Cytological Studies of Tumors, XXXII. Chromosome Analyses in Stomach and Uterine Carcinomas

By
Akira Tonomura

(Zoological Institute, Hokkaido University, Sapporo, Japan)

(*With 13 Text-figures*)

Recently the importance of karyological data on human tumors has aroused special interest in relation to clinical and pathological properties of the tumor. Since the genetic mechanisms involved in the development of tumors are reflected in numerical and structural changes of the chromosomes, it seems very significant to analyse in detail the stemline idiograms of human tumors for comparison with the normal idiogram. In one of recent papers of this series of studies, Makino, Ishihara and Tonomura (1959) have presented the chromosome conditions in thirty cases of primary human tumors, with special reference to the stemline idiograms in connection with clinical and pathological properties. The present paper is to describe in some detail the chromosome analyses in the stomach and uterine carcinomas, since the samples permitted rather sufficient survey of chromosomes.

The author wishes to express his sincere gratitude to Professor Sajiro Makino for his direction and for improvement of the manuscript for publication. The author is also indebted to Dr. M. Takahashi of the Sapporo Medical College in Sapporo, for friendly assistance in collection of the material.

Materials and Methods: Samples for this study were obtained in the Sapporo Medical College, by removal directly from patients before dragic administration or X-ray therapy.

1) *Gastric carcinoma*: The patient was a woman, 64 years old. The tumor was found in the pylorus of her stomach by physical examination. In Apirl of 1958, tumor cells appeared in the peritoneal fluid. Effusion was obtained directly from the peritoneal cavity. About 30 ml. of the fluid collected was centrifuged, and the deposit was stained with acetic dahlia after water pre-treatment (Makino 1957).

2) *Uterine carcinoma*: This tumor which originated from the uterine cervix was excised from a patient, 48 years of age, by operation in July of 1958. A piece of the tumor tissue was minced and treated with tap water about 30–60 minutes and then transferred into 10% acetic acid solution for 5–10 minutes. The shreds of tumor tissue were stained by the acetic dahlia squash technique.

---

1) Contribution No. 449 from the Zoological Institute, Faculty of Science, Hokkaido University, Sapporo, Japan.

Supported by a grant-in-aid from the Rockefeller Foundation to S. Makino for research in cytology.

Observations

1) *Gastric carcinoma*

Tumor cells occurring in the peritoneal fluid are generally round in shape; they show a variety of sizes. Dividing cells were observed rather frequently in this sample. Exact countings and camera-lucida drawings of the chromosomes were made in 42 clear metaphase plates. As shown in Figure 1, the chromosome number varied over a rather wide range from 62 to 136, with the most frequent variation lying between 68 to 79. In light of the fact that the chromosome number

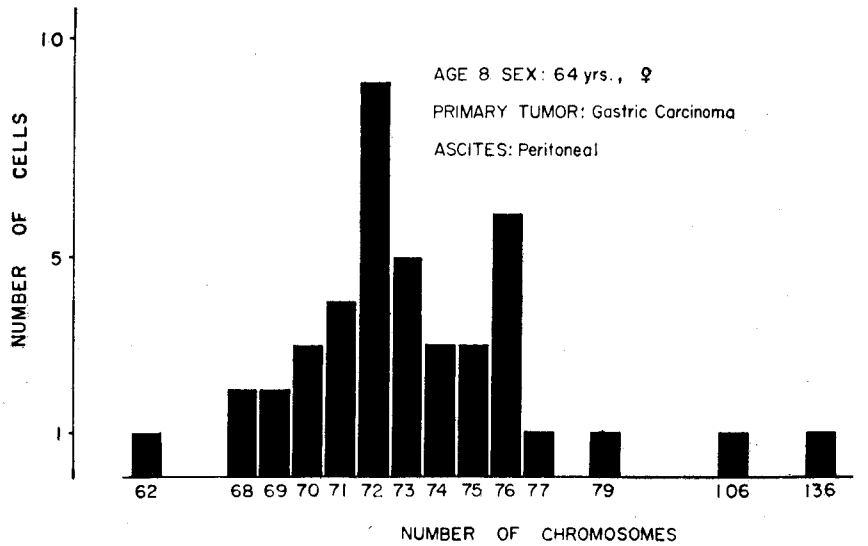
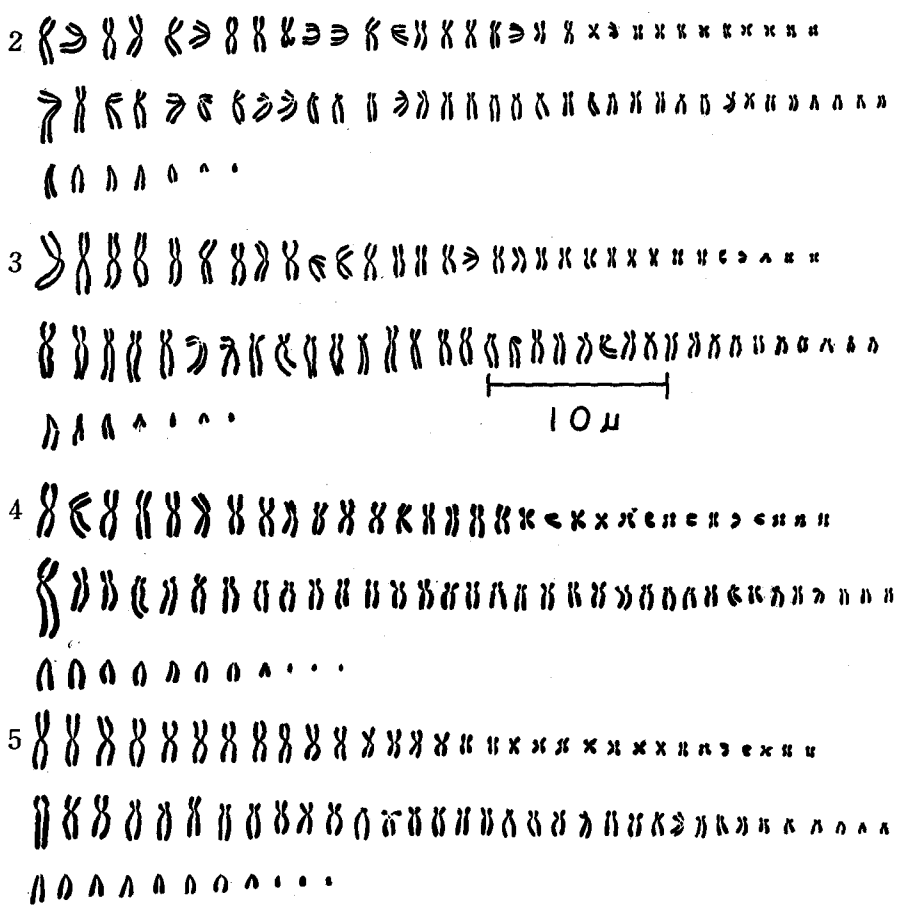


Fig. 1. Chromosome-number distribution observed in 42 tumor cells of the gastric carcinoma.

shows a frequent distribution in a hypertriploid range, the present tumor seems to be of a hypertriploid type. The chromosome-number distribution in that range offers two distinct peaks, the one formed by the cells with 72 chromosomes and the other by those with 76 chromosomes. Both modal cell-lines are very pronounced among other cell-lines of different chromosome numbers and of various frequency values.

The stemline idiogram was analysed in the two modal cell-lines with the use of five cells in each line. According to the chromosome classification of Tjio and Levan (1956a), the chromosome complements were divided into groups of median-submedian centromere (M), subterminal centromere (S) and terminal one (T). In each group , the individual chromosomes were arranged in order of their



Figs. 2–5. Idiogram analyses in four stem-cells of the gastric carcinoma. 2–3, 72 chromosomes. 4–5, 76 chromosomes.

decreasing lengths. Four of these idiograms are shown in Figures 2 to 5. In the cells with 72 chromosomes, there are 31 chromosome complements of type M which show gradual decrease in length from 5μ to 1μ. The S type includes 34 chromosomes, ranging from 5μ to 1.5μ, except the longest one which is about 6μ in length. This longest S type chromosome is very remarkable in being distinctly longer than the others : the ratio of long arm to short arm shows 2 to 1. The T type contains seven chromosomes in which six show lengths of 3μ to 1μ, while the remaining one is about 0.5μ (Fig. 10). On the other hand, the cells with 76 chromosomes contain 31 M, 34 S and 11 T chromosomes, respectively. There are no visible differences in the M and S type complements of cells of the two stemlines with regard to the

number and morphology of the chromosomes. The difference lies only in number and morphology of the T type chromosome complements. In the cells carrying 76 chromosomes, there are 11 T type chromosome complements, as mentioned above. In them, eight out of eleven chromosomes show length variation from 3 μ to 1 μ, while the remaining three are each 0.5 μ in length (Fig. 11). The smallest telocentric chromosomes which have about 0.5 μ length were identified as "minute chromosomes" (m).

Finally, the chromosome formulae of cells of the two stemlines of the present tumor are designated as follows;

72 chromosomes = 31 (M) + 34 (S) + 6 (T) + 1 (m)
76 chromosomes = 31 (M) + 34 (S) + 8 (T) + 3 (m)

The minute chromosomes are present in the majority of cells that could be analysed clearly. This seems to imply that the minute chromosomes take some part in the viability of the stemline cells of this tumor.

2) *Uterine carcinoma*

The tumor showed a rich content of dividing cells of which fifty-six clear metaphase cells permitted a close chromosome analysis in considerable detail. The chromosome count with those fifty-six cells revealed that the chromosome-number of this tumor shows a frequent distribution in a hyperdiploid range, most

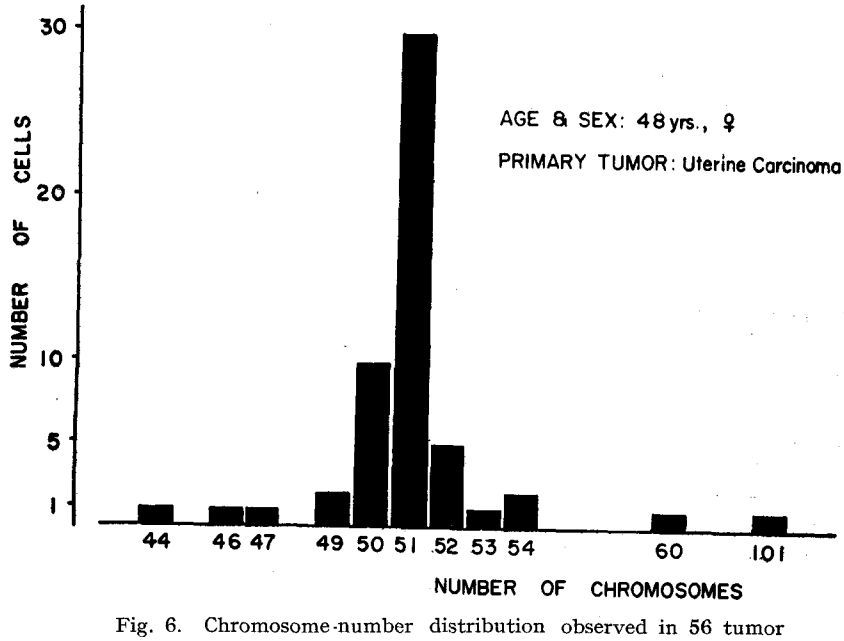
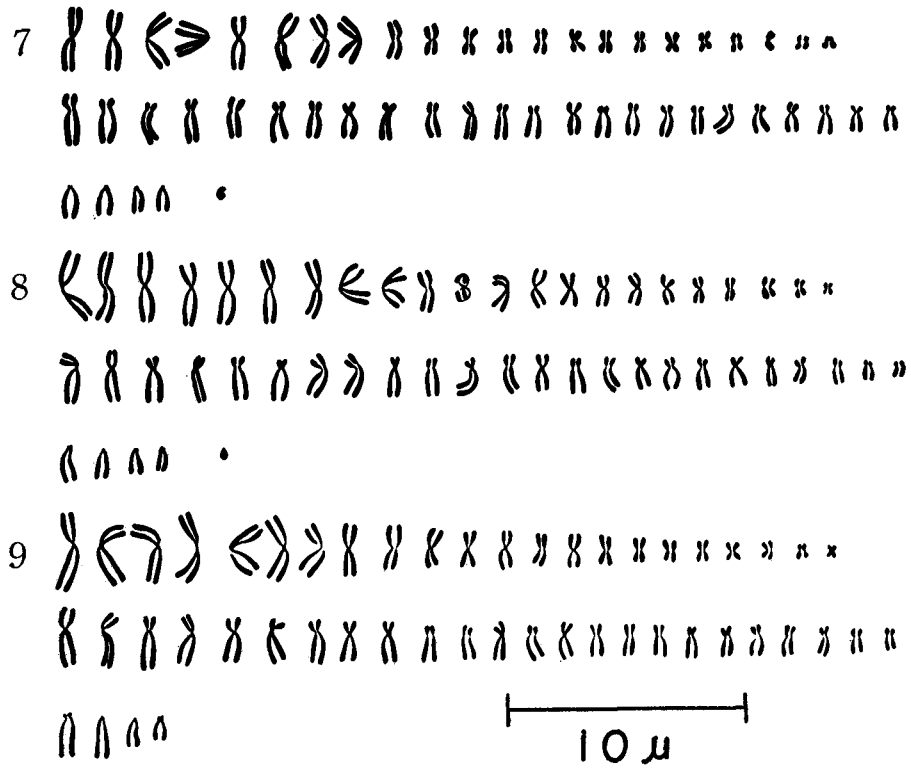


Fig. 6. Chromosome-number distribution observed in 56 tumor cells of the uterine carcinoma.

cells counted having 50 to 52 chromosomes. As seen in the histogram (Fig. 6), two peaks are strikingly prominent in the hyperdiploid range, the first one formed by the cells with 51 chromosomes (54% in frequency) and the second peak by those with 50 chromosomes (18%). It is then evident that this tumor has at least two remarkable stemline cell populations.

Idiogram analysis was made in two cell-lines according to the classification noted above. The cells with 50 chromosomes are characterized by containing in each 22 M, 24 S and 4 T chromosomes, while the cells with 51 chromosomes show in each the same chromosome constitution plus one extra minute chromosome (Figs. 7–9). In both cell-lines, the M type chromosomes show a gradual decrease from 5 μ to 1 μ in length. There occurs a variation of length in the S type chromosomes, from 5 μ to 2 μ in dereasing size order. The remaining four chromosomes are all of T type, ranging from 3μ to 2μ in length for both cell-lines. In addition to the four T type chromosomes, there is an extra minute chromosome (about 0.8 μ in

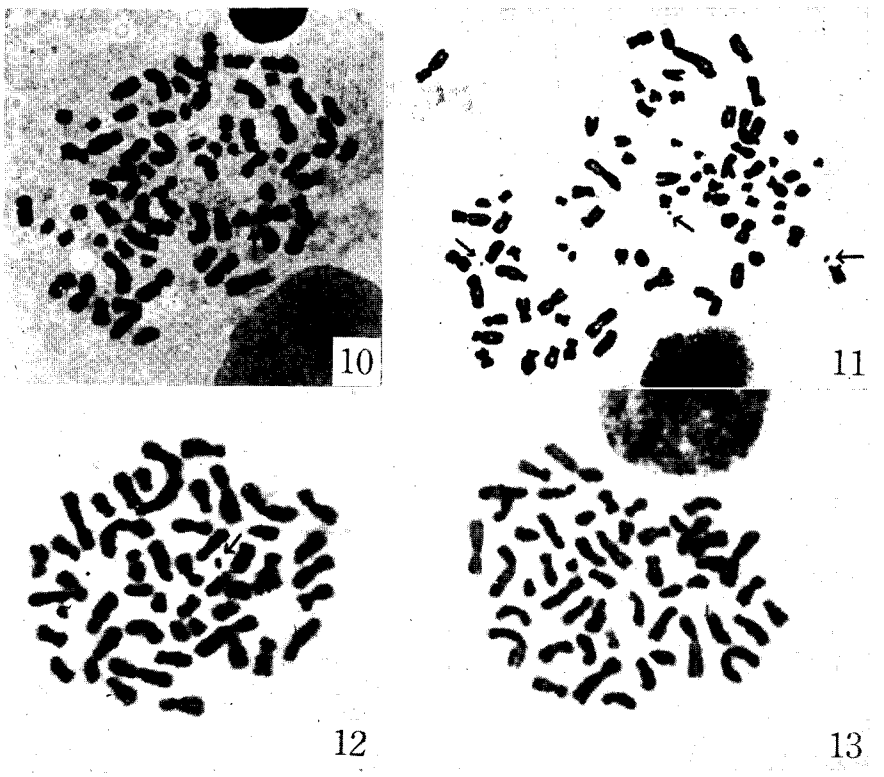


Figs. 7–9. Idiogram analyses in three stem-cells of the uterine carcinoma. 7–8, 51 chromosomes. 9, 50 chromsomes.

length) in the 51-cells (Figs. 12 and 13). Morphologically the minute chromosome seems to be similar in size and shape in every 51-cell under study. Consequently, the idiograms of the two stem-lines are represented as follows :

50 chromosomes = 22 (M) + 24 (S) + 4 (T)
51 chromosomes = 22 (M) + 24 (S) + 4 (T) + 1 (m)

The comparison between the chromosome complexs of the 50-cell and the 51-cell revealed that the M, S and T chromosomes are nearly identical in number, length and morphological details. At present, it is not clear whether the minute chromosome is self-perpetuating by having a normal centromere, or whether it is merely of occasional fragment origin. Whatever is the nature of the minute chromosome, it is apparent that the 50-cells and 51-cells are closely akin to each other in respect to their chromosome relationship.



Figs. 10–13. Photomicrographs of chromosomes of the gastric and uterine carcinomas. Arrows indicate the minute chromosomes. 10, gastric carcinoma, 72 chromosomes, ×1400. 11, gastric carcinoma, 76 chromosomes, ×1400. 12, uterine carcinoma, 51 chromosomes, ×2000. 13, uterine carcinoma, 50 chromosomes, ×2000.

Discussion

Recent progress in the field of chromosome cytology of transplantable ascites tumors of rats and mice have established an important fact that every neoplastic population is characterized by the presence of stemline cells which show a characteristic chromosome pattern in both number and morphology, and occur in high frequency as the progenitors of tumor growth (Makino 1957).

It is quite recently that valuable contributions have been made to the chromosome analysis of human tumors (Koller 1956. Hansen-Melander, Kullander & Melander 1956, Levan 1956, Ising & Levan 1957, Wakabayashi & Ishihara 1958, Ishihara 1959, Makino, Ishihara & Tonomura 1959). The results of the present investigation have revealed that the stomach and uterine carcinomas here studied are characterized by at least two distinct stemlines in each : the gastric carcinoma is remarkable for the hypertriploid stem-cells having 72 and 76 chromosomes, while the uterine carcinoma is furnished with the hyperdiploid cells having 50 and 51 chromosomes. Similar results were reported by Hansen-Melander *et al.* (1956) in a human ovarian cystocarcinoma in which were found two distinct hypotriploid stemlines with 58 and 63 chromosomes.

The analysed stemline idiograms were compared with the normal human idiogram. Tjio and Levan (1956b) and Makino and Sasaki (1959) have reported that the normal idiogram consists of 20 (M) + 20 (S) + 6 (T). If one follows this formula, the eu-triploid complex may be assumed as 30 (M) + 30 (S) + 12 (T). The hypertriploid stem-cells of the gastric carcinoma studied here contain T type chromosomes less in number than the normal pattern, though the M and S types are nearly similar or more in number. Such a tendency seems to occur in some other human tumors, such as a mammary carcinoma studied by Wakabayashi & Ishihara (1958).

It has been shown in rat and mouse tumors that numerical and structural changes occur in tumor chromosomes by which each tumor differs from the normal tissue as well as from each other (Makino 1957). There are several signs indicating certain structural alterations occurring in the chromosomes of the human tumors here under study. Specially remarkable is the fact of the cocurrence of the smallest chromosome which is about 0.5 μ in length. The normal human idiogram includes such a small one in the M and S groups, while the two tumors studied here contain similar ones in the T group. The normal chromosomes of man range in length from 8μ to 1μ in Tjio and Levan's idiogram (1956b), and from 6.8+1.4μ to 1.36+0.31 μ in the Tjio and Puck one (1958). The chromosomes of the two tumors here considered show a variation ranging from 5 μ to 0.5 μ, except one largest S type chromosome, about 6 μ in length, occurring in the gastric carcinoma as mentioned above. In an ovarian cystocarcinoma Hansen-Melander *et al.* (1956) also described a similar feature showing that the chromosomes vary from 4 μ to 0.5μ in length.

In conclusion, the two human carcinomas studied here present evidence that there occur numerical and structural changes of the chromosomes in the course of development of tumors, just as a feature established in animal tumors by Makino (1957).

Summary

The present paper deals with a morphological analysis of the stemline chromosomes in one each stomach and uterine carcinomas. The gastric tumor is characterized by the existence of two hypertriploid stemline cells bearing 72 and 76 chromosomes, respectively. In those two stemline cells, the M- and S-chromosomes are present in nearly the same number, being 31 in the M-chromosome group and 34 in the S-chromosome group, while the T-chromosomes are different in number : 8 in the 72-cells, 11 in the 76-cells. The uterine carcinoma is provided with two distinct hyperdiploid stemlines, one consisting of cells with 50 chromosomes, while the other including those with 51 chromosomes. Each of the 50-cells is characterized by 22 M, 24 S and 4 T chromosomes, while the 50-cells show in each the same chromosome constitution with addition of one minute chromosome.

The chromosome number and morphology of both tumors were discussed in comparison with those of the normal human idiogram and those of some other human tumors.

References

Hansen-Melander, E., Kullander, S. and Melander, Y. 1956. Chromosome analysis of a human ovarian cystocarcinoma in the ascites form. Jour. Nat. Cancer Inst. 16 : 1067–1081.

Ishihara, T. 1959. Some aspects on the chromosomes of a human gastric carcinoma in the ascites form. Jap. Jour. Genet. 34 : 28–33.

Ising, U. and Levan, A. 1957. The chromosomes of two highly malignant human tumors. Acta Path. Microbiol. Scandinavia, 40 : 13–24.

Koller, P.C. 1956. Cytological variability in human carcinomatosis. Ann. New York Acad. Sci. 63 : 793–816.

Levan, A. 1956. Chromosome studies on some human tumors and tissues of normal origin, grown *in vivo* and *in vitro* at the Sloan-Kettering Institute. Cancer 9 : 648–663.

Makino, S. 1957. The chromosome cytology of the ascites tumors of rats, with special reference to the concept of the stemline cell. Intern. Rev. Cytol. 6: 25–84.

Makino, S., Ishihara, T. and Tonomura, A. 1959. Cytological studies of tumors, XXVII. The chromosomes of thirty human tumors. Zeits. Krebsforsch. (in press).

Makino, S. and Sasaki, M. 1959. On the chromosome number of man (a preliminary note). Proc. Japan Acad. 35 : 99–104.

Tjio, J.H. and Levan, A. 1956a. Comparative ideogram analysis of the rat and the Yoshida rat sarcoma. Hereditas 42 : 218–234.

Tjio, J.H. and Levan, A. 1956b. The chromosome number of man. Hereditas 42 : 1–6.

Tjio, J.H. and Puck, T.T. 1958. The somatic chromosomes of man. Proc. Nat. Acad. Sci. 44 : 1229–1237.

Wakabayashi, M. and Ishihara, T. 1958. Cytological studies of tumors, XXVI. Chromosome analysis of a human mammary carcinoma. Cytologia 23 : 341–348.