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INDUCTION OF ENDOREDUPLICATION IN CHINESE HAMSTER V79 CELLS BY CYTOSINE ARABINOSIDE

チャイニーズ・ハムスターV79細胞における cytosine arabinoside による核内倍加の誘発

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SUMMARY

Endoreduplication (ER) could be induced effectively in Chinese hamster V79 cells exposed to cytosine arabinoside (1-β-D-arabinofuranosyl-cytosine: Ara-C). V79 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 Ara-C (5 μg/ml) release. The induction of ER also depends on Ara-C concentrations.

要約

チャイニーズ・ハムスター V79 細胞に cytosine arabinoside (1-β-D-arabinofuranosylcytosine; Ara-C)を処理することによって，核内倍加(ER)が非常に効率よく誘導された．V79 細胞は，Ara-C を含む培養液中で48時間培養した．ER の頻度は Ara-C 除去後，急激に増加する．Ara-C(5 μg/ml)除去後8－10時間目には，約60％の分裂中期細胞に核内倍加がみられた．ER の誘導はまた，Ara-C の濃度に依存している．

INTRODUCTION

Diplochromosomes, which are produced by twice passing DNA synthesis without an intervening mitotic stage, have been observed previously. The process resulting in diplochromosomes is called 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 appears 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 sister chromatid exchanges (SCE) in diplochromosomes within Bloom's syndrome cells. As mentioned above, ER is a cytogenetically important marker in cancer studies. Many attempts to effectively induce ER have been reported,[5-10] but an

緒言

分裂期を介在しないで2回の DNA 合成を経過することによって複糸染色体が生じることは以前から知られている．複糸染色体を生じる過程を核内倍加(ER)という．ER は非常にまれな現象であるので，複糸染色体を生じる機構はほとんど分かっていない．Levan ら[1,2]は，癌細胞の中にしばしば ER がみられることを報告している．Kuhn ら[3]は，Bloom 症候群や Fanconi 貧血症患者からの細胞，及び正常細胞中の核内倍加した分裂中期細胞にみられる mitotic chiasmata の頻度を調べた．白石ら[4]は，Bloom 症候群細胞中の複糸染色体内の単一姉妹染色分体交換(SCE)と，対(つい)SCE の頻度を調べた．以上のように ER は癌研究上，細胞遺伝学的に重要な指標である．ER を効率よく誘導するために多くの試みが紹介されているが，[5-10] 十分なシステム

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appropriate system has not yet been established. In the present study, it was confirmed that Ara-C efficiently induced ER in Chinese hamster V79 cells. The ER frequency increases at concentrations up to 7.5 μg/ml of Ara-C, but no increase is seen at higher concentrations.

はいまだ樹立されているとは言えない．我々は，Ara-Cがチャイニーズ・ハムスターV79細胞中にERを効率よく誘導することを確認した．ER頻度は，Ara-Cが7.5μg/ml濃度までは増加するが，それ以上の濃度域ではもはや増加はみられなかった．

MATERIALS AND METHODS

Cells and Culture Medium. V79 cells cultured in αMEM medium (GIBCO Co., USA) supplemented with 10% fetal bovine serum were used. Doubling time was 12 hours, in which 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 remaining cells (3.1%) showed hypoploidy (19 chromosomes), triploidy, and hyperploidy.

Chemical. Ara-C was given to RERF as a gift by Yamasa Shoyu Co. (Chiba, Japan). The Ara-C was dissolved in culture medium immediately before use. Suspensions of 5×10^5 cells in media containing various concentrations of Ara-C were planted on 5 cm plastic petri dishes (Falcon Co., USA). After culturing for 48 hours at 37°C in 5% CO_2, they were washed twice with serum-free αMEM medium.

Chromosome Analysis. The chromosome slides were prepared by the usual flame method,[11] 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. Then 1,000 metaphase cells were observed for each sample. Cells whose chromosomes had been pulverized (cells considered to be in prophase) and those which had more than 50 chromosomes were excluded.

材料及び方法

細胞と培養液． 10%胎牛血清を添加したαMEM培地(GIBCO社，米国)中で培養されたV79細胞を用いた．倍加時間は12時間であった．49.0%の分裂中期細胞は22本の染色体をもっていたが，35.9%は21本の染色体をもっていた．20本又は36～44本の染色体をもつ細胞は，各々6%であった．残り(3.1%)の細胞は低倍体(19本の染色体)，三倍体及び多倍体であった．

試　薬． Ara-Cは，ヤマサ醤油株式会社(千葉)から提供された．Ara-Cは使用直前に培養液中に溶解した．種々の濃度のAra-Cを含む培養液中に5×10^5個の細胞を懸濁し，5cmプラスチックシャーレ(Falcon社，米国)中に播種した．細胞は37°C，5% CO_2下で48時間培養後，無血清αMEM培地で2回洗浄した．

染色体観察． 染色体標本は，1時間のコルヒチン処理後，0.075 M KCl(2容)+1%クエン酸ナトリウム(1容)の緩衝液で低張処理した上，通常の火焰法で作成した．[11] 各検体について1,000個の分裂中期細胞を観察した．染色体が粉状化している細胞(これらの細胞は分裂前期にあると思われる)，及び50本以上の染色体をもつ細胞は除外した．

RESULTS

Endoreduplicated metaphase cells observed at 12 hours after Ara-C (5 or 10 μg/ml) release are shown in Figure 1. Two identical chromosomes form a pair and diplochromosomes are proximally aligned with one another. Conjunction of the two chromosomes at the centromere could not be observed microscopically. Aberrations such as gaps, breaks, rings, etc., were observed in both endoreduplicated and nonendoreduplicated cells.

ER frequencies at various times after Ara-C release are shown in Figure 2. ER sharply

結　果

Ara-C(5ないし10μg/ml)除去後12時間目に観察された核内倍加分裂中期細胞を図1に示す．2本の同一染色体が対をつくり，互いに近接して並ぶ複糸染色体を形成する．2本の染色体間の動原体部位での結合は光顕的には分からない．裂け目，切断，環状などの異常は核内倍加細胞及び核内非倍加細胞の両者に観察された．

Ara-C除去後の種々の時期におけるER頻度を図2

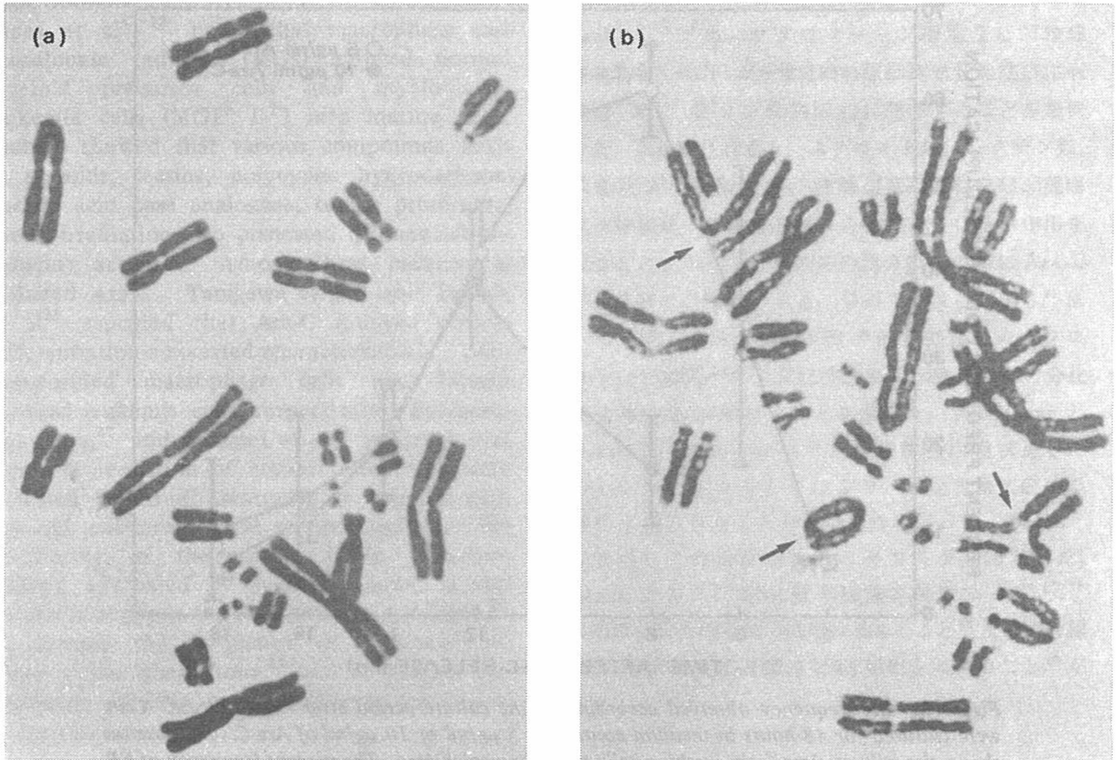

Figure 1. Endoreduplicated metaphase cells induced in Chinese hamster V79 cells by Ara-C. Cells were cultured for 48 hours in medium containing (a) 5 μg/ml or (b) 10 μg/ml of Ara-C. After washing, they were cultured for 12 hours in growth medium. Two identical chromosomes were paired and proximally aligned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

図1 Ara-Cによってチャイニーズ・ハムスターV79細胞に誘導された核内倍加分裂中期細胞．細胞は（a）5 μg/ml 又（b）10μg/ml のAra-Cを含む培養液中で48時間培養した．細胞は洗浄後，増殖培地内で12時間培養した．2本の同一染色体が対になり，非常に近接して平行に並ぶ複糸染色体が観察される．矢印は明確な異常を示す．

increased at four hours after Ara-C (5 μg/ml) release (after washing). At eight hours, about 60% of metaphase cells were endoreduplicated, but subsequently, the frequency sharply decreased. When processed with 10 μg/ml of Ara-C, ER frequency was investigated beginning at eight hours, because many metaphase cells then began to accumulate; the highest frequency was about 40% at 12 hours after Ara-C release.

The ER induction was dependent on the concentration of Ara-C (Figure 3). Colchicine was added at 12 hours after Ara-C release. ER increased in accordance with the increase of Ara-C concentration, but no increase was observed in concentrations over 7.5 μg/ml.

に示す．Ara-C（5 μg/ml）除去（洗浄）後4時間目からERは急激に上昇する．8時間目には，約60%の分裂中期細胞が核内倍加していた．続いて，その頻度は急激に減少した．10μg/ml のAra-Cで処理した場合には，8時間目から分裂中期細胞が多く集まるようになるので，この時期からER頻度を調べた．最も高い頻度はAra-C除去後12時間目の約40%であった．

ER誘導はAra-C濃度に依存していた（図3）．コルヒチンはAra-C除去後12時間目に添加した．ERはAra-C濃度の増加に伴って増加したが，その増加は7.5μg/ml 以上の濃度では見られなかった．

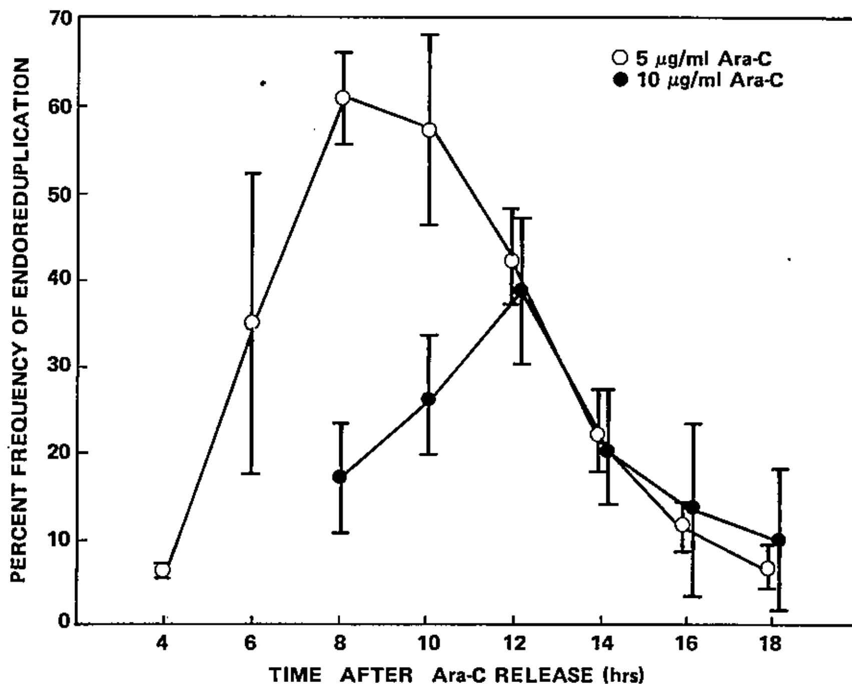

Figure 2. ER frequency observed according to the culture period after Ara-C release. Cells were cultured for 48 hours in medium containing 5 μg/ml or 10 μg/ml of Ara-C. The abscissa shows the culture time from washing cells till adding colchicine. The percent frequency of ER per 1,000 metaphase cells in each sample is shown. Bars indicate the mean and standard deviation of three independent experiments.

図2 Ara-C除去後，培養時間の経過に従って観察されたER頻度．細胞は5μg/ml又は10μg/mlのAra-Cを含む培養液中で48時間培養した．横軸はAra-C洗浄からコルヒチン添加までの培養時間を示す．核内倍加した分裂中期細胞の割合(百分率)は各検体中の1,000個の分裂中期細胞について調べた．縦棒は，3回の独立した実験の平均と標準偏差を示す．

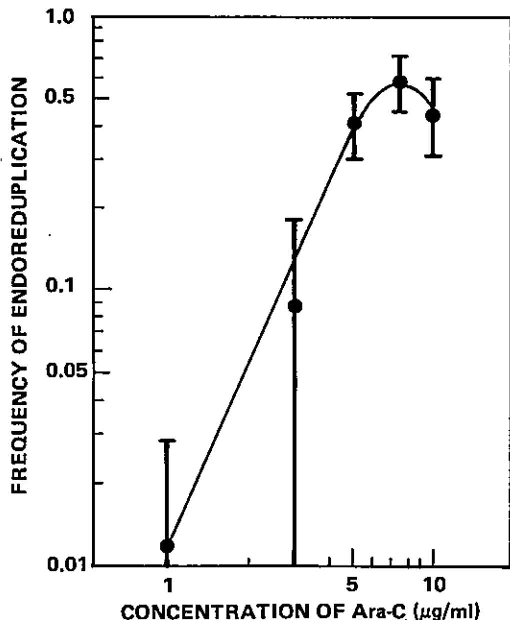

Figure 3. ER frequency observed after treatment of various Ara-C concentrations. Colchicine was added at 12 hours after Ara-C release. Bars indicate the mean and standard deviation of three independent experiments.

図3 種々の濃度のAra-C処理後に観察されたER頻度．コルヒチンはAra-C除去後12時間目に添加した．縦棒は，3回の独立した実験の平均と標準偏差を示す．

DISCUSSION

Sachs et al[12-18] noted that macrophage and granulocyte inducers (MGI) induced normal myeloid precursor cells and myeloblastic leukemia cells ($MGI^+ D^+$) into mature cells. Sachs[19] showed that various compounds, such as steroids, lectins, polycyclic hydrocarbons, nucleic acid base analogues, tumor promoters, and X-irradiation also possessed differentiation-inducing activity. Among these inducers is included Ara-C. Tanigawa et al[20] and Takeda et al[21] reported that Ara-C induced various differentiation-associated characteristics in SV40-transformed macrophage cells and human myeloid leukemia cells, respectively. Baccarani and Tura,[22] and Housset et al[23] 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.[19] Ara-C is also known to suppress the excision repair of DNA,[24] to increase the frequency of X-ray-induced chromosomal aberrations,[25] and to enhance the frequency of DNA breaks following irradiation or chemical treatment.[26]

Many diplochromosomes appeared in V79 cells treated with Ara-C (Figure 1). After Ara-C release, the frequency of ER increased rapidly (Figure 2). The highest frequencies appeared at 8 hours in the group treated with 5 μg/ml and at 12 hours with 10 μg/ml of Ara-C. After passing the peak, the frequency rapidly decreased. This suggests 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 (Figure 3). Highly concentrated Ara-C (10 μg/ml) may suppress the progression of endoreduplicated cells to metaphase, or have a lethal effect on endoreduplicated cells (Figures 2 and 3). Although many attempts to induce ER using chemical mutagens,[5] concanavalin A (ConA),[6,9] colchicine,[7] colcemid,[8] radiation,[8] β-mercaptoethanol,[27] or phytohemagglutinin (PHA)[28] were introduced, all of them were of low efficiency. However, it is interesting that ConA, radiation, PHA, and some chemical mutagens are included in the list of Sachs[19] as differentiation-inducing compounds. The use of Ara-C to induce ER may help to elucidate the mechanisms of ER production and differentiation.

考 察

Sachs ら[11-18]は，マクロファージ細胞及び顆粒球誘導物質(MGI)が正常骨髄前駆細胞や骨髄性白血病細胞($MGI^+ D^+$)を成熟細胞に誘導することを報告した．Sachs[19]はまた，ステロイド類，レクチン類，多環状炭化水素物類，核酸塩基類似物質類，発癌促進物質類，X線など種々の物質が分化誘導作用をもつことを示した．これらの誘導物質の中に Ara-C が含まれているのである．谷川ら[20]及び武田ら[21]はそれぞれ，Ara-C が SV40 で形質転換したマクロファージ細胞やヒト骨髄性白血病細胞に色々の分化関連特性を誘導することを示した．Baccarani と Tura[22]及び Housset ら[23]が，骨髄性白血病患者に Ara-C を投与することにより，完全な臨床症状の寛解が見られたことを報告するに至って，Sachs[19]の提唱した分化誘導療法の実用性が確認された．Ara-C はまた，DNA 除去修復を抑制すること，[24] X線誘発染色体異常の頻度を高めること[25]及び放射線又は化学物質処理後の DNA 切断を高めること[26]が知られている．

Ara-C で処理した V79 細胞に多くの複糸染色体が出現することを見いだした(図1)．Ara-C 除去後，ER 頻度は速やかに増加する(図2)．最も高い頻度は，5 μg/ml Ara-C で処理した群では8時間目に，10 μg/ml Ara-C で処理した群では12時間目に見られる．頻度は，ピークを過ぎてから速やかに減少した．すなわち，細胞周期上に ER 誘導を起こすための感受性の高い時期があると思われる．ER 誘導は Ara-C 濃度に依存していた(図3)．高濃度の Ara-C (10 μg/ml)は，核内倍加した細胞が分裂中期に進行するのを抑制するのかもしれないし，核内倍加した細胞に致死的に作用するのかもしれない(図2及び3)．ER を誘導するために化学変異剤，[5] コンカナバリンA(ConA)，[6,9] コルヒチン，[7] コルセミド，[8] 放射線，[8] β-メルカプトエタノール[27]又はフィトヘムアグルチニン(PHA)[28]を用いた多くの試みが紹介されているが，いずれも効果の低いものばかりであった．しかし，ConA，放射線，PHA 及び幾つかの化学変異剤は Sachs の言う分化誘導物質類[19]に含まれていることは注目に値する．ER を誘導するために Ara-C を用いることは，ER 産生及び分化の機構を明らかにする上で有用であると思われる．

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