Cytological Studies of Tumors. XLIV. Chromosomes of Friend Virus-induced Leukemias and Ascites Tumors of Mice[1)]

By

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(With 8 Text-figures)

Information on the chromosomal features in virus-induced tumor cells and virus-transformed cells *in vitro* has lately been accumulated, providing critical data on the significance of the chromsomal alteration in relation to malignant transformation and growth (Bayreuther and Thorell, 1959; Palmer, 1959; Bayreuther, 1960; Hellström *et al.* 1962, 1963; Pontén, 1963; Nichols, 1963; McMichael *et al.* 1963; Cooper and Black, 1963; Moorhead and Saksela, 1963; Tsuchida and Rich, 1964; and others). In the former papers, were presented preliminarily some chromosome conditions in Friend virus-induced mouse leukemias and two lines of Friend ascites tumors (Sofuni *et al.* 1967 a, b). Here I wish to present related data in some detail.

I wish to express sincere thanks to Professor Sajiro Makino for his direction and improvement of the mansucript. Thanks are due to Drs. Hiroshi Kobayashi, Nobuyuki Tanigaki and Masuo Hosokawa for preparing the material for this study with helpfnl advice.

Materials and Methods: 7 dd/0m mice were inoculated with Friend virus (Firned, 1957), and suffered a complex disease generally designated as "Friend disease" or "Friend leukemia", which was specially characterized by splenomegaly. Mice with this disease were injected at various intervals with a colchicine solution 2 hours prior to cervical dislocation, and their enlarged spleens supplied materials for chromosome studies. In addition, an enlarged liver from one mouse which received inoculation of Friend virus on the 49th and the 135th days after splenectomy and was killed on the 209th days after the 2nd virus-infection, was also used as material. Three non-infected mice provided control materials. Pieces of the spleen and liver tissues were minced in TC-199 tissue culture medium containing a 5% calf serum and treated with a hypotonic solution for 20 minutes.

A line of transplantable ascites tumor, established from Friend leukemic dd/0 m mice (Oboshi *et al.* 1964), has shown that Friend virus persists in this tumor during long-term successive transfers (Kobayashi, 1966). This line is designated as "virus producing Friend tumor" (VPFT). Another line of Friend tumor in which no virus has been detected

1) Contribution No. 790 from the Zoological Institute, Faculty of Science, Hokkaido University, Sapporo, Japan. Supported partly by a grant from the Damon Runyon Memorial Found for Cancer Research to S. Makino (DRG-889-AT) and partly by a grant from Ministry of Education for Co-operative Research (Cancer), No. 94033, 1966.

during 20 passages, was obtained from VPFT by serial transplantations of tumor cells, in a limiting dilution, previously neutralzied with Friend virus immune serum (Kobayashi *et al.* 1966). It is referred to as "non-virus producing Friend tumor" (NVPFT). These two lines of Friend ascites tumor were chromosomally studied here. The ascites tumors were drawn, incubated in culture medium containing colchicine for 2 hours at 37°C, and then treated with a hypotonic solution for 15 minutes. Chromosome slides were made according to the routine air-drying method.

Results

Friend leukemias: The modal number of chromosomes in cells of leukemic mice as well as of control animals showed 40, corresponding to the normal diploid number (Fig. 1). Morphologically, the karyotype of leukemic mcie showed no remarkable deviation from that of normal mice. It was noted, however, that the aneuploid cells slightly increased in leukemic mice (Fig. 1).

An increased incidence of cells having the chromosomes with secondary constrictions was observed to occur in leukemic mice: the average frequency was 37% in control cells, whereas 96% in leukemic cells (Figs. 1, 5). In addition, information

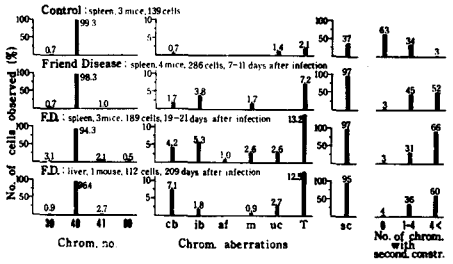


Fig. 1.

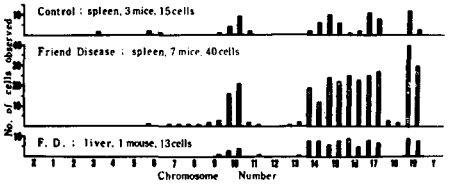


Fig. 2.

Fig. 1. Chromosome-number distribution, frequencies of chromosome aberrations, frequencies of cells having chromosomes with secondary constrictions and incidences of the number of chromosomes with secondary constrictions per cell in controls and Friend leukemias. cb: chromatid breaks or gaps, ib: isochromatid breaks or gaps, af: acentric fragments, m: minute chromsomes, uc: unusual chromosomes, T: total cells with chromosome aberrations, sc: cells having chromosome with secondary constrictions.

Fig. 2. Frequency in occurrence of the secondary constriction in chromsomes.

was obtained to show that the virus infection caused an increase in number of chromosomes with secondary constrictions per cell (Fig. 1). Karyotype analyses realized that the secondary constriction occurred in specific chromosome groups, most frequently in no. 10, nos. 14–17 and no. 19 chromsomes, and that cells from spleens and a liver of leukemic mice and those from spleens of control animals showed a similar tendency, except a low incidence observed in no. 16 chromosomes of the control series (Fig. 2).

An increase in the cells showing chromatid and isochromatid breaks or gaps was evident in leukemic mice (Fig. 1): the average frequency of cells with such aberrations was 7.5% in the leukemic mice, while 0.7% in controls (Figs. 1, 5).

Some unusual chromosomes, such as metacentric, submetacentric and minute chromosomes, were found to occur, though not frequently, in leukemic cells from mice killed 19 to 21 days after infection. The metacentric and submetacentric chromosomes were chracterized by secondary constrictions located near the centromere (Fig. 6). In the liver of a leukemic mouse, 3 cells were found to possess 41 telocentric chromosomes, while 3 cells with 40 chromosomes had one long telocentric element in each. In control specimens, two diploid cells each having one unusually long telocentric chromosome were found.

Friend ascites tumors: The chromosome counts in cells from two tumor lines, VPFT and NVPFT, are summarized in Fig. 3. The modal number was 40 in both lines. Karyotype analyses revealed that, in both VPFT and NVPFT, the modal cells forming their stemlines were of a quasi-diploid pattern, and included marker

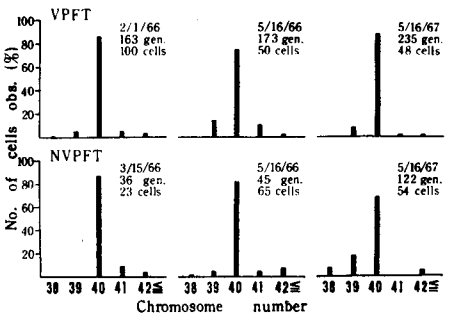


Fig. 3.

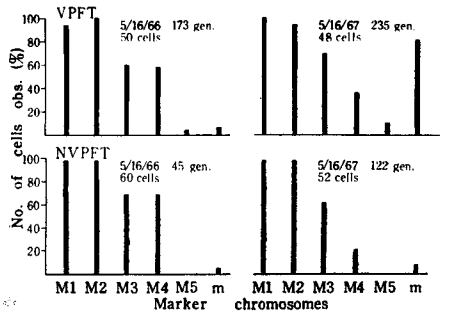


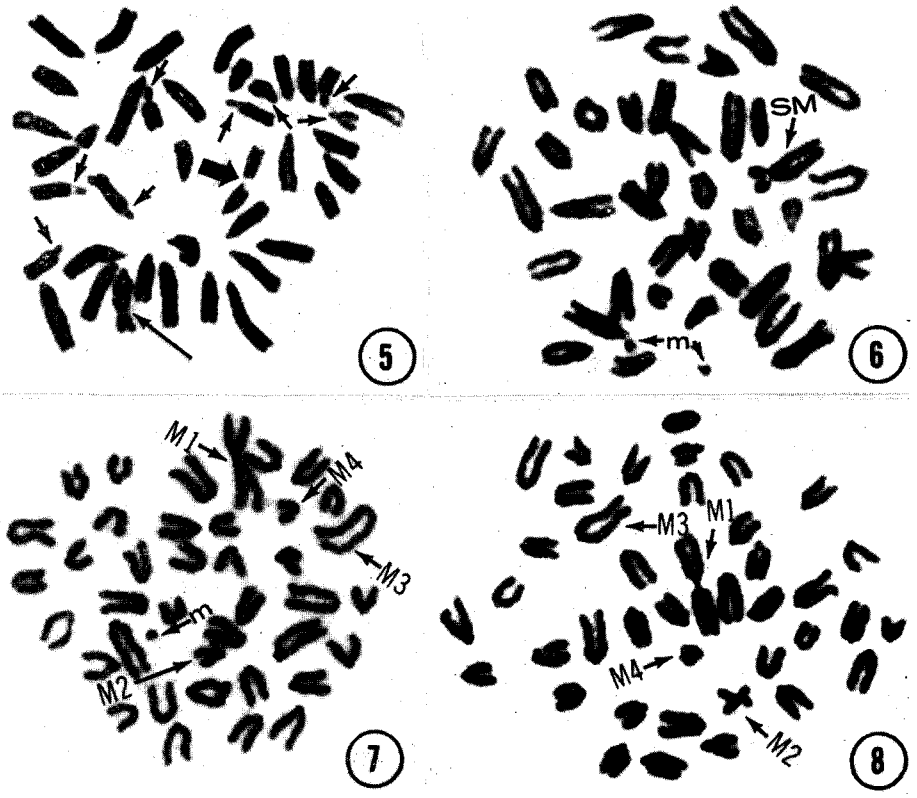
Fig. 4.

Fig. 3. Chromosome-number distribution in the virus producing Friend tumor (VPFT) and the non-virus producing Friend tumor (NVPFT).

Fig. 4. Frequencies of marker chromosomes in the diploid range in VPFT and NVPFT in 2nd and 3rd samples.

chromosomes. The markers were characteristically a large metacentric chromosome (M1), a small submetacentric one (M2), a large telocentric (M3), a small telocentric (M4), a medium-sized metacentric (M5), a minute chromosome (m) and some others (Figs. 7, 8). Based on the 2nd and 3rd samples, the frequency of the marker chromosome occurring in the diploid range was calculated in VPFT and NVPFT cells and is given in Fig. 4. The relative frequency of cells with the marker chromosomes in the VPFT and NVPFT was found to show no appreciable difference in the 2nd sample. However, in the 3rd sample, one year after the 2nd sampling, the frequency of cells with the minute chromosome showed a marked difference in

both lines; 81% in VPFT and 8% in NVPFT. It becames evident that the two lines are distinguishable with respect to the frequency of the minute chromosome. The frequency of polyploid cells calculated in 500 cells was slightly higher in NVPFT than in VPFT: 0.6% in VPFT and 2.6% in NVPFT in the 2nd sample, and 0.5% in VPFT and 3.8% in NVPFT in the 3rd sample. Aberrations such as chromatid breaks, acentric fragments, dicentrics and allied ones appeared in a low incidence in both lines.



Figs. 5–8. Metaphases from Friend leukemias and Friend ascites tumors. 5: a spleen cell in a male mouse with Friend leukemia, 11 days after infection. 40 chromosomes; 8 chromosomes having secondary constrictions (short arrow), one chromosome with an isochromatid gap (long arrow) and one chromosome with an isochromatid break (wide arrow). 6: a spleen cell in a male leukemic mouse, 20 days after infection, 40 chromsomes; one subtelocentric marker chromosome (SM) and 2 minute chromosomes (m). 7: a cell in the virus producing Friend tumor (VPFT), 40 chromosomes including 5 marker chromosomes (M1, M2, M3, M4, m). 8: a cell in the non-virus producing Friend tumor (NVPFT), 40 chromosomes including 4 marker chromosomes (M1, M2, M3, M4).

Discussion

The present findings on the chromosome features in Friend leukemias of mice agree with those in the majority of cases of Friend leukemias so far cytologically examined, in respect of the fact that leukemic cells were characterized by a normal chromosome constitution, with a variety of incidence of aneuploid cells and abnormal chromosomes case by case (Bayreuther 1960, Ohno and Hauschka 1960, Wakonig-Vaartaja 1961, Tsuchida and Rich 1964).

The features involving the secondary constriction observed in Friend leukemias correspond for the most part to those obtained by Tsuchida and Rich (1964). They described that the incidence of cells showing more than one secondarily constricted chromosome decreased as the chromosome number increased, and suggested the possibility that extra chromosome pairs might arise through the secondary constricition of existing chromosome pairs. I failed to find such an inverse relationship between hyperdiploidy and the incidence of the secondary constriction, due probably to a few hyperdiploid cells observed. However, unusual marker chromosomes observed in leukemic cells dealt here were worth recording on account of the fact that they consistently showed secondary constrictions near the centromere. This also suggests a possibility that the formation of the marker chromosomes may be closely associated with the elements carrying the secondary constriction. The above findings seem to lead the conclusion that the exaggerated secondary constriction in Friend leukemias may contribute importantly to arise chromosomal alteration, especially aneuploidization and chromosome rearrangment.

Ascites tumors originated from leukemic tissues of Friend virus-infected mice were found to have a quasi-diploid chromosome constitution, 40 in number and characterized by marker chromosomes. It is most probable that primary leukemic cells of a normal diploid complement might change into quasi-diploid constitution including marker chromosomes in the course of many passages. However, the marker chromosomes common in Friend ascites tumors did not show apparent secondary constrictions, though some acrocentrics beared secondary constrictions. It is difficult to inquire into the origin of the marker chromosomes in Friend ascites tumors on the basis of the findings of the present study.

Two Friend ascites tumor lines, a virus producing Friend tumor (VPFT) and a non-virus producing Friend tumor (NVPFT), showed that the stemline karyotype was visibly indistinguishable from each other in the 1st and 2nd samples. This suggests the occurrence of an identical cytogentic constitution between the VPFT and NVPFT lines, and that the pathogenic property of the two tumor lines may not always be associated with the chromosomal change. In the 3rd sample, one year after the 2nd samling, the increase in frequency of the cells with the m chromsome occurred in VPFT. It is apparent that the VPFT line had undergone a change of the stemline karyotype in the course of serial transfers. In spite of the fact that the stemline karyotype of the two lines is distinguishable with respect to the frequency

of cells having the m chromosome, it is needed to collect data for understanding the relationship between the karyological difference and the different property.

Summary

Chromosomes were studied in hypertrophied spleens and a liver of leukemic mice produced by Friend virus-infection, and in two lines of the Friend virus-induced ascites tumors of mice, one being a virus producing Friend tumor and the other a non-virus producing Friend tumor. The modal number of chromosomes in leukemic cells was 40, showing no remarkable deviation from that of normal mice. An increase in incidence of cells having the chromosomes with secondary constrictions and of those showing chromatid and isochromatid breaks or gaps was noted. Unusual marker chromosomes were especially remarkable in aneuploid cells from leukemic mice killed after a longer period of infection.

Friend tumor cells having a quasi-diploid chromosome constitution, 40 in number and characterized by marker chromosomes, were found to form stemlines of the two Tumor lines. The stemline karyotype of the two lines of tumors was the same morphologically and numerically in the 1st and 2nd samples. One year after the 2nd sampling, the two lines were different with respect to the frequency of the cells having the minute chromosome.

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