Further Data on the Common Origin of Various Stem-Lines in Human Tumors

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The stem-line concept in malignant neoplasms, first documented in rats and mice (Makino 1952, Makino 1956, Levan 1956) is supported both by experimental and clinical data (Makino *et al.* 1964). Although microspectrophotometrical investigations suggest that when a tumor exhibits several stem-lines, these are closely related, the filiation between the different lines of a tumor can be exactly established only by chromosomal studies; unfortunately, exact karyotyping of polyploid cells is not always possible for technical difficulties. In this paper, a human tumor with several stem-lines the relationship of which could be established by chromosome study is presented.

Materials and methods

The patient, aged 52 was admitted for a left ovary carcinoma with ascites and metastatic right pleurisy.

Chromosomes were studied by the direct technique of Slot (1967), slightly modified. Briefly, the ascitic fluid was centrifuged and the supernatant was removed. The cells were hypotonized for 30 min in potassium chloride 0.075 M at 37°C. After 5 fixations in a cold mixture of methanol: glacial acetic acid 3: 1, slides were made and stained with Giemsa dye.

Results

Seventy-nine metaphases were exactly counted. The results are shown in Table 1.

The majority of the cells are hypodiploid. The modal chromosome number was 38: 27 cells (37.2 per cent) had 37 or 38 chromosomes (s). A minor range between 66 and 75 chromosomes was found, with 10 cells (12.6 per cent) having 74 or 75 chromosomes (2 s). One cell had 138 chromosomes (4 s).

Karyotype analysis revealed four marker chromosomes in the hypodiploid cells: 2 large submetacentric chromosomes (M_1 and M_2) the second being somewhat longer than the first; a long acrocentric chromosome (M_3) and a ring chromosome (M_4) (Fig. 1). The majority of the cells exhibit all these marker chromosomes, but some cells were noted with only 3 marker chromosomes; the latter cells did not show the tendency for the preferential loss of a certain marker chromosome.

In the cells in the range of chromosome counts between 66 and 75, at least 3 out of the 4 marker chromosomes appeared in duplicate. Fig. 2 shows the karyotype of metaphase with 66 chromosomes; two M_1 -type chromosomes, two M_2 -type chromosomes, two M_3 -type chromosomes and one ring chromosome can be seen.

Fig. 1. Karyotype of a cell with 38 chromosomes. There are four marker chromosomes (M_1 , M_2 , M_3 , M_4).

The cell with 138 chromosomes (Fig. 3) exhibits one submetacentric M_1 -type chromosome, three submetacentric M_2 -type chromosomes, four long acrocentrics (M_3 -type) and four ring chromosomes (M_4 -type). Two dicentric chromosomes (Di) and an acrocentric chromosome slightly longer than M_3 -type chromosomes have also been observed.

The examination of the metaphases with 46 chromosomes revealed diploid karyotypes. The metaphases with 45 chromosomes and the metaphase with 44 chromosomes were probably normal "broken" cells, judging by the chromosome morphology.

Fig. 2. Karyotype of a cell with 66 chromosomes. There are two M_1 -, two M_2 -, two M_3 - and one M_4 -type chromosomes. A minute chromosome (m) can also be seen.

Fig. 3. Karyotype of a cell with 138 chromosomes. There are one M₁-, three M₂-, four M₃- and four M₄-type chromosomes. Two dicentric (Di) and a long acrocentric chromosome (T) can also be seen.

Discussion

Microspectrophotometric observations and cytogenetic studies strongly suggest the similarity of the cells belonging to the tumor stem-line (Atkin 1969 a, b, Atkin *et al.* 1967, Hauschka 1961, Hansen-Melander *et al.* 1956, Ising and Levan 1959, Levan 1956, Makino 1956, Makino *et al.* 1959, Makino *et al.* 1964, Sandberg and Yamada 1966, Sandberg *et al.* 1967, Sandritter 1968). These observations

Table 1. Chromosome counts in 79 metaphases

Chromosome counts	30	32	33	34	35	36	37	38	40	43
Number of cells	1	2	4	1	6	6	12	15	1	2
Chromosome counts	44	45	46	66	67	70	73	74	75	138
Number of cells	1	3	10	1	1	1	1	4	6	1

support the hypothesis that all the cells of a tumor arise from a single malignant cell (Atkin 1969 a, b, Atkin *et al.* 1967, Stich 1963 a, b).

The study of marker chromosomes suggests that the minor lines observed in some human tumors could have arisen from the cells belonging to the major line (Atkin 1967). In the case presented here the cells in the range of chromosome counts between 66 and 75 are clearly derived from the hypodiploid cells, by polyploidization. This fact is confirmed by the existence of a double number of marker chromosomes. Moreover, chromosome study could establish the relationship between the major line cells and the cell with 138 chromosomes. It is not clear why the doubling phenomenon does not involve in all cells, all the marker chromosomes. The probable cause, is represented by mitotic anomalies, but technical reasons ("broken metaphases") cannot be ruled out. Atkin (1970) observed that the incidence of dicentric and ring chromosomes in the tumor cells is generally low and suggested that their loss does not impair the tumor development. The constant presence of ring chromosomes in our case suggests that in some cases they "could carry genetic information necessary for the growth of the tumor" (Katayama and Masukawa 1968).

The diploid cells are probably leukocytes or mesothelial cells which appear as a consequence of an inflammatory reaction (Ishihara and Sandberg 1963).

The data presented, strongly favor the hypothesis of the single cell origin of human neoplasms. Recent studies based on the determination of glucose-6-phosphate dehydrogenase types also suggest that human tumors, both benign (Linder and Gartler 1965, Murray *et al.* 1971, Townsend *et al.* 1970) and malignant (Beutler *et al.* 1967, Fialkow *et al.* 1972, Park and Jones 1968) have, with few exceptions (Beutler *et al.* 1967, Smith *et al.* 1971), a clonal origin. The special situation of some genetically determined tumors which have enzyme patterns suggesting multiple cell origins (Fialkow *et al.* 1971, Gartler *et al.* 1966) could be explained by a common predisposition of these cells to malignant change (Fialkow *et al.* 1972).

Summary

Cytogenetic findings in a malignant effusion of ovarian origin are presented. Karyotype analysis revealed the existence of several cell lines. The relationship between these lines has been established by the study of the marker chromosomes; the cells belonging to the minor lines arose by polyploidization from the major line cells. These data support the concept of the single cell origin of various cell lines in human tumors.

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