101. *Frequencies of Sister Chromatid Exchanges in Cells from Patients with Neurofibromatosis Induced by Mitomycin-C*

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Neurofibromatosis (von Recklinghausen's disease; NF) is a kind of skin-nerve syndromes with the autosomal dominant inheritance. Patients with NF have been known to have a higher than normal incidence of the malignant neoplasms (D'Agostino *et al.* 1963a, b; Stay and Vawter 1977). *In vitro* study to the clastogenic effects of DNA damaging agents has been demonstrated in some malignancy high risk groups such as Down syndrome, ataxia telangiectasia, xeroderma pigmentosum, and Fanconi's anemia. NF is considered to have familial tendency toward malignancy. The present study was performed to examine the incidence of spontaneous and mitomycin-C (MMC) induced sister chromatid exchanges (SCEs) in the peripheral blood lymphocytes from patients with NF in order to clarify a possible relationship between the genetic state and the predisposition to the malignancy.

Materials and methods. Heparinized peripheral blood samples were drawn from four patients with NF and four healthy controls. Whole blood from each donor (0.3 ml) was added to 5 ml RPMI 1640 medium (GIBCO) containing 15% fetal bovine serum (GIBCO) and 3% phytohemagglutinin M (GIBCO). The medium also contained 40 μM bromodeoxyuridine (Sigma) for the entire culture period. For the treatment, MMC (Sigma) was first dissolved in sterile distilled water, and then aliquots of this freshly made solution were added to each culture to give the appropriate final concentrations (0, 3×10^{-9}, 1×10^{-8}, 3×10^{-8}, 1×10^{-7} M) for the entire culture period. The cultures were incubated at 37°C for 72 hr in the complete darkness. The colcemid (Wako) was added to each culture at the final 6 hrs (final concentration=2×10^{-7} M). The cells were then collected by the centrifugation, exposed to 0.075 M KCl hypotonic solution for 8 min, and fixed three times in ethanol:acetic acid (3:1). Air dried chromosome preparations were made, and a modification of the fluorescence-plus-Giemsa method was applied to obtain the sister chromatid differential staining (Goto *et al.* 1978). Cells dividing for the first(X1), second(X2), and third or more(X3+) time in the culture can be determined in such preparations (Tice *et al.* 1976; Morimoto and Wolff 1980).

Twenty five consecutive second-division metaphase cells were scored for SCEs and two hundred metaphase cells were scored for cell cycle kinetics per point per person.

Results. Results of the present study are summarized in Table I. The frequencies of the spontaneous SCEs in NF and the control cells were 6.7±0.4,

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7.5±0.5, respectively. There were no significant differences between them. The MMC treatment with NF and normal cells led to a clearly dose-related increase in frequency of SCEs. However, there were no significant differences in the frequencies of SCEs between NF and normal control cells in all MMC concentrations examined.

The analysis of the cell cycle kinetics by the sister chromatid differential staining method revealed that there were no remarkable differences between the cell cycle kinetics of NF cells and those of normal cells (Fig. 1).

Table I. The frequencies of SCEs in cells from patients with NF and normal controls treated with mitomycin-C

		Concentration of mitomycin-C (M)				
		0	3×10^{-9}	1×10^{-8}	3×10^{-8}	1×10^{-7}
Control	1	6.2±0.5*	9.9±0.9	15.0±1.0	25.7±1.4	63.3±4.7
	2	7.1±0.9	11.6±0.8	18.6±0.9	30.9±1.3	60.7±2.4
	3	8.7±0.7	11.5±1.1	16.2±0.8	31.0±1.9	67.9±3.3
	4	7.9±0.5	11.2±1.0	15.1±1.1	21.3±1.6	56.3±3.0
Mean		7.5±0.5	11.1±0.4	16.2±0.8	27.2±2.3	62.1±2.4
NF	1	6.5±0.7	9.4±1.0	14.9±1.7	28.1±1.6	60.3±3.7
	2	6.2±0.4	9.7±0.8	14.4±0.9	33.4±2.0	77.4±3.4
	3	7.9±0.7	12.5±0.6	18.5±1.5	—**	71.9±4.0
	4	6.0±0.7	10.5±0.9	15.9±1.0	28.8±1.5	63.1±2.5
Mean		6.7±0.4	10.5±0.6	15.9±0.8	30.1±1.4	68.2±3.4

* Mean ±S.E. ** No mitoses.

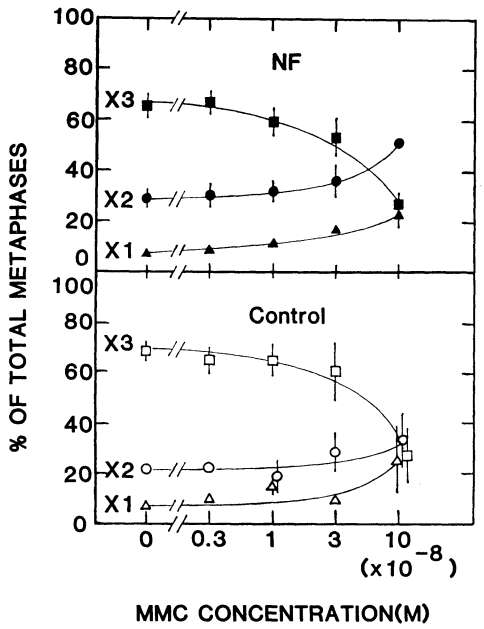

Fig. 1. Cell cycle kinetics in cells from patients with NF and normal controls treated with mitomycin-C.

Discussion. In this study, there were no significant differences in SCE frequencies between NF and normal cells. This result was in a good agreement with that obtained in the cells of tuberous scrlosis, one of the skin-nerve syndromes (Iijima *et al.* 1985). There is no report available for SCEs in cells from the patients with NF. However, Hafez *et al.* (1984) reported that there were significant increases in the incidence of chromosomal aberrations such as gaps, breaks and dicentrics in NF lymphocytes by X-ray irradiation when compared with normal cells. On the other hand, Mao *et al.* (1985) reported that quantitative parameters of the dose-dependent reduction in the colony-forming ability by the X-ray irradiation did not differ significantly between cells derived from peripheral neurofibromas of patients with neurofibromatosis and that of skin fibroblasts derived from healthy donors.

In conclusion, this study failed to reveal the significant induction of SCEs in NF cells. Further investigation should be performed to clarify a possible causal relationship between the genetic state of NF and its predisposition to malignancy.

Summary. Lymphocytes from four patients with NF and from four normal controls are studied for SCEs and the cell cycle kinetics in the MMC-treated cultures. The result reveals that NF lymphocytes have almost the same proliferative kinetics and SCE frequencies as normal cells both in untreated cultures and when exposed to MMC.

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References

D'Agostino *et al.*: Primary malignant neoplasms of nerves in patients without manifestations of multiple neurofibromatosis. Cancer, **16**, 1003–1008 (1963a).

D'Agostino *et al.*: Sarcomas of the peripheral nerves and somatic soft tissues associated with multiple neurofibromatosis. ibid., **16**, 1015–1020 (1963b).

Goto, K. *et al.*: Factors involved in differential-Giemsa staining of sister chromatids. Chromosoma (Berl.), **66**, 351–359 (1978).

Hafez, M. *et al.*: Evidence of chromosomal instability in neurofibromatosis. Cancer, **55**, 2434–2436 (1985).

Iijima, S. *et al.*: Spontaneous and mitomycin-C induced sister chromatid exchanges in cells from patients with tuberous sclerosis. Proc. Japan Acad., **61B**, 32–34 (1985).

Mao, R. *et al.*: Cell-culture studies on neurofibromatosis (von Recklinghausen's disease) III. Experiments on X-ray sensitivity. Arch. Dermatol. Res., **277**, 439–443 (1985).

Morimoto, K., and Wolff, S.: Increase of sister chromatid exchanges and perturbations of cell division kinetics in human lymphocytes by benzene metabolites. Cancer Res., **40**, 1189–1193 (1980).

Schwenn, M. R. *et al.*: Investigation of the cytotoxic effects of DNA damaging agents on neurofibromatosis cells. Mutat. Res., **142**, 55–58 (1985).

Tice, R. *et al.*: The utilization of bromodeoxyuridine incorporation into DNA for the analysis of cellular kinetics. Exp. Cell Res., **102**, 232–236 (1976).