THE INVESTIGATION OF A POSSIBLE MECHANISM OF TUMOUR PROMOTION BY A CYTOGENETIC ANALYSIS OF CULTURED MAMMALIAN CELLS

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In multistep carcinogenesis, tumour promoters act to complete the process begun by primary carcinogens and produce increases in tumour frequency and decreases in the lag time for tumour appearance. It has been suggested that tumour promoters act by inducing mitotic recombination which could lead to the expression of recessive carcinogen-induced mutations and the transformation of normal cells to tumour cells (Kinsella and Radman 1978). Sister chromatid exchange (SCE) is a form of intra-chromosomal somatic recombination, and agents that enhance mitotic recombination would be expected to increase the frequency of SCE. As part of an investigation into the mechanism of action of the tumour promoting phorbol esters we have examined the ability of 12-O-tetradecanoyl-phorbol-13-acetate (TPA) to increase SCE frequency in cultured Chinese hamster cells. We have also studied the effect of TPA on the induction of SCEs by two known carcinogens, the alkylating agent N-methyl-N^1-nitro-N-nitrosoguanidine (MNNG) and the DNA cross-linking agent Mitomycin C (MC).

Chinese hamster ovary cells CHO-K_1 grown as monolayers, were used in this work. 2×10^5 cells were inoculated into 5 ml of a modified Ham's F10 medium containing 10% foetal calf serum in a 25 cm^2 plastic tissue culture flask. Incubation was at 37.5° in 5% carbon dioxide in air. After incubation for 20-24 hours the growth medium was replaced with fresh medium containing 2×10^{-5} $Mol.L^{-1}$ 5-bromodeoxyuridine (BUdR) and the chemical(s) under test. After a further 23 hours incubation in the dark, the cells had been exposed to BUdR for two replication cycles and were then treated with colchicine at 1.25×10^{-6} $Mol.L^{-1}$ for 4 hours to accumulate cells at metaphase. Cells were harvested by centrifugation and treated with hypotonic 0.075 $Mol.L^{-1}$ KCl solution prior to fixing in 3:1 methanol/acetic acid. Air dried preparations were made and stained using the fluorescence plus Giemsa technique (Perry and Wolff 1974) for differential chromatid staining. A minimum of 30 differentially stained metaphase cells were examined on coded and randomised slides for each treatment and the number of SCEs per cell were recorded.

The mean number of SCEs per cell observed in control CHO-K_1 cells was 17.3 ± 0.74. The addition of TPA at concentrations of 1.6×10^{-7} $Mol.L^{-1}$ and 1.6×10^{-6} $Mol.L^{-1}$ to cultures of exponentially growing cells did not significantly increase the SCE frequency. The ability of TPA to modify SCE induction by primary carcinogens was examined in cells treated with either 1.36×10^{-7} $Mol.L^{-1}$ MNNG or 10^{-8} $Mol.L^{-1}$ MC. The test concentrations of MNNG and MC increased the mean number of SCEs per cell from the control level to 50.6 ± 2.8 and 31.0 ± 1.12 respectively. In neither case was the increase significantly altered when TPA was present at either of the two test concentrations.

These findings are in direct contrast to those of Kinsella and Radman who reported TPA-induced SCE formation in Chinese hamster V79 cells. Our SCE induction data cast serious doubts on the hypothesis that tumour promotion by TPA results primarily from enhancement of mitotic recombination.

Kinsella, A.R., Radman, M. (1978) Proc. Natl. Acad. Sci. U.S.A. 75, 6149-6153

Perry, P., Wolff, S. (1974) Nature (Lond.) 251, 156-158