Cytological Studies of Tumors, XVIII. A Chromosome Survey of Rat Ascites Tumors after Repeated Transfers in Mice[1)]

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(With 28 Text-figures)

Cytological investigations based on the chromosome analysis in ascites tumors of rats and mice have established the concept of stemline cells from which the tumor develops (Makino 1952 a, b, Makino and Tanaka 1953 a, b, Levan and Hauschka 1952, 1953, Hauschka 1953, Tjio and Levan 1954, 1956, Makino and Kanô 1953, 1955, Makino and Tonomura 1955, Sachs and Gallily 1955, Watanabe and Tonomura 1956, Watanabe and Azuma 1956, Makino 1956). This hypothesis is based on the occurrence of a population (or populations) of tumor cells that are characterized by a high frequency of occurrence and by characteristic chromosome patterns persistent through serial transfers with regular mitotic behavior (Makino 1952a,b, Makino and Nakahara 1953 a, b, 1955).

Makino (1952 a, b) and Nakahara (1952) have attempted morphological analyses of the chromosomes of the Yoshida sarcoma transplanted into mice, and revealed that there is a striking similarity between the chromosomes of the tumor cells transplanted in the heterogeneous hosts and those from the homoplastic transplantation. This cytological feature strongly supports the reality of the stem-cell hypothesis. But their observations are limited to the material derived from the first transfer generation. Further question is left whether the stemline chromosome pattern remains unaltered or undergoes changes in prolonged successive heteroplastic transplantations. This inspires the present author to take up the present study which deals with the chromosome analysis of tumor cells of rat ascites tumors transplanted into mice.

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Material and method

Two types of rat ascites tumors, the MTK-sarcoma II and MTK-IV tumor, were used as material for the present study. The MTK-sarcoma II was established in our laboratory by application of azo dyes in a Wistar inbred rat (Tanaka and Kanô 1951); serial transfers have succeeded for 309 generations (February 1956). The MTK-IV tumor is a new rat ascites tumor produced by Tanaka and Kanô through a similar experimental procedure; serial transfers have been successful for 150 generations (February 1956).

In the heteroplastic transplantation, the mouse strains used are B72, CBA, C57, dba/2, D and E. The tumor ascites which contain cells, about 0.2 cc in volume, derived from rats was injected into the peritoneal cavity of mice with the use of a fine glass pipette. The tumor cells thus inoculated can survive in the peritoneal cavity of mice for 5 to 7 days undergoing considerable proliferation with the accumulation of ascites in a more or less degree, but thereafter they disappear without killing the host. At 4 (or sometimes 5) day intervals, about 0.2 cc of the ascites containing tumor cells was successively transferred from mouse to mouse for 42 transfer generations in the case of MTK-IV tumor, and 10 transfer generations in MTK-sarcoma II.

The chromosomes of MTK-IV tumor cells in mouse transfers were observe in the samples taken from the lst, 3rd, 5th, 7th, 10th, 33rd and 42nd tranfser generations, and those of the MTK-sarcoma II were observed in the lst, 3rd, 5th, 7th and 10th transfer material. The material for preparation was sampled from the mouse peritoneal cavity at the 2nd (sometimes 3rd or 4th) day after transplantation. Microscopical observations were carried out with smear preparations of the tumor ascites stained with acetic dahlia after water pretreatment; droplets of the tumor ascites on slides were added in a nearly equal volume of tap or distilled water, left for 4 to 7 minutes, covered with acetic dahlia, and squashed under the cover-slip with a considerable pressure.

Cytological observations

The tumor cells inoculated in the peritoneal cavity of mice can survive for 5 to 6 (sometimes 7) days undergoing considerable proliferation. It was found in these experiments that the mitotic rate was highest on the lst day of inoculation with an increase in number of tumor cells. Towards the 3rd or 4th day after transfer the tumor showed a pure culture condition, whereas the mitotic frequency decreased to a considerable degree. On the 5th day the tumor cells began to show signs of degeneration. The sample taken on the 6th (or sometimes 7th) day after inoculation indicated many tumor cells undergoing degeneration; the tumor cells are damaged by phagocytic cells resulting in their complete disappearance without killing the host (Ohnuki 1956). It is remarkable that every mouse-transfer generation offers a similar pattern in behavior of tumor cells (Fig. 1, A & B).

The MTK-IV tumor is characterized by a neoplastic population in which subtriploid stemline cells are hihgly predominant. The modal variation of the chromosome number of the subtriploid cell falls between 58 and 62, the most frequent number being 60 (Table 1, A). The chromosome pattern is characterized by a specific combination of rod-like, V-shaped and J-shaped elements, among

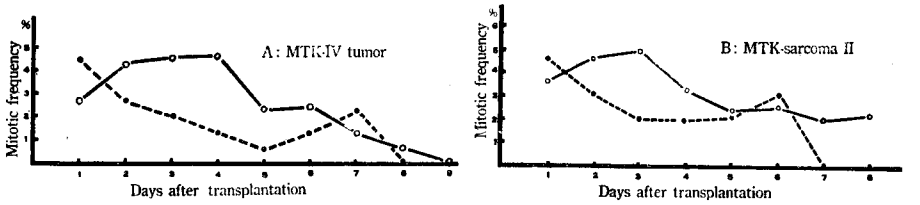

Fig. 1. Graphs showing the daily frequencies of dividing tumor cells of the MTK-IV tumor (A) and the MTK-sarcoma II (B). The solid lines and the broken lines denote the frequencies of dividing tumor cells in the peritoneal cavities of the rat and the mouse, respectively.

which the constant presence of two V-shaped ones of large size is prominent (Figs. 2, 7, 23). The study of the mouse transfer material has revealed that this characteristic stemline chromosome pattern is always observable in the samples taken from the 1st, 3rd, 5th, 7th, 10th, 33rd and 42nd transfer generations without exception (Figs. 3–6, 8–11). In the 42nd mouse transfer sample, the most frequent

Table 1. The variation of chromosome numbers of tumor cells in rat and mouse transfers.

A. MTK-IV tumor

Chromosome number		58	59	60	61	62	63	64	65	66	67	Total number of cells observed
Number of cells obs.	Rat transfer generation (87th)	2	4	13	1	3						23
	Mouse transfer generation (42nd)		4	10	3	2					1	20

B. MTK-sarcoma II

Chromosome number		35	36	37	38	39	40	41	42	43	44	Total number of cells observed
Number of cells obs.	Rat transfer generation (303rd)	1		5	7	36	12	1	1			63
	Mouse transfer generation (10th)		1	1	4	30	9	2	3			50

modal number was found to fall at 60, with the variation ranging from 59 to 67 (Table 1, A). From this evidence it can be stated that the stemline chromosome pattern in tumor cells of the MTK-IV tumor has remained unaltered during 42 heteroplastic successive transfer generations (for about 180 days).

A similar condition has been obtained in serial transmissions of the MTK-sarcoma II in mice. The neoplastic populations of MTK-sarcoma II are characterized by the subdiploid stem-cells which have the modal variation of chromosome number lying between 35 and 42, with the most frequent number of 39 (Table 1, B). The chromosome pattern of the MTK-sarcoma II is also characterized by a combination of rod-, V- and J-shaped elements, with a remarkable existence of two large V-shaped chromosomes (Figs. 12-13, 19–20, 24). The tumor has been transferred successively in mice for 10 generations and the material was sampled at the 1st, 3rd, 5th, 7th and 10th generations. In every sample there is a striking uniformity of the chromosomes in respect to possessing the stemline ideogram characteristic of the MTK-sarcoma II (Figs. 14–18, 21–22); the 10th transfer sample indicated the most frequent modal number at 39, the

Fig. 2. Chromosomes of the original stemline cell of the MTK-IV tumor. 60 chroms. Camera lucida drawing, ×1400. Figs. 3–6. Chromosomes of stem-cells of the MTK-IV tumor transplanted successivesly in mice, observed at the 3rd, 10th, 33rd and 42nd mouse transfer generations. 60 chroms. in each. ×1400.

variation being from 36 to 42 (Table 1, B).

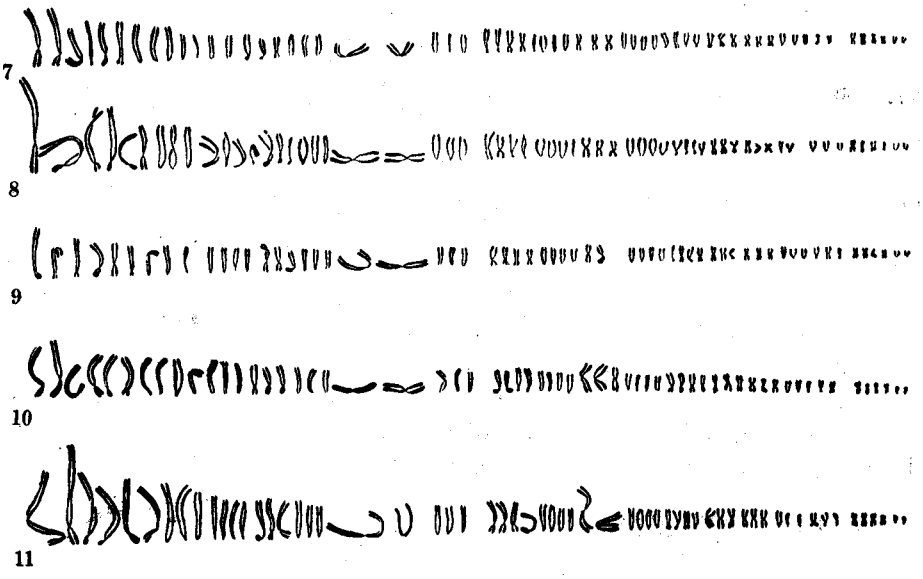

Fig. 7. Serial alignment of chromosomes of the original stemline cell in the MTK-IV tumor. 60 chroms. Figs. 8–11. Serial alignments of chromosomes of the MTK-IV tumor cells, from the samples at the 3rd, 10th, 33rd and 42nd mouse transfer generations.

Process of disintegration of stsm-cells of rat ascites tumors in mouse transfers

The tumor cells of rat ascites tumors (MTK-sarcoma II and MTK-IV tumor after inoculation in the peritoneal cavity of mice can survive for 5 to 9 days, but they then suddenly disappear without killing host animals. The process of degeneration of tumor cells in the heterogeneous host was investigated in the living condition

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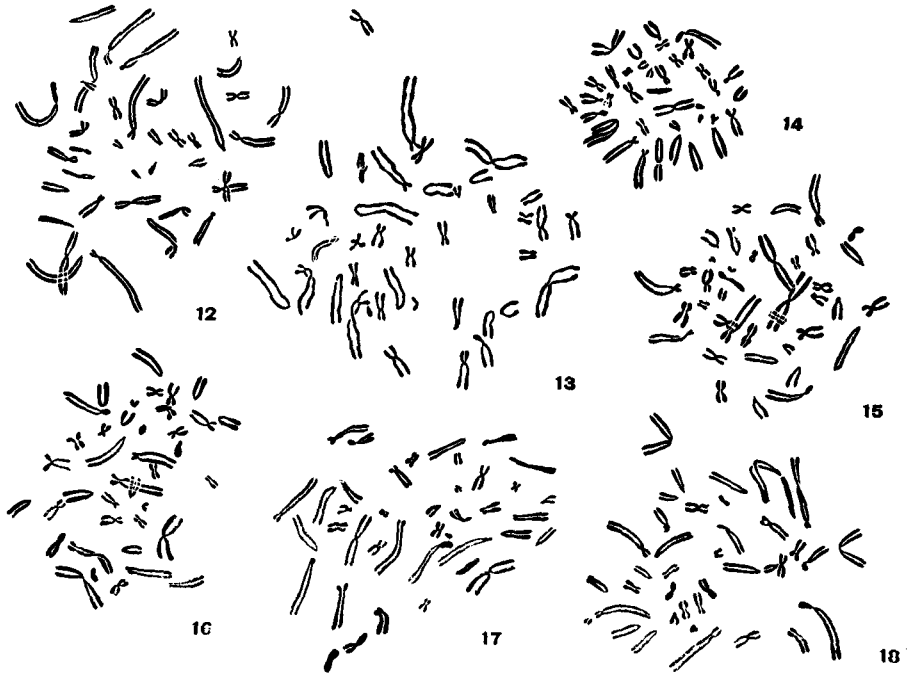
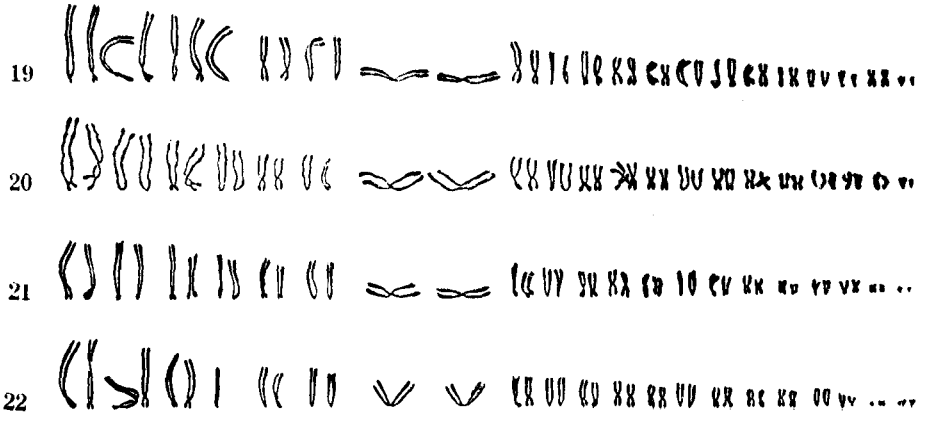
Figs. 12–13. Chromosomes of the original stemline cells of MTK-sarcoma II. 12, 39 chroms. 13, 40 chroms. Camera lucida drawings, ×1500. Figs. 14–18. Chromosomes of the MTK-sarcoma II cells transplanted successively in mice, from 3rd (Fig 14), 5th (Fig. 15), 7th (Figs. 16–17) and 10th mouse transfer generations (Fig. 18). ×1500.

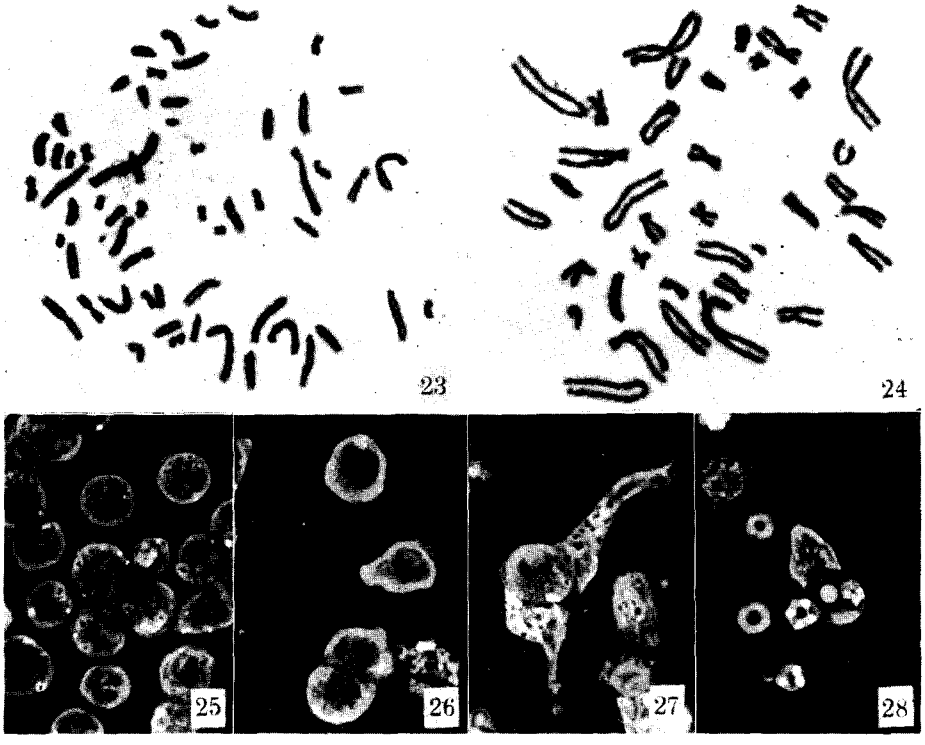
Figs. 19–20. Serial alignments of chromosomes of original stemline cells of the MTK-sarcoma II. Figs. 21–22. Serial alignments of chromosomes of the MTK-sarcoma II cells transplanted successively in mice. 21, from the 7th mouse transfer generation. 22, from the 10th mouse transfer generation.

with the use of phase optics and hanging drop preparations made in combination with liquid paraffin. The samples were taken at 2-hour-intervals during the latter part of a mouse transfer generation. As reported in the former paper (Ohnuki 1956), the tumor growth was found to be in a good condition in the mouse peritoneal





cavity on 5th to 6th day after transplantation, and thereafter the tumor cells suddenly underwent degeneration. On degeneration, they showed remarkable granular bodies and vacuoles in their cytoplasm (Fig. 25). Then these cells began to disintegrate with the irregular transformation of cell-form following the picnotic



Figs. 23–24. Photomicrographs of chromosomes of tumor cells. 23, the MTK-IV tumor. ×1200. 24, the MTK-sarcoma II. ×2000. Figs. 25–28. Photomicrographs showing the process of disintegration of tumor cells of rat ascites tumor in the mouse peritoneal cavity. 25, early stage of degeneration in tumor cells, showing bright granules and vacuoles in cytoplasm. 26, deformation of tumor cells. 27–28, disintegration of tumor cells, together with active leucocytes (macrophages).

degeneration of the nucleus (Fig. 26). The decrease in number of tumor cells is followed by the increase of the number of blood cells such as macrophages and lymphocytes. The tumor cells seem to be damaged by phagocytic leucocytes (Figs. 27–28).

Discussion

Many experiments have been performed to learn the behavior of tumor cells of rat ascites tumors inoculated into the mouse peritoneal cavity, without attention to the change of the chromosomes (Yosida 1952, Atsumi and Satoh 1952, Haga 1953). Cytological investigations involving both morphological and statistical analyses of chromosomes by Makino and his collaborators have revealed the presence of the stemline (or-lines) of tumor cells which primarily contribute to the growth of the tumor. For critical confirmation of the stemline concept, the chromosome analysis in tumor cells transmitted in the heterogeneous host has been attempted by Makino (1952 a, b) and Nakahara (1952). According to Makino (1952 a, b), the Yoshida sarcoma cells transferred into black rats, white mice, field mice, voles and guinea pigs survived for a short length of time with a few mitotic figures. Cytological observations of these mitotic figures revealed that the chromosomes of the Yoshida sarcoma cells remained unaltered in those heterogeneous hosts.

The present study deals with a chromosome analysis of tumor cells of rat ascites tumors after successive transfers in mice : the MTK-IV tumor was transferred for 42 generations in mice and the MTK-sarcoma II for 10 generations. This cytological investigation has revealed that the stemline ideograms characteristic to each tumor have been left unchanged in successive, repeated heteroplastic transfers. This seems then to show that the chromosome constancy has persisted after the transplantations of rat ascites tumors in mice such repeated over a long period. This cytological feature seems to have strengthened greatly the concept of stemline cells as progenitors of the neoplastic population. This idea has received further confirmation by the following experiments made by Makino and his co-workers : transplantation of the tumor into heterogeneous hosts, reciprocal transfer of the tumor from the peritoneal cavity to subcutaneous tissue, chemical treatment of the tumor, single tumor-cell inoculation and double inoculation with two different kinds of tumors in the same host (Makino and Tanaka 1953 a, b, Tonomura 1953, Makino and Tonomura 1955, Makino and Kanô 1955, Sasaki 1956, Makino 1956).

Summary

In order to ascertain whether the stemline chromosome pattern of tumor cells remains unaltered or undergoes changes in repeated heteroplastic transplantations, the chromosome analysis of rat ascites tumors has been undertaken using the material transmitted in mice. The MTK-IV tumor was transferred for 42 generations in mice and the MTK-sarcoma II for 10 generations. It has been shown that the stemline ideograms characteristic to each tumor have remained unchanged in successive, prolonged heteroplastic transfers. This result seems to offer confirmatory evidence for the hypothesis of stemline cells as progenitors of the

neoplastic population.

The process of disintegration of tumor cells of rat ascites tumor in the mouse peritoneal cavity is described.

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