92. *Alteration of Stemline Chromosomes
in Hypotetraploid Ehrlich
Carcinoma*^{*)**)}

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Although the constancy of the stemline is pronounced, the chromosome individuality of a neoplastic cell-population does not always remain unaltered even in highly homogeneous and uniform populations. It was observed in a certain transplantable tumor that for a long time continued serial transfers caused progressive changes both in number and structure of the stemline chromosomes (Levan 1956, Makino 1957, Makino and Sasaki 1958, Yosida 1959, 1960, Matano and Makino 1961).

Hypotetraploid Ehrlich carcinomas were investigated by Levan and Hauschka (1952), Yosida (1954), Ising (1955), Hauschka and Levan (1958); it was found that they were characterized by including one or two metacentric marker chromosomes in their karyotypes. The present paper deals with an alteration of the stemline chromosomes which were taking place in the course of serial transfers.

Material and Method. The hypotetraploid Ehrlich carcinoma (ELT tumor) was obtained in March 1960 from the Institute for Infectious Diseases, Tokyo. Since then this carcinoma was maintained by successive transfers to inbred Swiss albino mice. For the observation of chromosomes a pretreatment squash method proved to be useful.

Results. Samples derived from the tumor-bearing mice at the 11th and the 31st transfer generations showed that over 95 percent of tumor cells at metaphase were hypotetraploid as to chromosome number. The frequency of the chromosome numbers of stemline cells was in general in both samplings similar.

At the 11th transfer generation metaphase cells with 75 chromosomes occurred most frequently, and cells with 74 and 76 chromosomes ranked next. The karyotypes of stemline cells were remarkable by

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including two large metacentric chromosomes as markers (Figs. 1 and 3). One of them was larger than the other and had unequal arms, while the smaller carried a median centromere. The remaining chromosomes in those cells were telocentric. Out of 33 metaphase cells examined, two cells with 76 chromosomes had one strikingly large telocentric referred to as the LT chromosome. Other elements in the two exceptional cells with LT chromosome were apparently similar to those of stemline cells lacking this element (Figs. 2 and 4).

In the sample taken at the 31st transfer generation, the majority of 50 metaphase cells (82.0 percent) were found to have each one LT chromosome, but one cell was found with two LT chromosomes. Cells without LT chromosome occurred with a frequency of 16 percent. Although the chromosome number distribution was in general variable since the 11th transfer generation, the numbers 74, 75, and 76 remained as the stemline modes. The idiograms of cells with the LT element were quite similar to those observed at the 11th generation. Other idiograms showing 74 and 75 chromosomes were similar to those of 76 chromosome cells by having two metacentrics and one LT chromosome. The remaining elements in those cells were all of telocentric nature, being indistinguishable from each other as was observed at

Figs. 1-2. Metaphase chromosomes of hypotetraploid Ehrlich carcinoma. 1, unaltered cell with 74 chromosomes including two metacentric markers. 2, altered karyotype with two metacentric and one large telocentric markers, 76 chromosomes in all. LT: large telocentric chromosome. m₁: metacentric chromosome with unequal arms. m₂: metacentric chromosome with a median centromere.

Figs. 3-4. Idiogram analysis of hypotetraploid Ehrlich carcinoma. 3: cell of Fig. 1 (74 chromosomes). 4: cell of Fig. 2 (76 chromosomes).

the 11th transfer.

Concluding remarks. The present observations revealed that an alteration of karyotype occurred in the hypotetraploid Ehrlich carcinoma. In the early samplings the carcinoma was characterized by hypotetraploid stem cells with 75 chromosomes containing two large metacentric marker chromosomes. Since the 31st transfer generation the samples showed that the stem cells became characterized by the addition of one strikingly large telocentric chromosome, the LT. The reexamination of the preparations from the 11th transfer sample revealed that the LT chromosome was occurring in them in the tumor population at very low frequency. It is very likely that the conditions at the serial transfers were especially favorable to the LT cells to the disadvantage of cells lacking this chromosome. Thus, finally, the great majority of stemline cells were represented by LT cells.

A similar evidence was furnished by the Yoshida sarcoma of rats

(Yosida 1959); the stemline karyotype showed a complete change into a new karyotype during a period of about 20 transplant generations. In the present case, the karyotype alteration occurred in about 85% cells after about 20 transplant generations. According to Kurita *et al.* (1964), for one generation about 18.5 hours were required in the Yoshida sarcoma cells, while this takes about 38 hours for the Ehrlich tetraploid line (Defendi and Marson 1963).

The difference between the tetraploid Ehrlich and the diploid Yoshida sarcomas in the extent of the change from complete in the latter case to 85% alteration in the former can be attributed to the difference in the cell cycle time per generation.

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