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INDUCTION OF ENDOREDUPLICATION IN CHINESE HAMSTERS V79 CELLS BY CYTOSINE ARABINOSIDE

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Endoreduplication (ER) could be induced very effectively in Chinese hamster V79 cells exposed to cytosine arabinoside (1-β-D-arabinofuranosylcytosine; Ara-C). Cells were cultured for 48 hours in Ara-C containing medium. ER frequency increases rapidly after Ara-C release. About 60% of metaphase cells were endoreduplicated at 8-10 hours after release from Ara-C (5 μg/ml). Induction of ER also depends on Ara-C concentrations.

Diplochromosomes which are produced by passing DNA synthesis twice without interventing of a mitotic stage, have been observed from long ago. The process to produce diplochromosomes is called endoreduplication (ER). The mechanism involved in the forming of diplochromosomes is little known because ER is a very rare phenomenon. Levan et al (1, 2) reported that ER frequently appeared in cancer cells. Kuhn et al (3) investigated the frequency of mitotic chiasmata in endoreduplicated metaphase cells within Bloom's syndrome cells, Fanconi's anemia cells and normal cells. Shiraishi et al (4) studied the frequency of single and twin SCE in diplochromosomes within Bloom's syndrome cells. As mentioned above, ER is a cytogenetically important marker in the cancer study. Many attempts to induce ER effectively have been introduced (5-10), but an appropriate system has not yet been established.

We confirmed that Ara-C efficiently induced ER in Chinese hamster V79 cells. ER frequency increases till 7.5 μg/ml of Ara-C, but no increase is seen at higher concentrations.

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MATERIALS AND METHODS

Cells and culture medium Chinese hamster V79 cells (V79 cells) cultured in αMEM medium (GIBCO Co. USA) supplemented with 10% fetal bovine serum were used. Doubling time was 12 hours. 49.0% of metaphase cells had 22 chromosomes and 35.9% had 21 chromosomes. Cells having 20 or 36-44 chromosomes were 6% respectively. The rest showed hypoploidy (19 chromosomes), triploidy and hyperploidy. Chemical Ara-C was kindly gifted by Yamasa Shoyu Co. (Chiba, Japan). Chemical was dissolved in culture medium immediately before use. 5×10^5 cells were suspended in media containing various concentrations of Ara-C, and planted on 5 cm plastic Petri dishes (Falcon Co., USA). After culturing for 48 hours at 37°C, 5% CO_2, they were washed twice with serum-free αMEM medium. Chromosome analysis. The chromosome slides were prepared by the usual flaming method, with hypotonic treatment of the cells using 0.075 M KCl (2 volumes) + 1% Na citrate buffer (1 volume) solution after one hour colchicine treatment. 1000 metaphase cells were observed for each sample. Cells whose chromosomes had been pulverized (these cells are considered to be in prophase) and those which had more than 50 chromosomes were not counted.

RESULTS

Endoreduplicated metaphase cells observed at 12 hours after Ara-C release are shown in Fig. 1. Two identical chromosomes form a pair and are very proximally aligned with one another. Conjunction of the two chromosomes at the centromere cannot be observed microscopically. Aberrations such as gaps, breaks, rings etc. were observed in both of endoreduplicated and non-endoreduplicated cells.

ER frequencies at various times after Ara-C release are shown in Fig. 2. ER sharply increases from 4 hours after release from Ara-C (5 μg/ml). At 8 hours, about 60% of metaphase cells were endoreduplicated. Subsequently, the frequency sharply decreased. When processed with 10 μg/ml of Ara-C, ER frequency was investigated from the 8th hour when many metaphase cells were accumulated. The highest frequency was about 40% at 12 hours after Ara-C release.

ER induction was dependent on the chemical concentration (Fig. 3). Colchicine were added at twelfth hour after Ara-C release. ER increased in accordance with increase of concentration of chemical, but no increase was observed in concentrations over 7.5 μg/ml.

DISCUSSION

Sachs et al (11-17) noted that MGI (macrophage and granulocyte inducer) induced normal myeloid precursor cells and myeloblastic leukemia cells (MGI^+

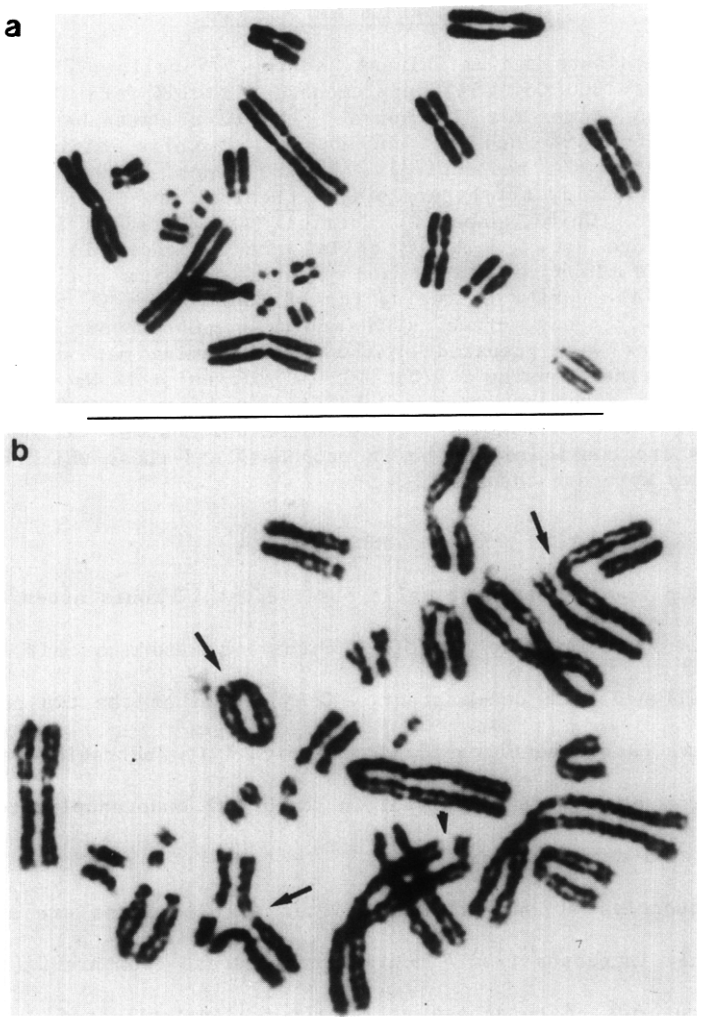

Fig. 1. Endoreduplicated metaphase cells induced in Chinese hamster V79 cells by Ara-C. Cells were cultured for 48 hours in medium containing 5 μg/ml (a) or 10 μg/ml (b) of Ara-C. After washing, they were cultured for 12 hours in growth medium. Two identical chromosomes were paired and proximately parrallelly aligned diplochromosomes were observed. Arrows show the definite aberrations.

$_{D}^{+}$) into mature cells. Sachs showed many substances in steroids, lectins, polycyclic hydrocarbons, radiation, nucleic acid base analogues, tumor promoters, etc. also possessed differentiation inducing activity (18). Among these inducers is included Ara-C. Tanigawa et al (19) and Takeda et al (20) reported that Ara-C induced various differentiation-associated characteristics

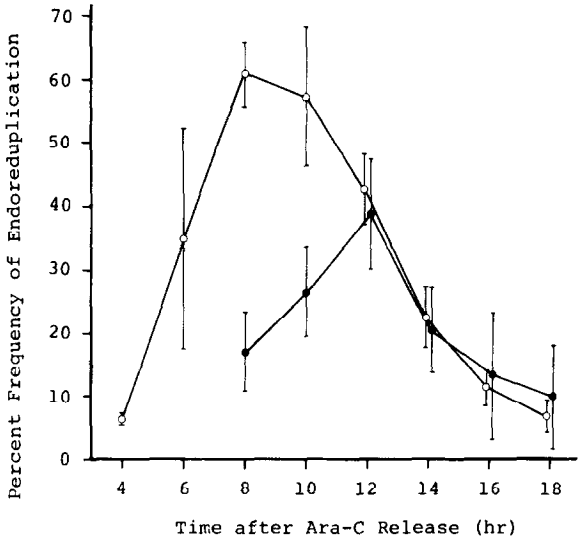

Fig. 2. ER frequency observed according to the culture period after Ara-C release. Cells were cultured in medium containing 5 μg/ml (o) or 10 μg/ml (o) of Ara-C for 48 hours. The abscissa shows the culture time from washing cells till adding colchicine. The percent frequency of ER per 1000 metaphase cells in each sample was shown. Bars indicate the mean and standard deviation of three independent experiments.

in SV-40 transformed macrophage cells and human myeloid leukemia cells, respectively. Baccarani and Tura (21), and Housset et al (22) reported that complete remission of clinical symptoms were obtained by Ara-C treatment in patients with myeloid leukemia. These reports confirmed the practicality of the differentiation induction therapy advocated by Sachs (18). Ara-C is also known to suppress the excision repair of DNA (23), increase the frequency of x-ray induced chromosomal aberrations (24) and enhance the DNA breaks following irradiation or chemical treatment (25).

We noted many diplochromosomes were appeared in V79 cells treated with Ara-C (Fig. 1). After removal of Ara-C, the frequency of ER increased rapidly (Fig. 2). The highest frequencies appeared at 8 hours in the group treated with 5 μg/ml and at 12 hrs with 10 μg/ml of Ara-C. After passing the peak, the frequency rapidly decreased. This suggest that there exists in the cell cycle a sensitive stage which causes induction of ER. ER induction was dependent on the concentration of Ara-C (Fig. 3). Highly concentrated Ara-C (10 μg/ml) may suppress the progression of endoreduplicated cells to

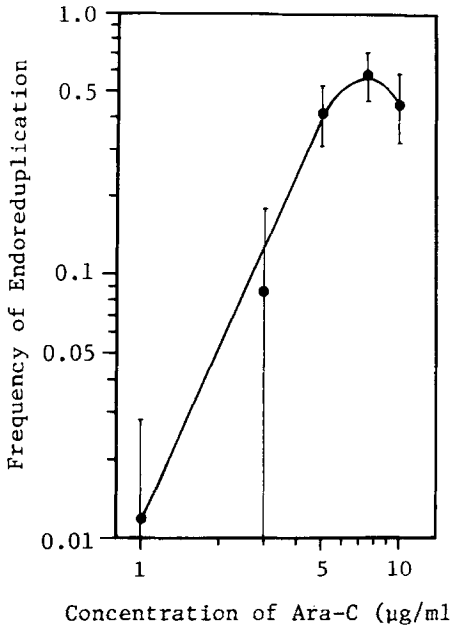

Fig. 3. ER frequency observed after treatment of various concentrations of Ara-C. Colchicine was added at twelve hours after Ara-C release. Bars indicate the mean and standard deviation of the three independent experiments.

metaphase, or have lethal effect on endoreduplicated cells (Figs. 2 and 3). Although many attempts to induce ER using chemical mutagens (5), concanavalin-A (Con-A) (6, 9), colchicine (7), colcemid (8), radiation (8), β-mercaptoethanol (26) or phytohaemaglutinin (PHA) (27) were introduced, all of them had low efficiency. However, it is interesting that Con-A, radiation, PHA and some chemical mutagens are included in Sachs's differentiation inducing substances (18).

The fact that Ara-C effectively induces ER is considered to be useful in the elucidation for the mechanism of ER and differentiation inductions.

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