosome change. It was frequently observed that plating efficiency increased along with the process of transformation (10–12). In this study, increased plating efficiency by treatment with 3′-Me-DAB as well as the appearance of large sized colonies seemed to be closely connected with chromosome change.

It is generally accepted that AFP is synthesized and secreated by hepatic cells of embryonic, neonatal and malignant rats. In the present study, it was shown that production of AFP by the cells was slightly enhanced by treatment with 3′-Me-DAB for 20 days in spite of its unknown mechanism. It is interesting that chromosome changes were also observed when such an enhancement of AFP production was shown.

The three properties examined here correlated well each other but not with tumorigenicity, since no tumors were observed 6 months after intraperitoneal injection of cells treated with 3′-Me-DAB. Further examinations are awaited to determine which of the cells or the procedures of transplantation were responsible for the lack of tumor production. A larger size of inoculum and much longer period of observation may be required to preserve tumorigenicity of the cells (4, 13).

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