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Deoxycytidine reverses the suppression of nucleolar organizer regions' activity caused by BrdU

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Summary. In this article we report that 5′-bromodeoxyuridine (BrdU) significantly suppresses the nucleolar organizer regions (NORs) activity of Chinese hamster cells (both dipliod cells and cell line Wg3-h) ($P<0.001$). One of the most obvious characteristics of the suppression is a significant decrease in the total number of the Ag-NORs per cell rather than in a frequency variation of the associated Ag-NORs. The decrease in the Ag-NORs number is mainly because of the decrease in number of chromosomes bearing 2 Ag-NORs. The degree of the suppression increases with increase in BrdU concentration in the culture medium. There is a close relationship between the suppression and the BrdU-treatment time, i.e. for a given concentration of the BrdU, the longer the BrdU-treatment time, the stronger the suppression. When the BrdU-treated cells are transferred into BrdU-free medium and allowed to grow in it for another 30 h, NORs activity can be restored. Therefore, the suppression of NORs activity may be due to BrdU toxicity. When deoxycytidine (dC) is added into medium containing 30 μg/ml of BrdU, the total number of both the Ag-NORs and the chromosomes bearing Ag-NORs per cell increases to the level of untreated cells. Our results thus indicate that the addition of dC reverses the suppression of the NORs activity caused by BrdU.

Key words: Somatic cell genetics – NORs activity – BrdU inhibition – dC reversion – Nucleolar organizer – Chinese hamster cells

Introduction

NORs are areas within a cell where rDNA transcription takes place. In situ DNA/RNA hybridization studies (Gall et al. 1969; Hsu et al. 1975) have shown that highly radioactive rRNA can be used as a probe in locating rDNA on the NORs. Silver-stained NORs (Ag-NORs) are believed to be sites of genes coding for 18s and 28s ribosomal RNA (rRNA) on the chromosomes (Goodpasture and Bloom 1975; Tantravahi et al. 1976; Stocker 1978). Ag-NORs number and location on the chromosomes are more or less constant within a given karyotype (Hsu et al. 1975; Yosida 1979). It has been shown in somatic hybrid cells (Miller 1976; Miller et al. 1976; Yan et al. 1983) that transcriptional activity of some rRNA genes of parental cell was suppressed, and thus the Ag-NORs reflects, in fact, the functionally active rRNA gene. Both Ag-NORs number and the frequency of the Ag-NORs association could be used to test the NORs activity (Hsu et al. 1975; Goodpasture and Bloom 1975; Jhanwar et al. 1981). It has been indicated in previous papers that the NORs activity could change in some cases, e.g. in the somatic hybrid cells mentioned above, spermatogenesis (Hofgärtner et al. 1979), some diseases (Rogers et al. 1975; Trent et al. 1981), and in interphase nucleus treated with a RNA inhibitor, actinomycin D (Hofgärtner et al. 1979).

As well known, BrdU is a very important efficient drug for either the mutation or the assay of sister chromatid exchanges. It has been demonstrated that BrdU can affect many cellular functions (see Discussion). It is the aim of the present paper to examine by means of silver-staining the influence of BrdU on the NORs activity of Chinese hamster cells, and the dC reversion of the suppression caused by BrdU.

Materials and methods

Cell source

1 Diploid cells. Some small tissue pieces from both the ears and tails of three healthy Chinese hamster were trypsinized

and cultured in Eagle essential medium (MEM) containing 15% new born calf serum at 37 °C for 3–5 generations.

2 Cell line. Chinese hamster cell line Wg3h is used in this experiment. These cells grew well in the MEM containing 15% new born calf serum at 37 °C.

Treatment of the cells and chromosome preparation

Twenty-four hours after inoculation the cells are transferred into MEM containing BrdU (Sigma) in concentrations of 9, 15, 24 and 30 μg/ml respectively for the Wg3h cells, and 30 μg/ml for the diploid cells. Wg3h cells were grown in the medium under dark conditions for 12, 24, 48 and 120 h; the diploid cells for 48 h. Two-three hours prior to cell harvest, colcemid (Sigma) was added into the medium to obtain a final concentration of 0.02 μg/ml. The preparation of the chromosomes was carried out according to routine air-dry method. Before the cells were fixed, all procedures were performed under a safe-light in a dark room.

In some cases Wg3h cells subcultured for 48 h in the MEM containing 30 μg/ml of BrdU were rinsed of the BrdU, and grown in BrdU-free medium for another 29 h before cell harvest.

In order to examine whether or not dC can reverse the suppression of NORs activity caused by BrdU, Wg3h cells were subcultured for 48 h in MEM containing 30 μg/ml of BrdU and the cells were then transferred into MEM containing both BrdU (30 μg/ml) and dC (20 μg/ml). These were then allowed to grow another 72 h in a dark room. Subsequent treatment is same as above described.

Silver staining

Six to ten day-old slides were covered with clean coverslips and incubated in 50% $AgNO_3$, in a moisture chamber either at 37 °C for 26 h or at 58 °C for 6 h. When the cells obtained a brown colour, the slides were washed with distilled water. Sometimes the slides were stained with 1 : 30 Giemsa for 1–3 min. The number of chromosomes bearing 1 Ag-NORs and 2 Ag-NORs were recorded, respectively, for each cell examined.

Results

Influence of BrdU on the NORs activity of the diploid cells

As shown in Table 1, the number per cell of both Ag-NORs and chromosomes bearing Ag-NORs in BrdU-treated cells is significantly lower than that of their control group ($P < 0.001$). The number of chromosomes bearing 2 Ag-NORs in the BrdU-treated groups decreases while the number of chromosomes bearing 1 Ag-NORs increases in comparison with the control groups. The number of chromosomes bearing Ag-NORs also decreases in the dipliod cells. In both the treated and control groups, there are very few cells (one or two cells among 50 cells examined for each group) with one associated Ag-NORs (Fig. 1).

Relationship between BrdU concentration and NORs activity

When the concentration of BrdU is 9 μg/ml or higher, the number of both Ag-NORs and chromosomes bearing 2 Ag-NORs per cell significantly decreases ($P < 0.001$) (Table 2) while the number of chromosomes bearing 1 Ag-NORs increases. When the BrdU concentration increases to 30 μg/ml (Table 3, group 3), the number of Ag-NORs and chromosomes bearing 2 Ag-NORs per cell is only 80% and 58%, respectively, of the control group (Table 2, group 1). For Wg3h cells, the number of chromosomes bearing Ag-NORs does not change, i.e. between 10.08 and 10.74 per cell (Fig. 2).

When the BrdU-treated Wg3h cells are rinsed of BrdU, and grown in BrdU-free medium, the number of either Ag-NORs or chromosomes bearing 2 Ag-NORs

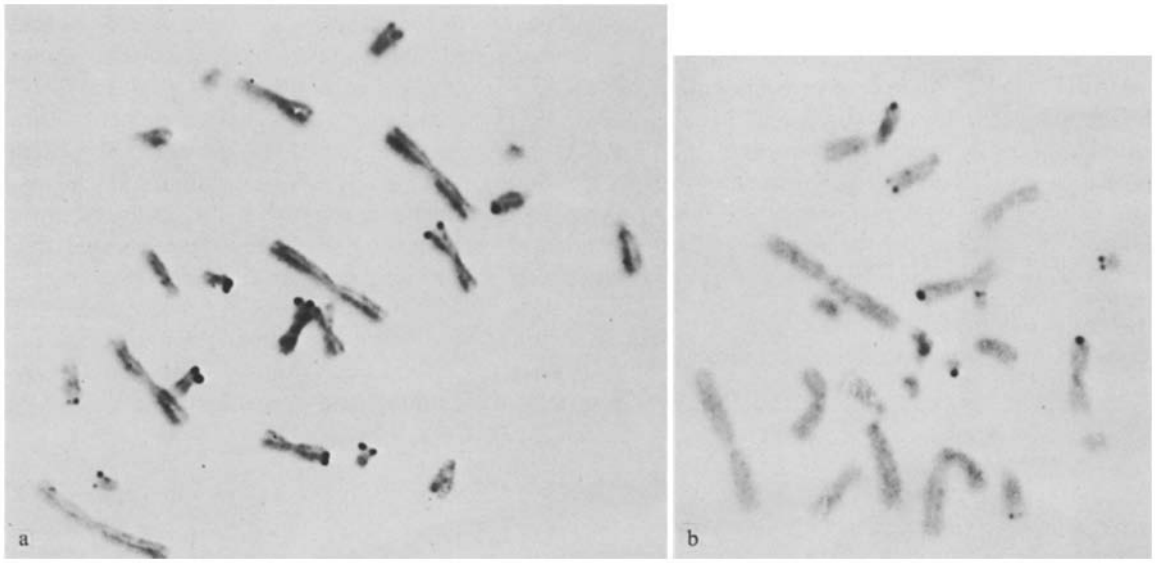

Fig. 1 a, b. Ag-NORs patterns of Chinese hamster diploid cells: **a** untreated cell; **b** BrdU-treated cell

Table 1. Influence of BrdU on the Ag-NORs of Chinese hamster diploid cells

No. of animals	Concentration of BrdU (μg/ml)	No. of cells examined	No. of chromosomes bearing Ag-NORs per cell (mean ± SE)			No. of Ag-NORs per cell (mean ± SE)
			Total	1 Ag-NORs	2 Ag-NORs	
No. 1	0	53	8.42 ± 0.26	1.49 ± 0.19	6.96 ± 0.40	15.33 ± 0.59
	30	50	7.52 ± 0.53	3.00 ± 0.48	4.48 ± 0.56	11.96 ± 0.90
No. 2	0	52	9.50 ± 0.34	1.85 ± 0.30	7.71 ± 0.50	16.98 ± 0.82
	30	50	8.32 ± 0.48	6.00 ± 0.61	2.37 ± 0.58	10.64 ± 0.87
No. 3	0	42	8.79 ± 0.25	1.26 ± 0.27	7.52 ± 0.49	16.36 ± 0.64
	30	30	7.23 ± 0.58	3.60 ± 0.71	3.63 ± 0.71	10.87 ± 1.10
Mean of No. 1, 2 and 3	0	147	8.90 ± 0.16	1.54 ± 0.15	7.40 ± 0.27	16.22 ± 0.39
	30	130	7.69 ± 0.30	4.20 ± 0.32	3.49 ± 0.36	11.16 ± 0.54

Table 2. Influence of different concentrations of BrdU on the Ag-NORs of Wg3H cells[a]

No. of groups	Concentration of BrdU (μg/ml)	No. of cells examined	No. of chromosomes bearing Ag-NORs per cell (mean ± SE)			No. of Ag-NORs per cell (mean ± SE)
			Total	1 Ag-NORs	2 Ag-NORs	
1	0	52	10.42 ± 0.57	2.12 ± 0.19	8.18 ± 0.40	18.53 ± 0.94
2	9	50	10.74 ± 0.44	3.86 ± 0.33	6.94 ± 0.42	17.58 ± 0.83
3	15	50	10.20 ± 0.50	5.04 ± 0.33	5.18 ± 0.59	15.40 ± 1.00
4	24	51	10.73 ± 0.50	5.26 ± 0.59	5.47 ± 0.59	16.22 ± 0.99
5	0[b]	50	10.12 ± 0.41	4.10 ± 0.33	6.02 ± 0.47	16.12 ± 0.77

[a] The cells were grown in MEM containing BrdU for 48 h before harvest
[b] After growing in the medium containing 30 μg/ml of BrdU for 48 h, the cells are rinsed of the BrdU and grown in the BrdU-free medium for another 29 h prior to harvest

Table 3. Effects of treatment time of dC and BrdU on the Ag-NORs of Wg3H cells

No. of groups	Treatment time (h)		No. of cells examined	No. of chromosomes bearing Ag-NORs per cell (mean ± SE)			No. of Ag-NORs per cell (mean ± SE)
	With BrdU (30 μg/ml)	With a mixture of dC and BrdU[a]		Total	1 Ag-NORs	2 Ag-NORs	
1	12	0	50	10.96 ± 0.45	3.12 ± 0.28	7.64 ± 0.63	18.74 ± 0.97
2	24	0	50	10.20 ± 0.44	4.20 ± 0.34	6.02 ± 0.51	16.26 ± 0.85
3	48	0	50	10.08 ± 0.71	5.40 ± 0.42	4.74 ± 0.51	14.86 ± 1.09
4	120	0	51	8.47 ± 0.66	3.24 ± 0.43	5.22 ± 0.60	13.70 ± 1.20
5	48	72	50	10.64 ± 0.48	3.10 ± 0.43	7.54 ± 0.58	18.18 ± 0.97

[a] The cells grow in a medium containing both 20 μg/ml of dC and 30 μg/ml of BrdU

significantly increases ($P < 0.001$) (Table 2, group 5 and Table 3, group 3). It is obvious that the activity of the NORs in the BrdU-treated cells can be restored after rinsing away the BrdU.

As in the dipliod cells, there are one or two cells with one Ag-NORs association among the 50 cells examined in each group of Wg3h cells.

Correspondance between BrdU-treatment time and NORs activity

It is shown in Table 3 that if the BrdU-treatment time only is 12 h, namely one cell cycle, the number of either Ag-NORs or chromosomes bearing 2 Ag-NORs per cell does not obviously differ from that of the control group

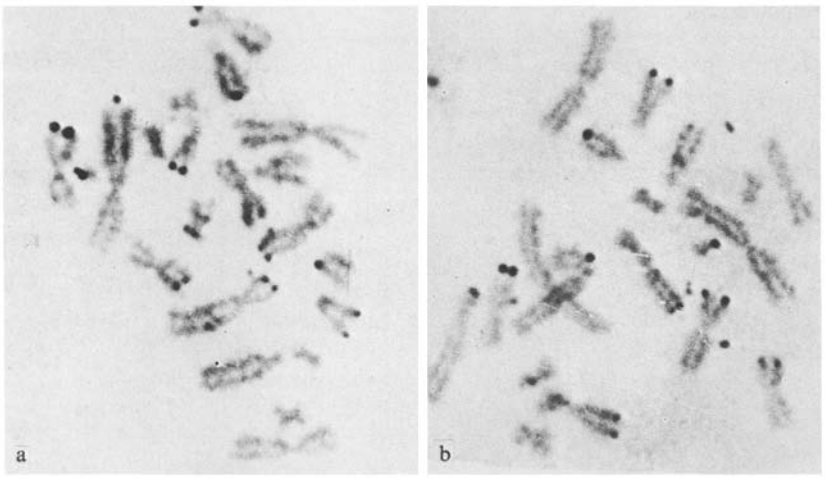

Fig. 2a, b. Ag-NORs pattern of Wg3h cells: **a** cell treated with BrdU (30 μg/ml) for 5 days; **b** both BrdU und dC-treated cell (see methods)

(Table 2, group 1). However, if the BrdU-treatment time is 24 h or more, the number of the Ag-NORs per cell decreases with the increase in treatment time, and the total number of chromosomes bearing Ag-NORs does not significantly decrease until the BrdU-treatment time increases to 120 h. For each of these groups, only one or two cells with one Ag-NORs association could be found.

Effects of dC on Ag-NORs

As mentioned above, if the BrdU-treatment time increases to 120 h, the number per cell of both Ag-NORs and chromosomes bearing Ag-NORs significantly decreases. However, if dC is then added into the medium containing 30 μg/ml of BrdU (Table 3, group 5) for another 72 h (i.e. for this group, the total BrdU-treatment time in also 120 h), the number per cell of both Ag-NOrs and chromosomes bearing Ag-NORs significantly increases in comparison with that in group 4 (Table 3) and rises to the level of untreated cells (Table 2, group 1).

Discussion

BrdU is a analogue of thymidine. During DNA replication, BrdU can substitute for thymidine in the replicating DNA. It is thus important to study the influence of BrdU on gene expression. There have been many papers published on this subject during recent years: for instance, BrdU can suppress cell differentiated functions (Rogers et al. 1975; Wrathall et al. 1975), interfere with DNA repair (Rommelaere et al. 1974), inhibit activity of ribonucleotide reductase (Meuth et al. 1974), affect the binding of regulatory proteins to DNA (Lin and Riggs 1972), induce mutation (Litman and Parduee 1956). Our data in present paper indicate that BrdU obviously suppresses the NORs activity of Chinese hamster cells. In other words, the expression of the genes coding for 18s and 28s rRNA can be significantly suppressed by adding BrdU. An obvious characteristic of the suppression is the significant decrease in Ag-NORs number per cell because the number of the chromosomes bearing 2 Ag-NORs decreases. When the BrdU (30 μg/ml)-treatment time increases to 120 h, the number of chromosomes bearing Ag-NORs also decreases. If the BrdU-treatment time is only one cell cycle, BrdU suppression does not occur even though the concentration of BrdU is as high as 30 μg/ml. When the BrdU-treatment time increases to 48 h, the lower concentration of BrdU (15 μg/ml) also obviously causes a decrease in Ag-NORs number ($P < 0.001$). In all these cases, the decrease in number of chromosomes bearing 2 Ag-NORs accompanies the increase in number of chromosomes bearing 1 Ag-NORs. It implys that under BrdU effects, one of the sister chromatids either lacks an adequate number of gene copies, leading to minute amounts of stainable materials which might be difficult to detect in the silver staining technique, or that genes, though present, fail to express. Because the frequencies of Ag-NORs association in both the treated and control groups are extremely low, there is no obvious difference between the two groups-BrdU does not seem to influence the Ag-NORs association.

Since the BrdU-treated cells growing in the BrdU-free medium can restore NORs activity, the suppression of the NORs activity might be due to BrdU toxicity. The toxicity increases with increase in either BrdU-treatment time or BrdU concentration in the medium.

Our results demonstrate that dC reverses the suppression of the NORs activity caused by BrdU, in which the increase in number of chromosomes bearing

2 Ag-NORs results in an increase in number of the chromosomes bearing Ag-NORs, leading to an increase in the Ag-NORs number per cell. The mechanism by which the BrdU suppresses the NORs activity remains to be further investigated.

References

Goodpasture C, Bloom SE (1975) Visualization of nucleolar organizer regions in mammalian chromosomes using silver staining. Chromosoma 53:37–50

Gall JG, Pardue ML (1969) Formation and detection of RNA-DNA hybrid molecules in cytological preparation. Proc Natl Acad Sci USA 63:378–383

Hofgärtner JF, Schmid M, Krone W, Zezes MT (1979) Pattern of activity of nucleolus organizer during spermatogenesis in mammals as analyzed by silver-staining. Chromosoma 71:197–216

Hofgärtner FJ, Krone W, Jain K (1979) Correlated inhibition of ribosomal RNA synthesis and silver staining by actinomycin D. Hum Genet 47:329–333

Hsu TC, Spirito SE, Pardue ML (1975) Distribution of 18s+28s ribosomal genes in mammalian genomes. Chromosoma 53:25–36

Jhanwar SC, Prensky W, Chagnt RSK (1981) Localization and metabolic activity of ribosomal genes in Chinese hamster meiotic and mitotic chromosomes. Cytogenet Cell Genet 30:39–46

Lin S, Riggs A (1972) Lac operator analgues: bromodeoxyuridine substitution in the Lac operator affects the rats of dissociation of the Lac represor. Proc Natl Acad Sci USA 69:2574–2576

Litman R, Parduee A (1956) Production of bacteriophage mutants by a disturbance of deoxyribonucleic acid metabolism. Nature 178:529–531

Meuth M, Gree H (1972) Induction of a deoxycytidineless state in cultured mammalian cells by bromodeoxyuridine. Cell 2:109–112

Miller OJ, Miller DA, Dev VG, Tantravahi R, Croce CM (1976) Expression of human and suppression of mouse nucleolus organizer activity in mouse-human somatic cell hybrids. Proc Natl Acad Sci USA 73:4531–4535

Miller DA (1976) Suppression of human nucleolus organizer activity in mouse-human somatic hybrid cells. Exp Cell Res 101:235–243

Rommelaere J (1974) The influence of BrdU on DNA repair in Chinese hamster cells exposure to UV radiation. Biochim Biophys Acta 340:388–398

Rogers J, Ng SKC, Coulter MB, Sanwal BD (1975) Inhibition of myogenesis in a rat myoblast line by 5-bromodeoxyuridine. Nature 256:438–439

Stocker AJ (1978) Correspondence of silver banding with rRNA hybridization sites in polyten chromosomes of Rhyn chosciara hollaender. Exp Cell Res 114:429–434

Silagi S, Bruce SA (1970) Suppression of malignancy and differentiation in melanotic melanoma cells. Proc Natl Acad Sci USA 66:72–78

Tantravahi R, Miller DA, Dev VG, Miller OJ (1976) Detection of nucleolus organizer regions in chromosomes of human, chimpanzee, gorilla, orangutan and gibbon. Chromosoma 56:15–27

Trent JM, Carlin DA, Davis JR (1981) Expression of silver-staining Ag-NORs in human cancer. Cytogenet Cell Genet 30:31–38

Wrathall JR, Oliver C, Silagi S, Essner E (1973) Suppression of pigmentation in mouse melanoma cells by 5-bromodeoxyuridine. J Cell Biol 57:406–423

Yosida TH (1979) A comparative study on NORs in 7 rattus species with special emphasis on organizer differentiation and species evolution. Proc Jpn Acad 55:481–489

Yan Yongshan, Qian Jin, Xi Xihui (1983) Suppression of nucleolar organizer regions activity of Chinese hamster cells in mouse-hamster somatic cell hybrids. Acta Genet Sin 10:35–39