Cytological Studies of Tumors, XXVI Chromosome analysis of a human mammary carcinoma

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One of the most important contributions in the understanding of many fundamental aspects of cancer problems may be offered by the cytological study of tumors, since either growth or inhibition of the tumor is essentially connected with the mechanism of cell division. The recent discovery of ascites tumors in rats and mice, or the conversion of solid tumors into ascites phase have furnished evidence for understanding of the genetic pattern of tumors and chromosomal mechanism in malignant growth (Makino 1957b). Working with rat ascites tumors in recent years, Makino has developed the concept of stemline-cells which emphasizes that there do exist stem-cells as principal contributors to the neoplastic growth maintaining the characteristic genetic pattern of each tumor (Makino 1951, 1952a, 1952b, 1956, 1957a, 1957b, Makino and Kanô 1955). Tumor cells most frequently occurring with characteristic chromosome-number mode along with a specific chromosome ideogram form a stem-lineage (or lineages) of tumor cells which primarily function as the progenitors of the neoplastic population. The existence of stem-cells has been established in many mouse ascites tumors (Levan and Hauschka 1952, 1953, Tjio and Levan 1954, 1956, Sachs and Gallily 1955), and in venereal tumors of the dog (Watanabe and Azuma 1956, Takayama 1958). Recent investigations going on with human neoplastic cells have presented valuable data which serve to supplement the stem-cell hypothesis (Hansen-Melander, Kullander and Melander 1956, Levan 1956, Ising and Levan 1957, Manna 1957). In the present paper the authors describe the results of the chromosome analysis of a human mammary carcinoma which favor and strengthen the theory of the stemline-cell.

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Method. The material for the present study consists of tumor cells obtained from the pleural fluid occurring in a patient with mammary carcinoma in the University Hospital, Sapporo. The diagnosis of the tumor was made by experts of the Pathological Department. The tumor cells were obtained by centrifuging the pleural fluid for five minutes at 700 rpm. The material was smeared and stained with acetic dahlia subjected to a water pre-treatment method (Makino 1957b).

Records of the tumor patient. The patient who furnished the present materials was a woman, 41 years old. The mammary tumor was first found on her right breast in 1953, being in size as large as a pigeon's egg. Mastectomy performed in January 1957. In February of the same year, the patient received radiation therapy at 6000 r irradiation. She showed much exudation in the middle of September. After microscopical examinations it was found that the exudation contained a large number of tumor cells. In

Fig. 1. Diagram showing the variation of chromosome numbers and the distribution of cells with different numbers.

the middle of October, tumor cells were also found in the peritoneal fluid. She died of disease on the 9th of November.

The result of autopsy showed re-multiplication of the tumor on the operated portion. Metastases were observed on the right 7th rib, epicardium, liver, right pancreas, right ovary pleura, peritoneum and mesentery.

Type of the tumor. Histologically the present tumor was proved to be a flat cell carcinoma of an undifferentiated type.

Observations

1. *Tumor cells.* Tumor cells, round in shape, occurring in the pleural fluid show a variety of size (Fig. 2). The mitotic rate of tumor cells was

Figs. 2-3. Photomicrographs of tumor cells in a human mammary carcinoma. 1, showing tumor cells in the pleural fluid. 2, tumor cells in the peritoneal fluid.

found to be 1 to 1.3 per cent. Noticeably, most of them are characterized by normal mitotic behavior, though there are a few cells which display the

following mitotic abnormalities; stickiness and coalescence of chromosomes, disintegration of chromosomes, lagging of chromosomes and chromosome bridges. Tumor cells occurring in the peritoneal fluid are exactly the same in every morphological aspect as those in the pleural fluid, but show a low mitotic rate of about 0.01 per cent. Most of dividing cells show disintegrating configurations (Fig. 3).

2. *Chromosome numbers.* The chromosome count was carried out in 100 cells with samples derived on different dates, namely on the 9th, 11th, 16th and 25th of October. The results of examinations are summarized in Table 1 and Figure 1. The chromosome numbers show a wide range of

Table 1. Chromosome number distribution in four samples obtained at different days

Sample No.	Chromosome												
	33...	41....	52	53...	64....	67	68	69	70	71	72	73	74
I (Oct. 11)	1				1	1		1			3	1	
II (Oct. 16)		1	1	1	1								1
III (Oct. 19)					1			2	1	2	1	1	
IV (Oct. 25)					1	1			1	1		1	
Total	1	1	1	1	4	2		3	2	3	4	3	1
	(1)	Diploid range (3)					Triploid						

number											No. of cells obsv.
75	76	77	78	79	80	81...	134....	145....	147...	>200	
16	2	1	1	1		1			2	1	33
15	2	1					1	2	3	1	30
9		1							3	1	22
8									1	1	15
48	4	3	1	1		1	1	2	9	4	100
range (80)						Hexaploid range (12)				(4)	

variation from 33 to 147, while the modal ploidy of this tumor is near-triploid, since in the above four samplings, 80 per cent of metaphase cells are nearly triploid. Frequent variation falls between 71 and 77, the modal number being 75 which corresponds to a hypertriploid number of human chromosomes. Indeed, 48 per cent of the cells under study showed 75 chromosomes. The diploid range was observed in only three cells, though none of them showed human basic chromosome number. There were 12 tumor cells with the chromosomes in a hexaploid range, nine of which were hyperhexaploid cells with 147 chromosomes. Based on these results, it is highly reasonable to consider that the stem-cells as the principal contributors

to the neoplastic growth of this tumor are represented by hypertriploid cells which are characterized by having 75 chromosomes (Figs. 4, 5, 6, 14,

Figs. 4-8. Chromosomes of a human mammary carcinoma in the pleural fluid. Camera lucida drawings, $\times 1000$. 4-6, chromosomes of hypertriploid stem-cells, 75 chromosomes. 7-8, chromosomes of hyperhexaploid stem-cells, 147 chromosomes.

Figs. 9-11. Ideogram analysis in three hypertriploid steam-cells, 75 chromosomes in each.

15, 16, 17, 18). Further, the evidence presented suggests that there is another additional line of tumor cells which possess a hyperhexaploid chromosome number, 147 (Figs. 7, 8, 19, 20).

3. *Chromosome ideogram.* As stated above, the most frequently occurring tumor cells are characterized by a hypertriploid complex with 75 chromosomes. According to Tjio and Levan (1956), the human chromosomes were morphologically classified into three groups as follows; M (the one with a median-submedian centromere), S (the one with a subterminal centromere), and T (the one with a terminal centromere). In comparison with the normal ideogram presented by them, it is highly probable that the present tumor ideogram is not to be a simple euploid duplication of the human complex.

Ideogram analysis was made in five ideal metaphase plates which show 75 chromosomes in each with indisputable clearness. Three of these ideograms are illustrated in Figures 9, 10, 11. Analysis made it clear that each of them is composed of approximately 37 M-chromosomes, 28 S-chromosomes and 10 T-chromosomes. It is noticed that the remarkable feature characteristic of these cells is the presence of a large M-chromosome and a long S-chromosome.

Figs. 12-13. Ideogram analysis in two hyperhexaploid stem-cells, 147 chromosomes in each.

Ideogram analysis was further carried out in three hyperhexaploid tumor cells which show 147 chromosomes in each (Figs. 12, 13). Each of them is composed of about 63 M-chromosomes, 63 S-chromosomes and 21 T-chromosomes. The particular character to be noticed is that two large M-chromosomes and a long S-chromosome characterize this type of cell. It is thus evident that the hyperhexaploid tumor cell has almost twice as many chromosomes as those of the hypertriploid cell, though there is a possibility that the hyperhexaploid complex is not a simple duplication of the hypertriploid complex.

Discussion

The results of the present investigation have clarified that the mammary carcinoma here under consideration are provided with the stem-cells each of which is characterized by a hypertriploid complex, on the basis of the facts that the hypertriploid cells occur in the highest frequency, divide with

a regular mitotic manner and show a characteristic ideogram. The frequency in variation of chromosome number is most usual between 71 and 77, a modal number being at 75 which is a hypertriploid number of the normal human chromosomes.

Figs. 14-20. Photomicrographs of chromosomes of a human mammary carcinoma. $\times 1000$. 14-18, hypertriploid stem-cells, 75 chromosome in each. 19-20, hyperhexaploid stem-cells, 147 chromosome in each.

Several human near-triploid tumors have already been reported by Hansen-Melander, Kullander and Melander (1956) in ovarian cytsocarcinoma, Ising and Levan (1957) in tumors in stomach and lung, and Hsu and Moorhead (1957) in a tumor in tissue culture. Recently, the junior author (T.I.) has had an opportunity to study the chromosomes of three maxillary tumors which

showed near-triploid numbers such as 69, 71 and 73 (unpublished). It is very interesting to see that there are human tumors existing in a triploid range of chromosomes.

Ising and Levan (1957) have suggested that the optimum chromosome number region of human tumors is wide; it starts from hypodiploid, having hyperdiploid at maximum, and gradually slopes down to a region above the triploid number. They have stated that the hypertriploid region is an upper outpost of the optimum region.

Though no sufficient analysis has been made, the hyperhexaploid tumor cells with 147 chromosomes seem to contribute as an additional type of stem-cells to the growth of the present tumor. It is probable that the hypertriploid complex is not a simple duplication of the somatic complex, and further that the hyperhexaploid complex is not a simple duplication of the hypertriploid complex. Apparently, as pointed out by Makino (1957b), there may exist in the tumor chromosomes many possible structural and mutational changes involving rearrangements and adjustments which may be associated with the neoplastic condition.

Summary

The present paper describes the results of a chromosomal analysis in tumor cells of a human mammary carcinoma. The material for study was derived by centrifuging from the pleural fluid from a patient. It was established that there is present at least a stemline of tumor cells which are characterized by a hypertriploid complex of chromosomes, primarily contributing to the neoplastic growth. A possible item of evidence has been presented that there may be an additional existence of tumor stem-cells with a hyperhexaploid complex.

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