Cytological Studies of Tumors, XX. A Chromosome Analysis in the MTK-IV Tumor, A Hypo~~tetra~~tri ploid Rat Ascites Tumor[1)]

By

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(With 10 Text-figures)

Inquiries into the chromosome cytology of ascites tumors of the rat and mouse during the last few years have established indisputably the reality of the stem-cell hypothesis that there do exist stem-cells which function as the principal progenitors of the neoplastic population maintaining the genetic pattern of each tumor. There no longer seems to be any doubt that the tumor cells most frequently occurring with characteristic chromosome-number mode together with specific chromosome ideogram form a stem-lineage (or-lineages) of tumor cells which primarily contribute to the growth of the neoplasm, and further that the distinct pattern of each tumor is closely associated with the genotype determined by stem-cells (cf. the review by Makino 1957). The present paper deals with the rise and further development of the hypotriploid stemline of the MTK-IV tumor of the rat, with some information about the general trends on the chromosomes of rat ascites tumors.

Observations

The MTK-IV tumor here under study is one of the transplantable ascites tumor lines in the white rat; originally this tumor was produced artificially by prolonged administration of azo dyes: in the course of the experimental production of the hepatoma in many rats with azo dyes, Drs. Tanaka and Kanô found a rat which showed a tumorous growth in its liver. Small pieces of the tumorous liver tissue were crushed and injected in the peritoneal cavity of new rats. A few rats which had received injections showed the propagation of the tumor cells in their ascites. The average life-span of tumor-bearing rats was 11.5 days for 1-100 transfer generations, 9.4 days for 101-200 generations, and 8.6 days for 201-250 generations. The type of this tumor — sarcoma or carcinoma — has remained undetermined as yet. The serial transfer has been continued since its start in February 1954 for over 250 generations (March 1957). Details on the character of this tumor will be given in another paper by Tanaka and Kanô.

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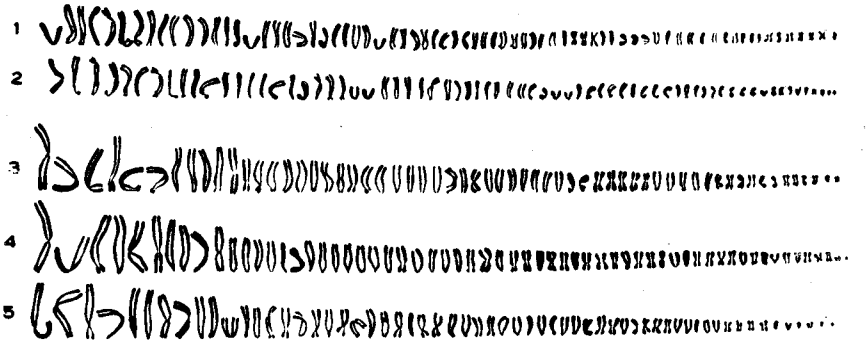
On the basis of the chromosome count, this tumor was described as a tumor of a hypertriploid line, on account of the neoplastic population provided with near-triploid cells in high frequency (Makino 1956). In the samples derived from 30th to 50th transfer generations (July to October 1954), over 85 per cent of dividing cells were nearly triploid.[1] The frequent variation of the chromosome number fell between 62 and 68, with the modal number at 67. These frequent cells were provided with a characteristic complement consisting of rod-like, V- and J-shaped chromosomes, among which a V-shaped element of remarkable size was very prominent (Figs. 1–2). This chromosome constitution was maintained without visible shift through the 60th to 80th transfer generations (January to March 1955).

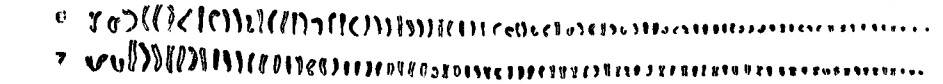


Figures 1-5. Idiograms of MTK-IV in later transfer generations. Serial alignments of chromosomes according to approximate order of size ; the members do not indicate the homologous mates. 1-2, two examples of 1V-type (hypertriploid) cell, from samplings in 30th-50th transfer generations (1 ; 71 chroms. 2 ; 68 chroms.). 3, an example of 2V-type (hypotriploid) cell, from sampling in the probably 100th transfer generation (60 chroms.). 4-5, two examples of 2V-type (hypotriploid) cell, from samplings in 240th-250th transfer generations (4 ; 61 chroms. 5 ; 60 chroms.).

Observations on samples obtained from the recent transfer generations have furnished interesting evidence that the stem-cells of this tumor have undergone certain transitions in the course of successive transfers. In the samples from the 100th to 120th transfer generations (June to September 1955), and those from the 150th to 160th transfer generations (February to March 1956, see Ohnuki 1956), an appreciable reduction in the range of the modal chromosome number has occur-

1) In addition, a few subdiploid cells appeared, together with some aberrant cells. In the present status of investigation, it remains unknown whether these subdiploid cells are neoplastic cells or not.

red; a frequency between 58 and 62 is most common showing the modal value of 60 in about 60 per cent of the observations.



Figures 6–7. Idiograms of MTK-IV in early transfer generations. Serial alignments of chromosomes according to approximate order of size. 6, an example (hypotetraploid cell) from sampling in the 3rd transfer generation (76 chroms.). 7, an example (hypotetraploid cell) from sampling in the probably 5th transfer generation (75 chroms.).

Another remarkable feature to be noticed is a visible change occurring in the stem-line chromosome complement. The cells of modal number have been furnished by a new chromosome type which is provided with two pronouncedly large V-shaped elements of slightly dissimilar size (Figs. 3–5, 9–10). This new chromosome type has been maintained in the most recent samples obtained from the 240th to 250th transfer generations (February to March 1957). The transfer line characterized by this new chromosome type somewhat differes from the stock tumor in the character of disease produced.

Discussion

The main matter of interest in dealing with the MTK-IV tumor lies in the question whether this tumor may have been triploid from the start. Investigations have revealed that in earlier transfer generations of this tumor shortly after

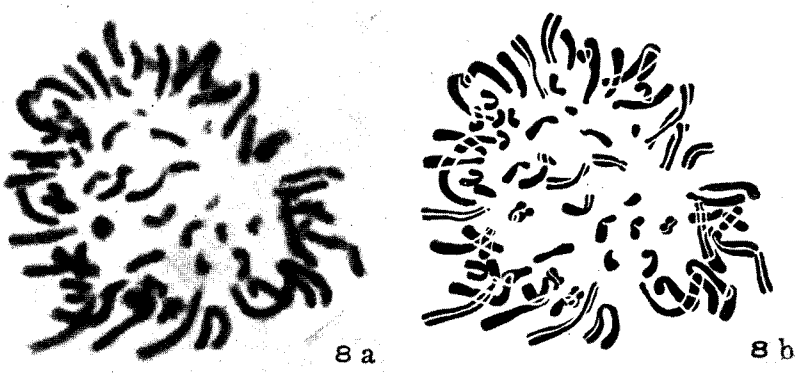
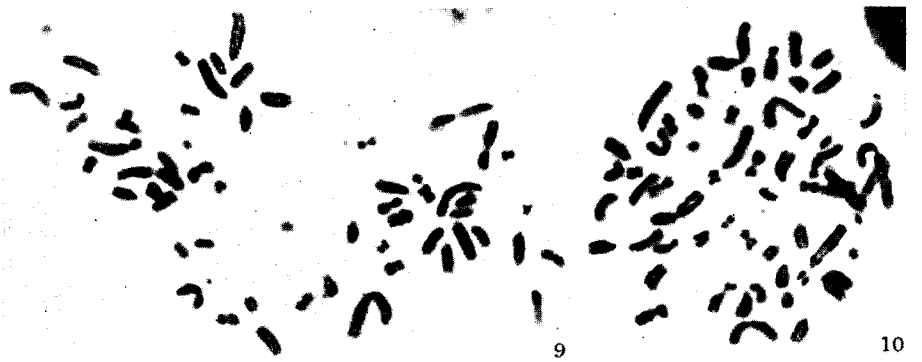


Fig. 8a, metaphase plate of MTK-IV, from the sampling in the 3rd transfer generation, March 1954 (76 chroms., hypotetraploid); ordinary acetocarmine smear. Fig. 8b, drawing of Fig. 8a. The same as Fig. 6.

its original development ranging from the 3rd to 5th transfer generations, hypotetraploid cells, 75 or thereabouts in chromosome number, showed a predominant occurrence. Figures 6, 7 and 8 represent examples. With the increase of transfer generations the chromosome number showed a gradual decrease toward triploidy through elimination of certain chromosome elements. It is then evident that the MTK-IV may have originated from hypotetraploid predecessors, and that



Figs. 9-10. Metaphase plates (hypotriploid) of MTK-IV, from recent samplings (February, 1957); pre-water treatment squash technique with acetic dahlia. 9; 61 chroms. 10; 60 chroms. × *ca.* 1500.

the cell population of this tumor may have gradually shifted as a whole toward hypotriploidy through hypertriploidy from the hypotetraploid start. It is, therefore, most probable that the hypotriploid cells now representing the stemline of this tumor are not a mere triple combination of a basic complex of the rat. Furthermore, most of the chromosomes of the stem-cells differ in shape from the chromosomes of normal tissue cells. Certainly, a large number of structural changes and other adjustments should take place in the tumor chromosomes in association with the development of the tumor since its origin from ordinary tissue.

Based on the experiments with drastic applications, cold storage, heteroplastic transplantation and some other procedures, it is emphasized that tumors generally show a very pronounced constancy maintaining their cytological and physiological characteristics during prolonged serial transfers (Ohnuki 1956, Makino 1957a). In the light of the present findings it can be stated that the stability of the stemline is, nevertheless, not always a permanent one, and tends to undergo transitions after its origin. Under certain conditions, transitions will occur in the stemline chromosomes and such transitions may lead to changes in the property of the tumor. Instances favoring this view have been reported in several other ascites tumors (Hauschka 1953, Levan & Hauschka 1953, Levan 1956, Makino 1956, 1957a,b). The shifts in the chromosome complement may involve structural rearrangements and mutational changes, and such shifts result in the rise of the new type of tumors. Since the tumor pattern is to be controlled by the tumor genotype, it seems justified to state that the development of a new tumor type with a change in property is closely associated with chromosome changes in stem-cells.

Summary

The conclusion may be possible that the hypotriploid stemline occurring in the MTK-IV tumor population is induced from hypotetraploid predecessors passing through hypertriploidy by gradual elimination of certain chromosome elements, and that the stability of the stemline idiogram is not necessarily a permanent one. The tumor generally has undergone, during prolonged serial transfers from its origin, numerical and structural chromosome alterations involving genotypic changes in stem-cells.

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