Theor Appl Genet (1987) 74:247–252



# Sister chromatid exchange in vivo, chromosomal characterization and NORs activity of leukemia cells during 5FU-treatments

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Received November 9, 1986; Accepted January 30, 1987
Communicated by Hu Han

**Summary.** A transplantable mouse leukemia model, the leukemia cell of which has a marker chromosome and the XX genome type which differ obviously from their male host cells provides a possibility to precisely identify the leukemia cells among their male host cells cytogenetically. A sister chromatid exchange (SCE) plus chromosomal C-banding technique that we report here is very useful. The SCE frequencies in vivo of both leukemia cells and host cells were twice as high as the normal mouse cells. The higher SCE frequencies of the host cells in the leukemia mice may be due to some toxicities from the leukemia cells or some biological large molecule exchanges between the leukemia cells and the host cells. There was no significant difference in SCE frequencies between cells from the spleen and from the bone marrow of the leukemia mice. The percentages of leukemia cells in both spleen and bone marrow were more than 90% when the mice had been injected with the leukemia cells for five days. The host cells in the leukemia mice did not become leukemia cells. The 5FU-treated leukemia mice survived very well for more than twenty-three days. After the 5FU-treatments, most of the leukemia cells died, subsequently, SCE frequencies decreased to a normal level. Both the number of Ag-NORs per cell and the number of chromosomes bearing Ag-NORs per cell in the leukemia mice decreased to 60% and 40%, respectively, of the level found in normal mouse cells.

**Key words:** Leukemia cells – Marker chromosome – SCE in vivo plus C-banding – Anticancer drug treatments – Decreased NORs activities

## Introduction

It has been suggested that sister chromatid exchange (SCE) may be involved in DNA repair processes (Latt et al. 1975; Kato 1975; Solomon et al. 1975). SCE frequency could indicate diminished DNA repair capabilities. This technique is more sensitive than measurement of chromosome aberrations in detecting DNA damage induced by a wide variety of chemical compounds (Perry et al. 1975).

Since culture medium components, when exposed to fluorescent light, produce photo-products which may induce SCE (Monticone et al. 1979) and as the sera in the medium may also induce SCE (Kato et al. 1976), in vivo SCE analysis is preferable. In addition, in vivo SCE analysis accounts for host mediation of the drug and thereby affords several advantages for mutagenesis trials. Multiple tissue analysis permits assessment of a tissue-specific level of risk for genetic damage. Physiological factors that might influence the distribution of genetically active substances among different tissues include DNA-repair capabilities and metabolic reactivity. The in vivo SCE analysis is complementary to in vitro trials and often provides information which is otherwise unobtainable. For example, in contrast to findings in vitro, the intercalating substances proflavine, methylene blue and chlorpromazine did not induce SCE in vivo (Speit 1982). It is clear that the use of intact animals provides a highly relevant test system which takes into account host metabolism and possible activation of the drugs, especially for detecting mutagenic carcinogens and for investigating neoplasia after therapeutic drug administration, or certain hereditary diseases that predispose to neoplasia.

In situ DNA/RNA hybridization studies (Hsu et al. 1975; Gall et al. 1969) have shown that silver-stained nucleolar organizer regions (Ag-NORs) are believed to be the sites of genes coding for 18s and 28s rRNA on chromosomes. The Ag-NORs number could be used to test the NORs activity (Goodpasture et al. 1975; Jhanwar et al. 1981). It is therefore of interest to investigate the NORs activity in cancer cells growing in their host.

In the present paper we report a transplantable mouse leukemia model in which all the leukemia cells belong to the XX genome type with a marker chromosome which differs from their male host cells. This model provides a possibility to investigate both leukemia cells and host cells cytogenetically. The SCE frequencies

in vivo during 5FU-treatments, using SCE plus the chromosomal C-banding technique, and the NORs activity of the leukemia cells, are reported here.

## Material and methods

*Leukemia cell proliferation*

The leukemia model was provided by the Institute of Materia Medica, Chinese Academy of Medical Sciences. The leukemia cells could proliferate in syngenetic inbred mouse strain no. 615 by the injection of a spleen cell suspension of the leukemia mice. All mice die of leukemia approximatively six days after injection of the leukemia cells. In the present experiment only male mice were used for the leukemia cell proliferation except for the female groups (see Table 1). Briefly, the spleen of the leukemia mice was removed on the fifth day after injection of the leukemia cells ond one-third of it was suspended in 10 ml of PBS. About 0.2 ml of the cell suspension were injected into the peritoneal cavity of healthy male mice. For the female groups, 0.2 ml of the cell suspension from male leukemia mice were injected into healthy female mice intraperitoneally. On the third and fifth day after injection, most of the leukemia mice were killed (spinal dislocation) for chromosomal analysis. At least 5 mice were used from each group.

*Anticancer drug treatments*

5FU (Shanghai, China) dissolved in PBS to a final concentration of 2 mg/ml was injected intraperitoneally into the mice with a dosage of 30 mg 5FU/kg of body weight soon after the injection of the leukemia cells. The mice were repeatedly injected with the 5FU solution in the same dosage for seven days (once a day). If the mice had been kept alive for another sixteen days, they were sacrificed for chromosome preparation; some mice were killed on the fifth day after 5FU-injection for chromosome analysis.

*Chromosomal analysis*

For the chromosome preparation each of the mice was injected intraperitoneally with 2 μg colcemide. Three hours later the bone marrow and spleen cells were suspended in PBS and incubated in a 0.075 M KCl solution at room temperature for 20 min. Finally, the cells were treated 3 times in 3 : 1 methanol/glacial acetic acid. The chromosome preparation was performed according to the air-dry method.

Chromosomal G- and C-banding were carried out according to the method described in our previous paper (Yan Yongshan et al. 1987).

Silver-staining was performed as described in a previous report (Yan Yongshan et al. 1985).

*Treatments for SCE in vivo analysis*

Each of the mice was injected with 9 mg BrdU (Sigma) intraperitoneally every 2 h for 25 h. Colcemide was injected three hours before the mice were killed. The bone marrow cells and spleen cells were suspended in PBS, incubated in 0.075 M KCl solution and fixed in the 3 : 1 fixative solution for the chromosome preparation. The procedure was performed under a safelight before cell fixation.

The SCE treatments and analysis were carried out as described by Yan Yongshan et al. 1987. Since all leukemia cells belong to the XX genome type, i.e., each cell contains the two smallest chromosomes (no. 19) and the male host cell contains the three smallest chromosomes (two chromosomes no. 19 and the Y chromosome), the SCE frequencies of the leukemia cells and the host cells were scored, respectively.

To test our observation, some SCE slides were photographed under the microscope and then underwent the chromosomal C-banding treatment.

## Results

*Cytogenetic characterization of the leukemia cells*

As shown in Table 1, in both the bone marrow and spleen of the male leukemia mice which had been injected with the leukemia cells for five days more than 90% of the cells belonged to the XX genome type and less than 10% were the male host cells. In the bone marrow of the male leukemia mice which had been injected with the leukemia cells for three days, however, only 16% were female cells while the remaining 84% were male host cells. The percentage of female cells in their male host increased with time after leukemia cell injection. In the female groups there were no male cells except one among two hundred cells examined. As mentioned above, the leukemia mice die of the leukemia by the sixth day after leukemia cell injection. On the fifth day after the injection the weight of a spleen of the leukemia mice was about 0.36 g, but it was 0.033 g for a normal spleen of the mice, i.e., ten times higher than the latter (Fig. 3). It is clear that the female cells in the male host leukemia mice should be leukemia cells.

Chromosomal C-banding analysis indicated that all leukemia cells had a marker chromosome, i.e., on the long arm end of one of the chromosome no. 19 there was a dark stained C-banding. The marker chromosome was named the Bj chromosome. In addition, one of chromosome no. 4 of the leukemia cells had a large secondary constriction (Fig. 1).

There was no aberration in the chromosomal G-banding pattern of the leukemia cells except that at the long arm end of the Bj chromosome there was a clear dark G-banding (Fig. 2).

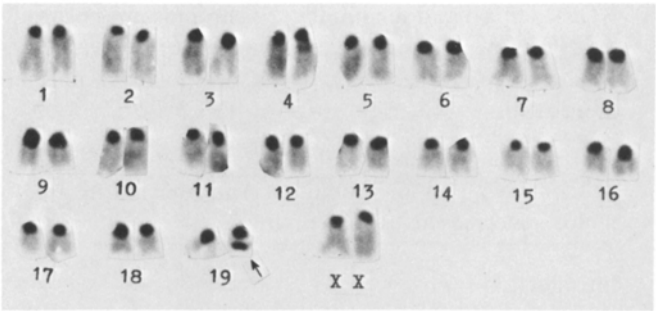


**Fig. 1.** Chromosome C-banding of the leukemia cells. *Arrow* indicates the Bj chromosome

**Table 1.** Percentage of the leukemia cells and their host cells in leukemia mice

| Item | Time after leukemia cell injection (days) | Tissue type | Sex of animals | No. and % of the cells | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Total | Host cells (XY genome type) | | Leukemia cells (XX genome type) | | | |
| | | | | | | | With Bj chromosome | | Without Bj chromosome | |
| | | | | | No. | % | No. | % | No. | % |
| Leukemia mice | 5 | Bone marrow | Male | 52 | 4 | 7.7 | 48 | 92.3 | 0 | 0 |
| | | | Male | 56 | 6 | 10.7 | 50 | 89.3 | 0 | 0 |
| | | | Average | 108 | 10 | 9.2 | 98 | 90.8 | 0 | 0 |
| Leukemia mice | 3 | Bone marrow | Male | 50 | 42 | 84.0 | 8 | 16.0 | 0 | 0 |
| Leukemia mice | 5 | Spleen | Male | 109 | 7 | 6.4 | 102 | 93.1 | 0 | 0 |
| | | | Male | 54 | 8 | 14.8 | 46 | 85.2 | 0 | 0 |
| | | | Average | 163 | 15 | 9.2 | 148 | 90.8 | 0 | 0 |
| Leukemia mice | 5 | Spleen | Female | 104 | 1 | 0.9 | 97 | 93.3 | 6 | 5.8 |
| | | | Female | 104 | 0 | 0 | 104 | 100 | 0 | 0 |
| | | | Average | 208 | 1 | 0.5 | 201 | 96.7 | 6 | 2.9 |
| 5FU-treated leukemia mice | 5 | Bone marrow | Male | 46 | 46 | 100 | 0 | 0 | 0 | 0 |

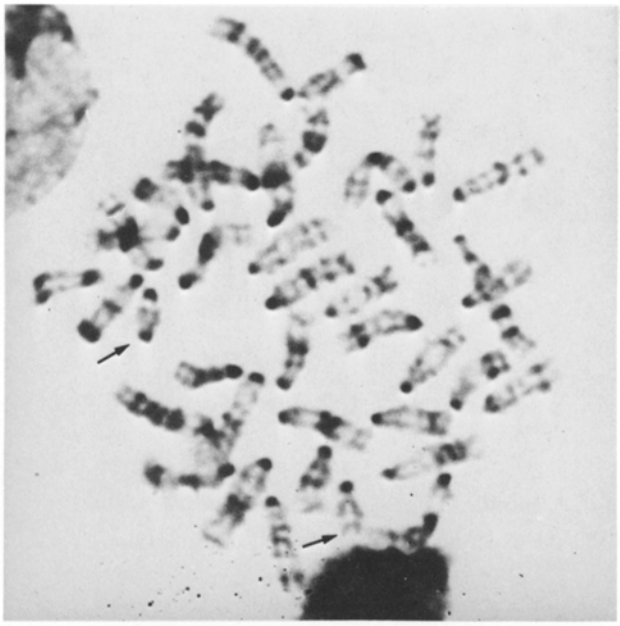

**Fig. 2.** Chromosome G-banding of the leukemia cells. *Arrows* indicate the Bj chromosome (*bottom*) and chromosome no. 19

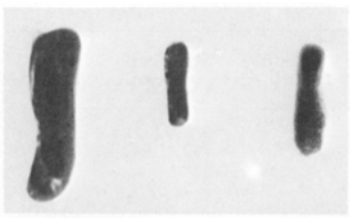

**Fig. 3.** Comparison of spleen size among leukemia mice (*left*), normal mice (*middle*) and 5FU-treated leukemia mice which had lived for 23 days (*right*)

**Table 2.** Comparison of SCE frequencies in vivo between leukemia mice and normal mice[a]

| Item | Tissue examined | No. of groups | No. of cells | SCE/cell (X±SE) |
|---|---|---|---|---|
| Normal mice | Bone marrow | 1 | 54 | 3.65±0.22 |
| | | 2 | 42 | 3.74±0.26 |
| | | Average | 96 | 3.70±0.24 |
| Leukemia mice | Bone marrow | 3 | 78 | 8.39±0.32 |
| | | 4 | 42 | 7.74±0.30 |
| | | Average | 120 | 8.07±0.31 |
| Leukemia mice | Spleen | 5 | 61 | 9.28±0.43 |
| | | 6 | 63 | 8.21±0.36 |
| | | 7 | 52 | 8.50±0.39 |
| | | Average | 176 | 8.66±0.39 |
| Leukemia mice treated with 5FU | Bone marrow | 8 | 53 | 4.40±0.31 |

[a] All leukemia mice had been injected with the leukemia cells for five days

*Analysis of SCE frequencies in vivo*

As shown in Figs. 4–5, very clear SCE patterns were obtained under our experimental conditions. It can be shown in Table 2 that the SCE frequencies in vivo in the bone marrow and spleen were 8.07±0.31 and 8.66±0.39, respectively. There was no significant difference in SCE frequencies between the bone marrow and spleen in the leukemia mice ($P > 0.05$). However, in the bone marrow of the normal mice it was 3.70±0.24. It was obvious that the SCE frequencies in vivo of the leu-

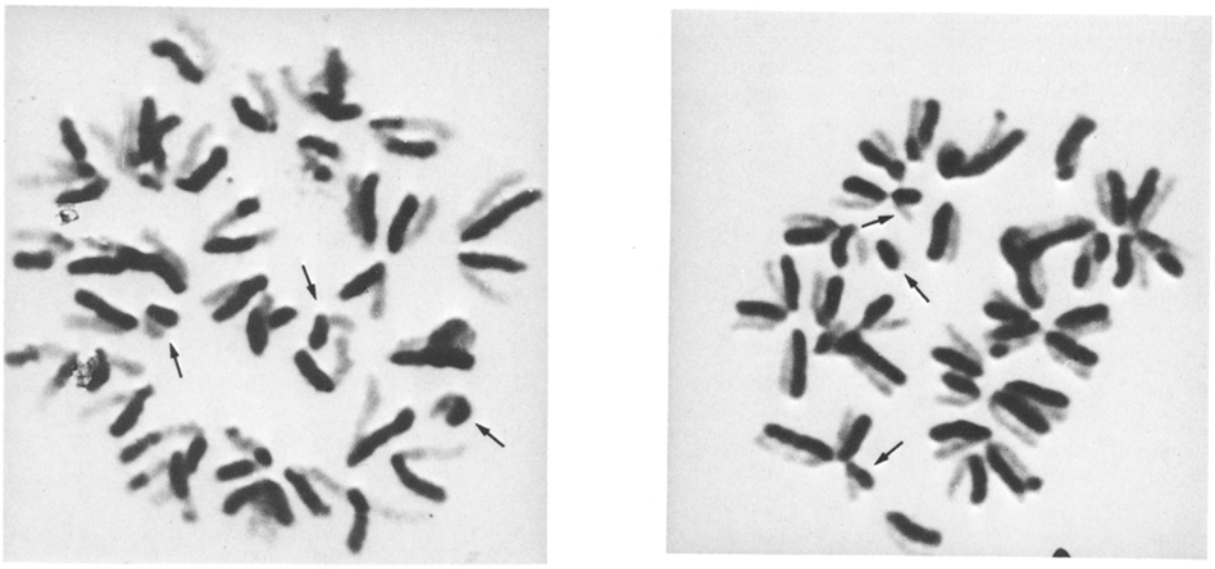

**Fig. 4.** SCE patterns of host cells in the leukemia mice with 5FU-treatment (*right*) and without 5FU-treatment (*left*). *Arrows* indicate the three smallest chromosomes

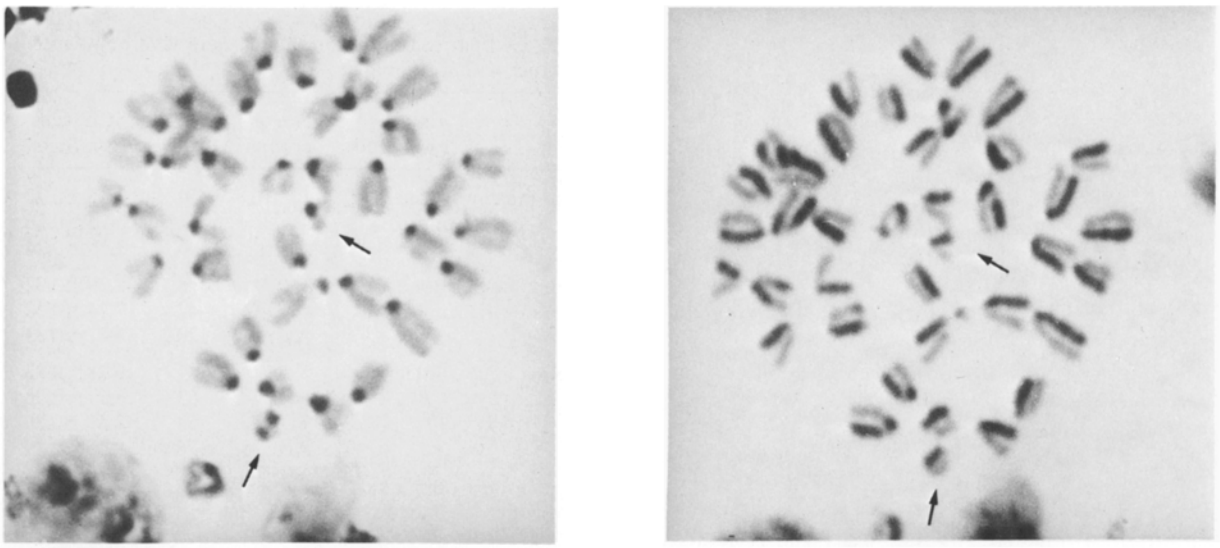

**Fig. 5.** SCE and C-banding patterns of the leukemia cell. *Arrows* indicate the Bj chromosome and chromosome no. 19

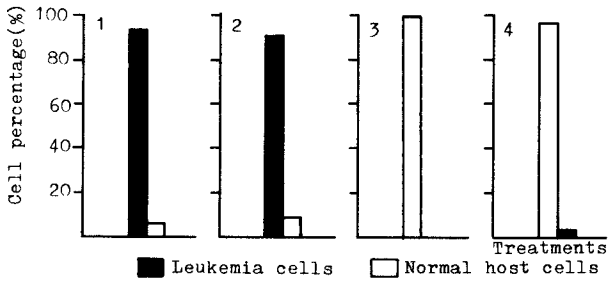


**Fig. 6.** Proportion of leukemia cells and their host cells in leukemia mice five days after leukemia cell injection: *1* spleen cells; *2* bone marrow cells; *3* bone marrow cells of leukemia mice treated with 5FU for five days; *4* bone marrow cells of 5FU-treated leukemia mice which had lived 23 days

**Table 3.** SCE frequencies in vivo of leukemia cells and normal host cells of same leukemia mice

| Cell type | Tissue examined | No. of cells | SCE/cell (X±SE) | *P* |
|---|---|---|---|---|
| Host cells (XY genome type) | Bone marrow | 31 | 8.81±0.52 | |
| Leukemia cells (XX genome type) | Bone marrow | 47 | 8.10±0.46 | > 0.05 |

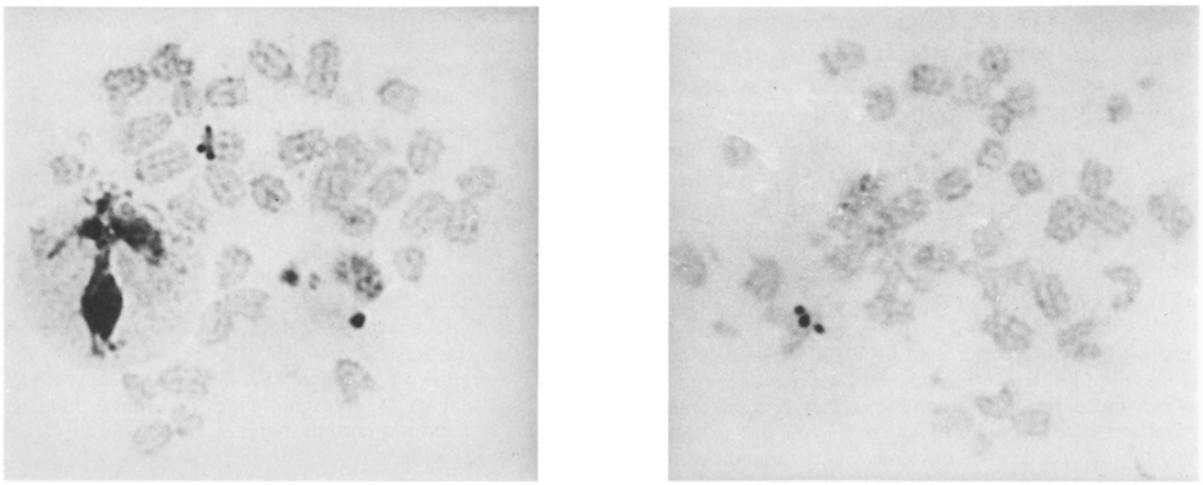

**Fig. 7.** Ag-NORs distribution of leukemia cells

kemia mice were significantly higher than those found in the normal mice ($P < 0.001$).

Our SCE plus chromosomal C-banding technique evidenced that the cells which contained only two of the smallest chromosomes were the leukemia cells (Fig. 5).

We also can observe in Table 3 that in the same leukemia mice, the SCE frequency of the leukemia cells was $8.10 \pm 0.46$ while that of the normal host cells was $8.81 \pm 0.52$. There was no significant difference between them ($P > 0.05$).

The SCE frequency of the 5FU-treated leukemia mice reduced to $4.40 \pm 0.31$ (Table 2). That did not significantly differ from that of the normal mice ($P > 0.05$). The SCE patterns of the host cells in the leukemia mice with and without the 5FU-treatments are shown in Fig. 4.

*NORs activity of leukemia cells and normal mouse cells*

The Ag-NORs number per leukemia cell was $2.96 \pm 0.16$ in which about 60% of the Ag-NORs were located on one of chromosome no. 4. This chromosome bore 2 Ag-NORs but often bore three Ag-NORs, one of which was located on it's centromere and another two on the large secondary constriction (Fig. 7). The number of the chromosomes bearing Ag-NORs per leukemia cell was $1.83 \pm 0.11$. However, the number of Ag-NORs per cell and the number of chromosomes bearing Ag-NORs per cell for the normal mouse cells were $4.93 \pm 0.35$ and $4.13 \pm 0.29$, respectively: both were higher than the leukemia mice ($P < 0.001$).

## Discussion

SCE frequencies in different types of neoplasias have been observed. SCE frequencies prior to therapy are slightly elevated in patients with lymphoma (Otter et al. 1979) and depressed in patients with acute myelogenous leukemia (Abe et al. 1979). When patients go into remission SCE frequencies in both instances return to normal levels. However, generally speaking, the tumor cells had to be cultured in the presence of BrdU in vitro. It has been indicated that SCE frequencies in vivo could quite different from SCE frequencies found in vitro (Speit 1982; Monticone et al. 1979; Kato et al. 1976; Ghosh et al. 1979). If the tumor cells have no obvious marker in their SCE patterns, it is impossible to identify them once among the host cells. Since the leukemia cell in our model has only *two* of the smallest chromosomes and the host cell three, it is easy to identify the leukemia cells among their host cells. Our results have been confirmed by observations of the SCE plus chromosomal C-banding.

Our results indicate that in vivo SCE frequencies of leukemia mice were twice as high as those of the normal mice: this differs from the patients with acute myelogenous leukema (Abe et al. 1979) and in patients with lymphoma (Otter et al. 1979).

It is of interest that there was no significant difference in the SCE frequencies in vivo between the leukemia cells and the host cells in the leukemia mice. Since in the female leukemia mouse groups it is extremely difficult to find the male cell, it is implied that the male host cells did not become leukemia cells. When the leukemia mice were treated with 5FU and went into remission, the SCE frequencies decreased to a normal level. The chromosomal C-banding analysis showed that in the bone marrow of the 5FU-treated leukemia mice there was only 3.6% the leukemia cells, and 96.4% the host cells. Why were the SCE frequencies of the normal host cells in the leukemia mice twice as high as in the normal mouse cells and did they return to the normal level after the 5FU-treatments? One possible explanation is that the increase in SCE frequencies of the host cells in the leukemia mice might be due to effectors from the leukemia cells, e.g., some toxicities or exchanges of some large molecular between the leukemia cells and the host cells. When the leukemia cells were

killed by 5FU, the effective factors were so weak that they could not significantly increase the SCE frequencies of the host cells. Our results agree with the SCE frequencies found in Bloom's syndrome cells where SCE frequencies could be reduced by cocultivation with Chinese hamster cells (Van Buul et al. 1978). Such a metabolic cooperation has been demonstrated in other combinations (Hooper et al. 1981; Gilula et al. 1972; Azaria et al. 1974; Yan Yongshan 1986).

Kanda et al. (1979) reported that in vivo SCE in spleenic cells was higher than that found in bone marrow in normal mice – a result which differs from ours. This difference may be due to the material difference. For instance, we used leukemia mice in which the percentages of the leukemia cells in both bone marrow and spleen were more than 90%.

Because of irregularities in nucleoli found in malignant cells, a study of the cytological expression of ribosomal cistrons would be of great interest. Trent et al. (1981) indicated a proportionate increase in the number of Ag-NORs with increasing number of total chromosomes and an increasing number of acrocentric chromosomes in human cancers. Huffell et al. (1977) found that despite increases in NOR-bearing chromosomes, human cancer cells expressed the same number of Ag-NORs as diploid control cells. Miller et al. (1978) demonstrated that a proportionate increase in Ag-NORs was observed with increasing chromosome number. However, it was evidenced in our leukemia model that both the number of Ag-NORs per cell and number of the chromosomes bearing Ag-NORs per cell decreased significantly to 60% and 40% of the level found in normal mouse cells. In addition, about 60% of the Ag-NORs of the leukemia cells were located on one of chromosome no. 4. It seems that Ag-NORs distribution could be quite different for different malignant cells

The mechanism for the SCE formation still remains to be resolved. It is clear that in the SCE patterns of the leukemia cells, the pale stained region at the long arm end of the Bj chromosome could appear a dark C-banding after subsequent chromosomal C-banding treatment. It implied that most heterochromatin in this region of the Bj chromosome remained even though the differential methods involved light exposure and hot salt treatments.

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