Cytological Effect of Chemicals on Tumors, XII. A Chromosome Study in a Human Gastric Tumor following Radioactive Colloid Gold (Au^{198}) Treatment[1),2)]

By

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(With 2 Tables, 8 Text-figures and 2 Plates)

Recent investigations with the application of modern cytological techniques have revealed that in human tumors there occurs a stem-line (or -lines) of tumor cells, which have metabolic superiority and contribute principally to the neoplastic growth, quite comparable to those found in transplantable rat and mouse tumors (Hsu 1954, Levan 1956a, Hansen-Melander *et al.* 1956, Manna 1957, Wakabayashi and Ishihara 1958, Awano and Tuda 1959, Ishihara 1959, Makino *et al.* 1959, Tabata 1959, Tonomura 1959a, b, 1960). It is thus remarkable that the reported evidence in human tumors supports the stemline hypothesis established for animal tumors. Recently Ising and Levan (1957) attempted to discover any chromosome conditions particular to the pathological features in some human tumors. Makino *et al.* (1959) investigated the chromosome conditions in thirty human primary tumors with special consideration of the stem-line idiograms in relation to the types of the tumors. A good deal of interest has increasingly been aroused in the medical field with particular attention directed towards the genetic constitution and clinical and pathological properties of human tumors.

The present paper deals with a study of the chromosome condition in a human tumor which was characterized by two stem-lines of tumor cells, with special reference to certain functional responses of tumor cells to a radio-therapeutic treamtent.

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Material and method : The patient was a man aged 37 years, with the career of a coal-miner. The tumor was originally found in his stomach by physical examination at

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the Sapporo Medical College. The tumor growth was active and produced a rich serous fluid in his peritoneal cavity. There were a large number of tumor cells in his peritoneal exudate. During a period from the 22nd October, 1959 to the 19th June, 1960, the patient was placed under radiotherapeutic treatment (Table 1). For the present study, five samplings were made before and after the administration of radioactive colloid gold, Au^{198}. The Au^{198} which emits radiation of low penetrating power has long been known to be effective to certain human tumors. The 1st sampling of the peritoneal fluid was made about thirty minutes before the intraperitoneal administration of 50μc of Au^{198}, and the 2nd, 3rd, 4th and 5th samplings were obtained 17, 23, 37 and 40 days after the administration. In each sample, tumor cells floating in the peritoneal fluid were collected by means of centrifugal selection at 1000 r.p.m. for 10 minutes. The slides for chromosome study were prepared according to the water pretreatment technique, in combination with the acetic dahlia squash method (Makino 1957).

Table 1. Data on radio-therapies and samplings.

Date	Radio-therapies	Doses	Samplings
Oct., 22, 1959	Co^{60}	6,000r	
Feb., 12, 1960	X-ray	2,600r	
March, 1, 1960	Co^{60}	3,300r	
April, 7, 1960	X-ray	3,200r	
April, 15, 1960	Co^{60}	3,000r	
April, 27, 1960	.....	.....	1st sampling
April, 27, 1960	Au^{198}	50μc	
May, 15, 1960	.....	.....	2nd sampling
May, 21, 1960	.....	.....	3rd sampling
June, 4, 1960	.....	.....	4th sampling
June, 17, 1960	.....	.....	5th sampling
June, 19, 1960	died		

The present material provided a considerable number of tumor cells in mitotic stages available for adequate chromosome analyses. Chromosome counts were made in reliable metaphasic cells, with an immersion 100× objective and 20× ocular.

Normal somatic chromosomes of man were exclusively investigated in tissue culture with the hypotonic pretreatment squash method.

Results of observations

Number of chromosomes: The results of chromsome countings made in five samples are shown in Figure 1. In the 1st sample, most tumor cells counted in the ascites exudate were of uniform size, though there occurred a few giant cells. Chromosome numbers exhibited a major range of variation from 59 to 73, showing two modes, one at 63 (28.0 per cent) and the other at 65 (31.3 per cent). The 2nd sample was obtained 17 days after the administration of Au^{198}. It was found that the tumor cells were variable in size and that a great number of giant cells were present (43.8 per cent), together with damaged cells in large number (Fig. 18). Of five metaphasic plates which allowed exact countings of chromosomes,

three had 65 chromosomes and other two showed 64 and 130 chromosomes, respectively. The 3rd sample showed a sudden decrease of the giant cells as well as of the damaged cells, indicating a regrowth of the tumor with many mitotic cells. The chromosome number in this sample varied widely from 45 to 130. The cells having 65 chromosomes occurred most frequently showing 37.4 per cent, being domi-

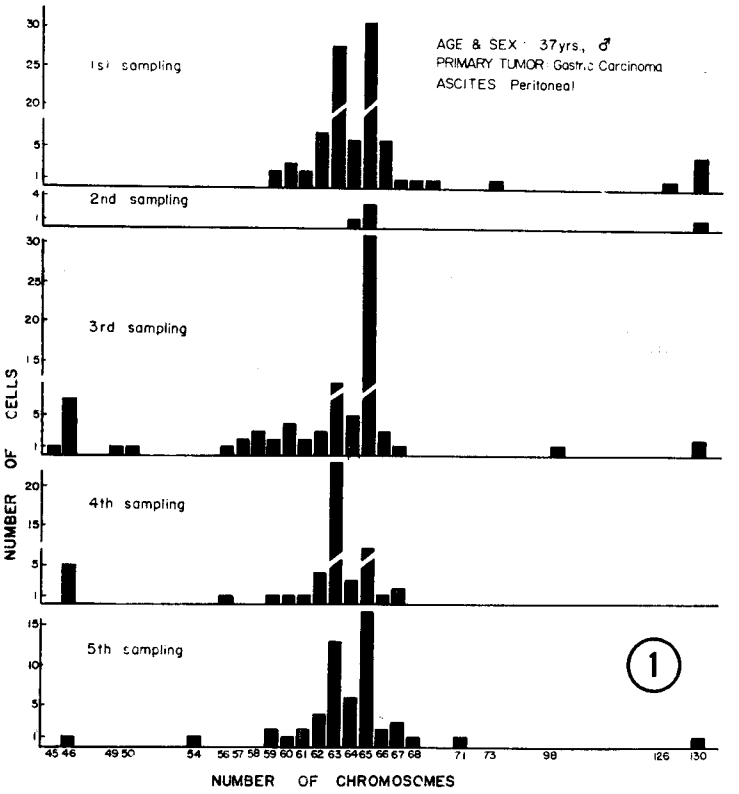

Fig 1. Distribution of chromosome number in five samplings from a human gastric tumor.

nant over the cells having 63 chromosomes at 14.4 per cent. Some cells showed the number of 46 (8.5 per cent). The ascites fluid as the 4th sample was hemorrhagic and showed a further decrease of the giant as well as of damage cells. In this sample, the cells with 63 chromosomes dominated over the 65-cells in occurrence, being 35.5 per cent for the former and 23.6 per cent for the latter, while the cells with 46 chromosomes showed 9.1 per cent in occurrence. The ascites fluid obtained by the 5th sampling was considerably hemorrhagic and contained many tumor cells of uniform size like the 1st sampling. Few damged cells were observed in

this sample. The cell population was again characterized in this sample by the coexistence of two stem-lines, one represented by 63 cells and the other by 65 cells at 23.2 per cent and 30.3 per cent respectively. A few metaphasic cells having 46 chromosomes were observed in this cell population.

Morphology of the stem-line chromosomes: To facilitate a critical comparison of the idiograms between normal and neoplastic cells, and among different tumor lines, the chromosomes were divided into five morphological groups as follows: group A comprising large and medium sized chromosomes with approximately median centromeres, group B including large chromosomes with subterminal centromeres, group C presenting medium sized acrocentric elements, group D containing relatively small chromosomes with approximately median and submedian centromeres, and group E consisting of very short acrocentric chromosomes (Figs. 2–8). The chromosomes in each group were distinguishable from those of another group by their characteristic size and/or morphology. In reference to the above classification, the number of chromosomes of normal human male complex are 21 for group A, 4 for group B, 6 for group C, 10 for group D and 5 for group E, as shown in Figure 8. The cells having 65 chromosomes showed that groups A, B, C, D, E comprised 32, 6, 8, 15 and 4 chromosomes, respectively (Figs. 2 and 3). The cells with 63 chromosomes, on the other hand, consisted of 35, 4, 6, 14 and 4 chromosomes for groups A, B, C, D and E, respectively (Figs. 4 and 5). It is thus evident that, in addition to the difference of chromosome number, the two stem-line cells differ in their chromosome constitution.

The cells having 46 chromosomes found in the present material were found to have a chromosome constitution similar to normal somatic cells of man, since there were observed 21, 4, 6, 10 and 5 chromosomes in group A, B, C, D and E, respectively, with a morphological similarity of each element, so far as the superficial feature of each element is concerned (Figs. 6 and 7).

Further it was shown that there was no difference of the chromosome constitution in the stem-line cells before and after the administration of Au^{198}. It is also noteworthy that a sign of endoreduplication was observed rather frequently in tumor cells of this tumor. The frequency of the metaphasic cells with endoreduplicated chromosomes were 9.3, 10.3, 0.59 and 0.89 per cent in the 1st, 3rd, 4th and 5th samples, respectively. Each chromosome count in thirteen endometaphasic plates has shown that eleven endometaphasic cells included 65 diplochromosomes, while the remaining two contained 130. The endometaphasic cells having 65 diplochromosmes showed the chromosome constitution quite similar to the 65-cells in the morphology of the chromosomes. Around the hexaploid range, in which the chromosome counts were made, cells having 130 chromosomes were most frequent. It is highly probable that they have originated from the 65-cells through the endoreduplication mechanism.

Chromosome abnormalities: In addition to the tumor cells with the normal chromosome configuration, there occurred many cells showing chromosome abnor-

Figs. 2–7. Idiogram analyses in the stem-line cells of the gastric tumor. 2–3, 65 chromosomes. 4–5, 63 chromosomes. 6–7, 46 chromosomes.

Fig. 8. Idiogram analysis of a normal tissue cell cultured (male lung).

Table 2. Summary of data on the frequency of chromosome abnormalities around three ploidy ranges.

Samplings	Diploid range		Hypotriploid range				Hexaploid range			
			Without chrom. ab.		With chrom. ab.		Without chrom. ab.		With chrom. ab	
	Without chrom. abnor.	With chrom. abnor.	Regular metaphase	Endo-metaphase	Regular metaphase	Endo-metaphase	Regular metaphase	Endo-metaphase	Regular metaphase	Endo-metaphase
1st sampling	—	—	204	21	—	—	11	1	—	—
	..... Radioactive colloid gold, Au^{198} (50μc)									
2nd sampling	5	1	3	—	22	—	1	—	14	—
3rd sampling	8	1	77	7	26	—	2	1	8	—
4th sampling	6	1	78	3	2	—	3	—	1	—
5th sampling	3	—	98	8	1	—	4	1	—	—

malities in the samples obtained from the peritoneal fluid following the administration of Au^{198}. Data are given in Table 2. Most metaphasic cells obtained from the 2nd sample showed various types of chromosome abnormalities in very high frequency: they are represented by stickiness of chromosomes, chromatid breaks, somatic translocations and bead-like elongation of chromosomes (Figs. 20–22). They are rather common in highly-ploid cells. In the 3rd sample, the cells with chromosome abnormalities decreased in frequency showing 25.8 per cent. In the 4th sample, chromosome abnormalities showed the frequency of 0.8 per cent. However, no chromosome abnormalities were observed in the endometaphasic cells in any one of the five samples under study. Cells showing very highly-ploid numbers were observed in the samples following the administration of Au^{198}. Some contained more than 600 chromosomes. The 5th sample exhibited no cells with chromosome abnormalities.

Discussion

Thanks to recent advance of cytological techniques for chromosome analyses in normal and malignant cells both *in vivo* and *in vitro*, accurate and critical knowledge on the human chromosomes has increasingly been accumulated facilitating analysis of the correlation between the genetic constitutions and their functional properties (Hauschka 1953, 1957, Hauschka and Levan 1953, 1958, Sachs and Gallily 1955, Levan 1956, Makino 1956, 1957, Hauschka and Amos 1957, Tonomura and Sasaki 1957, Makino and Sasaki 1958, Sasaki 1958, 1961, Matano and Makino 1961). It has been emphasized that under any certain prevailing condition cells with a certain genetic constitution are most efficient and represent the so-called "stem-line cells" which primarily contribute to the malignant growth. Cells with other genetic constitutions may grow in response to other environmental

conditions. Some experimental procedure could induce shifts of cell populations reflecting the altered environmental conditions (Ising 1955, Levan 1956b, Sasaki 1961, Matano and Makino 1961).

A human gastric tumor in the ascites form on which the present study was based was evidently characterized by two stem-lines. On the basis of the veiw that a certain genotype has its own environmental optimum, Hansen-Melander *et al.* (1956) and Ford and Mole (1959) emphasized that the tumor cell population with a bimodal or multimodal distribution was in the transitional site. In the gastric tumor here under consideration, however, the bimodal distribution was maintained for 40 days without shift of the stem-lines. Most probably, the two stem-lines of this tumor seem to be very stable, having become adapted to the physiological condition of the host.

It is most likely that the chromosomes of the tumor cells have undergone various structural changes involving the "cryptostructural changes" of Tjio and Levan (1956). The two cell-lines, 63- and 65-cell lines, in the gastric tumor presently under study showed a marked difference in their chromosome constitution beyond the numerical difference. The chromosome analysis revealed that the difference in chromosome constitution in the two cell-lines seems not to be simply accounted for by non-disjunction mechanism. It seems probable that the difference may refer to the difference in functional property of the two stem-lines. The 63- and 65-cell lines showed in the 1st sample different responce to the Au^{198} while in the 3rd sample the 65-cells were efficient and dominated over the 63-cells. In the 4th sample the 63-cells dominated in turn over the 65-cells. It is then evident that the radio-therapeutic treatment has pointed out a difference in property of the two stem-lines. Further, the difference in functional property between the two cell-lines seems to have connection with the different incidence of the endoreduplication, since most endometaphasic cells were found to contain 65 or 130 diplochromosomes. The above feature seems to supplement the data presented by Levan and Hauschka (1953) showing that c-mitosis and endoreduplication are connected with the functional property of the tumor.

The behaviour of cell population was studied by Koller (1956) in a human malignant effusion after administration of Au^{198}. He reported an increase of cells with the diploid chromosome number (stem-line number in his material). In the present study, a high frequency of cells with diploid chromosome number was observed to occur in the samples under the influence of Au^{198}.

On the other hand, the chromosomes of 46-cells showed no visible difference from those of normal male cells and further the incidence of 46-cells decreased under the effect of Au^{198}. It is highly probable that 46-cells found in the ascites fluid following the administration of Au^{198} are normal tissue cells which have recovered their mitotic activity under the effect of Au^{198}. A similar feature has been observed by the present author in a human tumor following the administration of Mitomycin having a radiomimetic effect on tumor cells.

Summary

The chromosome conditions of a human gastric tumor were studied before and after the administration of radioactive colloid gold, Au^{198}, with special regard to the genetic constitution and phenotypic property of stem-line cells. This gastric tumor was characterized by two marked stem-lines, one represented by tumor cells with 63 chromosomes and other by those having 65 chromosomes. The chromosome constitutions of these two stem-line cells differ highly from one another. Also the two cell lines differ in response to different amounts of Au^{198}.

It was found that an alteration occurred in cell population following the Au^{198} treatment.

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Explanation of Plate XIII

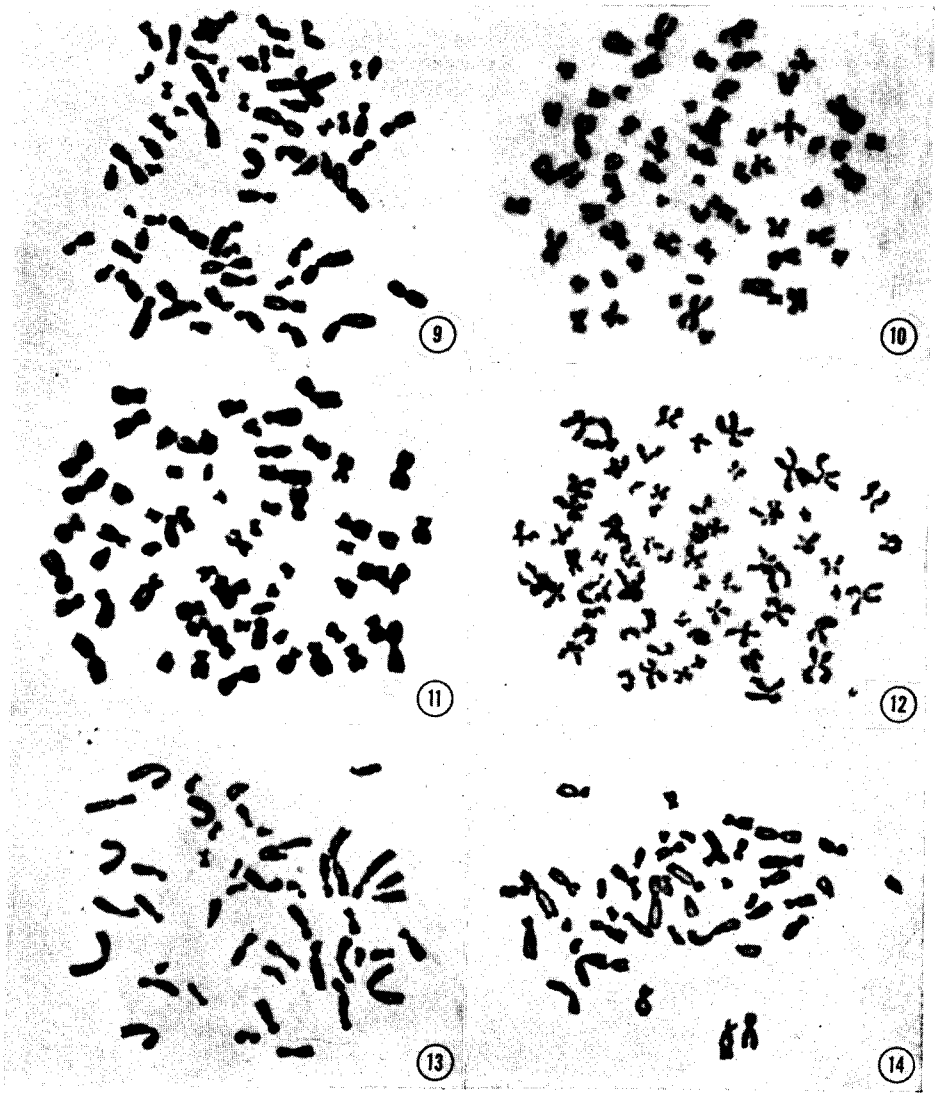
Figs. 9–14. Photomicrographs of chromosomes in the stemline cells of the gastric tumor. 9–10, 65 chromosomes. 11–12, 63 chromosomes. 13–14, 46 chromosomes.

Explanation of Plate XIV

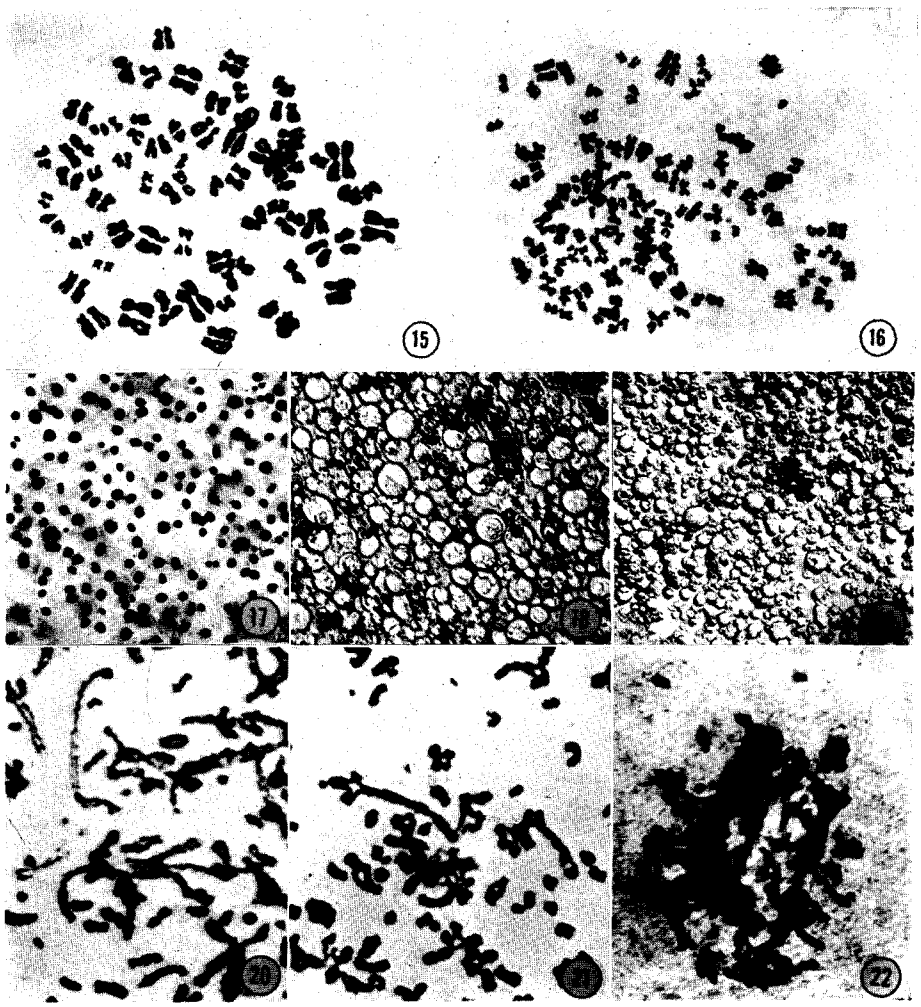
Figs. 15–16. Photomicrographs showing endoreduplication of chromosomes. 15, 65 diplochromosomes. 16, 130 diplochromosomes.

Figs. 17–19. Photomicrographs showing cell populations in the different three samplings from the gastric tumor. 17, 1st sampling. 18, 2nd sampling. 19, 3rd sampling.

Figs. 20–22. Photomicrographs showing chromosome abnormalities caused by Au^{198} treatment. 20, fragmentation and bead-like elongation of chromosomes. 21, translocation of chromosomes. 22, stickiness of chromosomes.



M. S. Sasaki: *Chromosomes of a Gastric Tumor following* Au^{198} *Treatment*



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