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On the Chromosome Distribution of Uterine Carcinoma in Culture[1]

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배양한 자궁암세포의 염색체 구성에 관하여

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적 요

두 케이스의 자궁암 조직을 배양하여 그들의 염색체를 구룹별로 분석한 결과는 아래와 같다.

1. 증족세포의 염색체수가 한 경우는 46이었고, 다른 한 환자에서는 60으로서 저 3배성을 나타내었다.

2. 후자의 핵형을 구룹별로 분석한 결과, 구룹간의 염색체 분포는 non-random인 것으로 나타났다.

INTRODUCTION

Within the past two decades, great methodological improvements were made in the field of mammalian cytogenetics. These advances have facilitated precise and reliable analyses of chromosomes in cancer cells *in vitro* or *in vivo*. Thanks to the laborious works of Ishihara et al. (1963) and Makino et al. (1964), it is generally accepted that chromosomal anomalies exist in many different cancers of human, and it is often possible to find a variety of different karyotypes and modal numbers within a given cancer. They found a very wide variation in stem-line number from 39 to 133 in gastric, uterine, mammary and ovarian carcinomas. In addition, it was revealed that no chromosomal features correlated to their different tissue origin existed.

But Steenis (1966) and Levan(1966) found some interesting phenomena concerning the non-random distribution of the chromosomes over seven groups of the Denver classification with the data of Ishihara et al. and Makino et al. Thus, it was revealed that the chromosomes of group C tend to increase in number, while group D and G decrease.

This paper deals with chromosome compliments in clutured cells from 2 cases of uterine carcinoma in solid phase, with particu-

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lar interest to the distribution of chromosome numbers and variation of karyotypes in relation to non-randomness. The karyotypic patterns will be discussed whether there exist stability/or unstability in some group (s) or not.

MATERIALS AND METHODS

Cells were obtained from solid cancer of uterine taken surgically from patients. Some clinical and pathological data for each case are listed in Table 1.

The samples were stored in TC-199 medium for transport to our laboratory, and then throughly rinsed 2 or 3 times with $Ca^{++}$and Mg^{++} free PBS. Tissues were prepared for digestion by mincing with fine scissors in a small beaker of trypsin solution which composed of 0.25% trypsin (Difco) in PBS at room temperature. Then, tissue fragments were transferred to the fresh digestion mixture in Erlenmeyer flask, and were agitated on a magnetic stirrer at room temperature. After 30 to 40 minutues, the trypsin solution was changed with TC-199 medium to prevent excess digestion, and magnetic dispersion was resumed for 20 to 30 minutues. Mixture was then washed 3 times with fresh medium by means of centrifugation. After last washing, a pellet of cells at the bottom resuspended by manual shaking. Depending on the cell concentration, one to four milk dilution bottles containing 8 ml medium each were seeded with the cell suspension. Culture medium was supplemented with 50% bovine serum. The fresh medium was changed 3 or 4 times during 7-day culture. About 4 hours before harvesting of the cultures, colchicine mixed in serum free medium was added at a final concentration of about 0.1 mg/ml.

Chromosome preparations were made from cultures fixed in acetic-alcohol, air dried and stained with Giemsa, as described previously (Kang et al., 1968).

The counting of chromosomes was made from sketching or photographing under a 100x objective and 15x ocular. Only the well-spread and clean metaphases were selected for analysis. Karyotype analyses were done with suitable size of photographs of reliable metaphase chromosomes. For classification and identification of individual chromosomes, Denver-London system(1960, 1963) was used.

OBSERVATION

Summary of chromosome constitution of each case is presented in Table 1, together with clinical and pathological data obtained.

Case 1. Nine dividing cells gave counts in the range of 38-47. The chromosome numbers were distributed normal diploid range with the mode at 46. About 56% of metaphases had chromosome numbers in 46. But, there are some trouble to suggest the modal number of this population due to the small metaphases scored. The result of karyotypic classifications is given in Table 2, and it can be seen that no cell resembles another with respect to its chromosomal distribution. Owing to the low mitotic index of this culture, we excluded this data from statistical analysis.

Case 2. The preparation from this sample provided well spread 42 cells. The chromosome numbers varied from 27 to 122. Major range of chromosome number distribution falls into the range of 53-62. This range inc-

Table 1. A summary of clinical, pathological and karyological data obtained

Case No.	Tumor type	Patient's age	Sampling date	Sampling from	Histo-pathol. structure	Grade of differentiation	Therapy (chemo. or radio.)	Major range of chormosome No.	Modal No.	Cells scored
1	Cervix carcinoma	46	8/19/71	Solid tissue	Squamous cell carcinoma (spindle cell type)	Stage IA	None	44-47	46 (55.6%)	9
2	〃	44	2/2/72	〃	Squamous cell carcinoma (transitional cell variety)	Stage IB	None	53-62	60 (23.8%)	42

Table 2. Distribution of chromosomes in case 1

Chromosome No.	Group						
	A	B	C	D	E	F	G
38	2	2	16	7	4	3	4
44	5	4	16	6	5	4	4
46	6	4	17	6	5	4	4
46	8	4	14	8	5	4	3
46	4	4	20	6	6	4	2
46	6	5	15	6	8	4	2
46	5	6	14	6	6	6	3
47	6	5	16	6	5	5	4
47	5	3	18	7	6	4	4

Table 3. Distribution of chromosomes in case 2

Chromosome No.	Group						
	A	B	C	D	E	F	G
27	3	2	9	5	4	2	2
28	9	2	11	0	3	3	0
30	4	2	6	7	7	4	0
44	4	4	16	7	8	4	1
47	5	4	18	7	8	3	2
49	6	4	16	6	11	4	2
50	8	3	12	8	9	6	4
53	9	1	19	8	8	4	4

53	7	4	15	10	6	7	4
54	6	4	20	7	9	6	2
58	8	4	24	5	9	6	2
58	5	2	28	8	8	4	3
58	9	2	18	8	13	4	4
59	6	4	23	6	12	4	4
59	6	4	20	8	9	7	5
59	4	4	22	8	10	6	5
60	6	2	19	10	14	4	5
60	6	1	28	8	9	4	4
60	3	4	18	9	9	10	7
60	10	4	16	10	10	6	4
60	4	2	26	7	8	8	5
60	8	2	22	10	8	8	2
60	7	1	24	6	8	6	8
60	8	4	24	8	6	7	3
60	8	2	20	8	12	8	2
60	6	4	23	8	12	5	2
61	9	4	18	8	12	7	3
61	5	5	21	6	13	5	6
62	6	6	24	8	9	5	4
62	10	4	20	7	14	4	3
62	6	4	23	8	12	5	4
62	10	4	18	8	12	6	4
62	10	4	18	10	10	8	2
62	5	3	22	10	15	6	1
62	12	2	20	10	8	8	2
74	12	4	24	11	6	8	9
77	10	4	28	9	10	10	6
114	8	4	48	18	14	14	8
117	14	4	35	16	36	6	6
118	22	8	34	18	16	10	10
119	15	6	36	20	16	10	16
122	8	4	46	22	22	10	10

ludes about 66% of total metaphases, with a hypotriploid mode of 60 (Table 3). The cells with stem-line number of chromosomes made 23.8% of the metaphases. Cells with large chromosome number (up to 114) were observed, which were presented in 11.9% of the spreads. In this case, due to the large number of chromosomes, their identification and distribution in various groups as shown in Table 3 may not be quite accurate. In

Table 4. Significance test applied to the data of Table 3.

	Group														Total
	A		B		C		D		E		F		G		
	O*	E*	O	E	O	E	O	E	O	E	O	E	O	E	
Chromosome No.	327	350	146	233	932	934	381	350	456	350	261	233	180	233	2683
O—E	−23		−87		−2		+30		+106		+23		−53		0
P for deviation from expected	>0.3		<0.001**		>0.95		>0.05		<0.001**		>0.05		<0.001**		

*O, observed; E, expected **highly significant

contrast to case 1 , this patient showed a remarkable variation of chromosome numbers of a wide range. The cells with small chromosome number below modal region was 11.6% of the metaphases. Some of them have near haploid number.

From the data of Table 3 they seemed to be karyotypically heterogeneous. In all cells the chromosomal distribution was not identical with each other. In the case of cells which have equal number of chromosomes, their karyotypic patterns were not identical. For instance, the chromosomal distributions of the stem-line cells were different (Figs. 1&2).

In order to investigate the stability of certain groups of chromosomes, the chromosome numbers expected in each class were calculated for each chromosome number under the assumption of the same proportions between the classes as in normal female cells.

For the statistical significance of the data, the Chi-square method was applied. The result of these tests is shown in Table 4. It can be seen from the result that chromosomes are not randomly distributed over the groups ($P<0.001$). Thus groups B and G were remarkedly decreased, while opposite trend was observed for group E.

Of particular interest was the finding that whole missing of group D occurred in some cells and missing of G in another (Figs. 3 & 4).

DISCUSSION

It is well known that chromosomal anomalies are common feature of cells of malignant tumors. Most often solid tumor that have been investigated are well-established cancers having undergone extensive chromosome rearrangements. In 1962 Spriggs et al. observed that variations from diploid compliments were present in uterine carcinoma. Makino et al. (1959, 1964) analyzed stem-line karyotypes of 18 patients with uterine carcinoma *in vivo*. They found that the modal-numbers varied from 43 to 86, but with preferential range from 44 to 60. Recent extensive studies of Granberg (1971) are consistent with the earlier findings. Kang et al. (1968) reported that the modalities of uterine carcinoma in Koreans were recorded as 45 or 46 from cultured materials. In the present study one patients showed 60 modal

chromosome number. This data indicates that the possibility of a wide variation in stem-line number might occur in our situation.

On the point of view of the development of cancerous state, gross chromosomal changes appeared in early stage prior to the onset of invasion (Spriggs et al., 1962). There was no correlation between the grade of malignancy and the modal number (Ishihara et al., 1963; Makino et al., 1964). Hence, it is difficult to state that the difference of modality may be concerned with their pathological conditions between case 1 and 2 (Table 1).

Karyotypic analyses in human tumors have often given the general impression that certain group(s) of chromosomes occurred in higher proportion than in the normal karyotype, and other group(s) in lower proportion. Levan et al. (1963) reported that chromosomes of C group including X were often overpresented in leukemia, and D and G groups were underpresented (Moorhead and Saksela, 1963). The same phenomena of non-randomness were suggested by Levan (1966) and Steenis (1966) in stem-line karyotypes of solid or effusion cancers. Recent report of Granberg (1971) agreed, in general, with the above facts. However, he suggested that deficits of chromosomes were also appeared in group B. The materials used by him were unique species of cancer, uterine carcinoma. As reliable mechanisms of this tendency, Levan and Steenis argued that the formation of reciprocal translocations between the D and G groups would give rise to chromosome types that morphologically fall into the C group. Finally, this type of rearrangements may be causes of abnormal chromosome compliments of widely different tumors. Although the data presented in Table 4 are few and preliminary, owing to the only one case, the tendency of non-randomness exsist. Further analysis of cases will furnish the detailed tendency between various groups.

The significance of karyotypic changes in relation to mechanism of carcinogenesis and to the maintenance of malignancy is now in debate.

But in the recent series reported by Rabinowitz and Sachs(1970) and Hitotsumachi et al. (1971, 1972) the chromosomal control mechanism of carcinogenesis was proposed. They reported that malignant cell transformation and its reversion were controlled by the balance between chromosomes that determine the expression (E) of malignancy and chromosomes that determine its suppression(S) in golden hamster. Balance between E and S was changed by chromosomal rearrangements. They pointed out that chromosomes in group 5 (submetacentrics) carried factor for E and chromosome in groups 6, 7 and 9 (all acrocentrics except three) contained factor for S. Hence, there may be some correlation between non-random distributions of chromosomes of human cancer and their hypothesis. But one wonders whether these can be merely coincidental facts or not.

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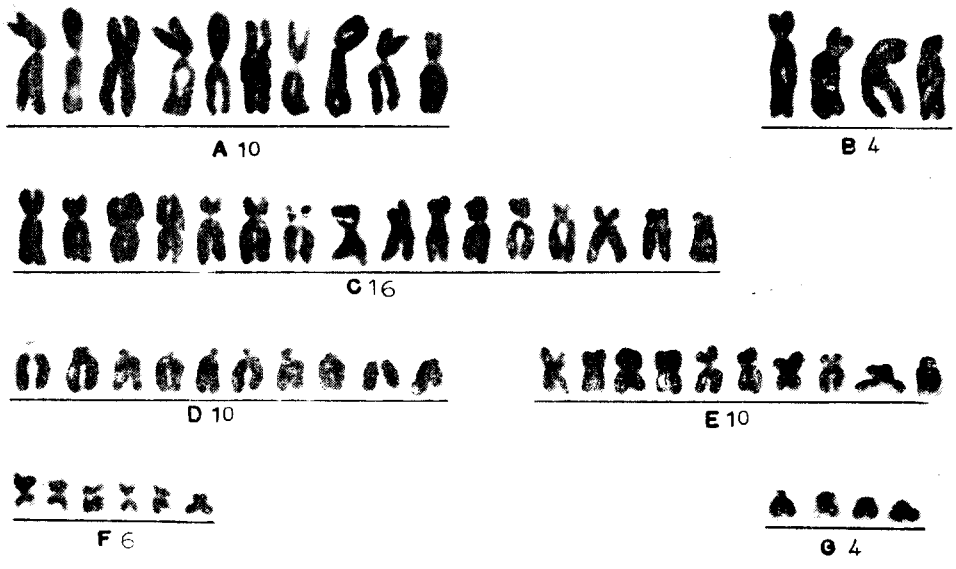

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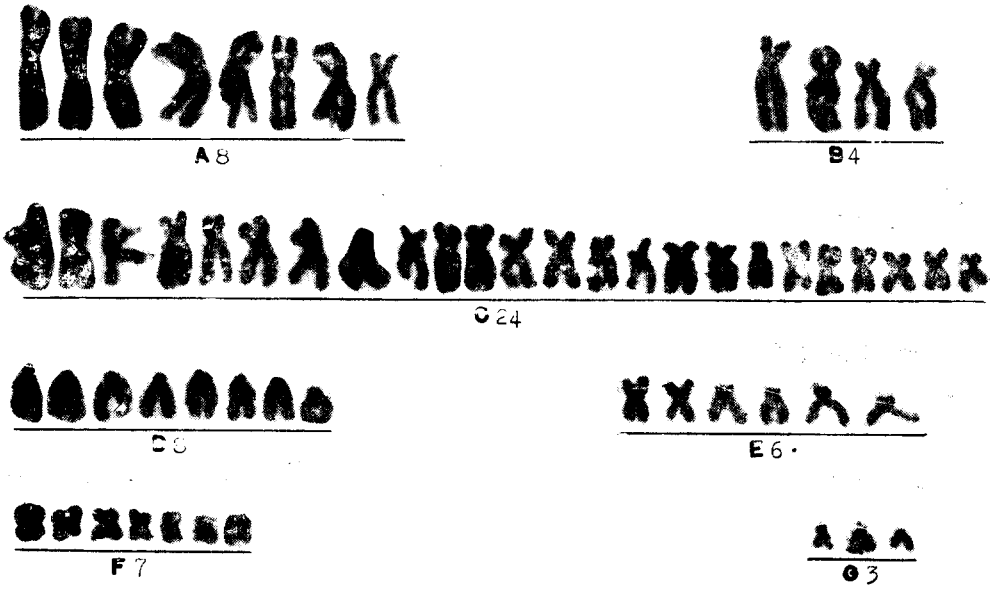
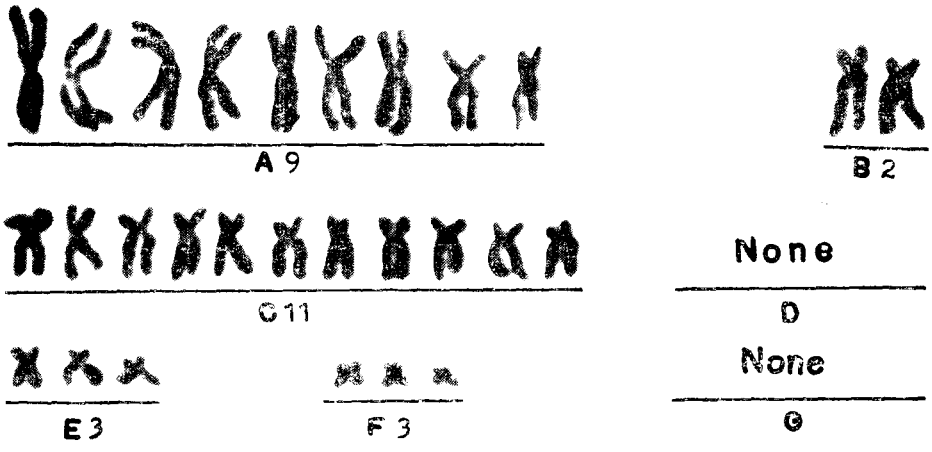


2

Figs. 1 & 2. Karyotypes of cells with modal chromosome numbers (60) in case 2.



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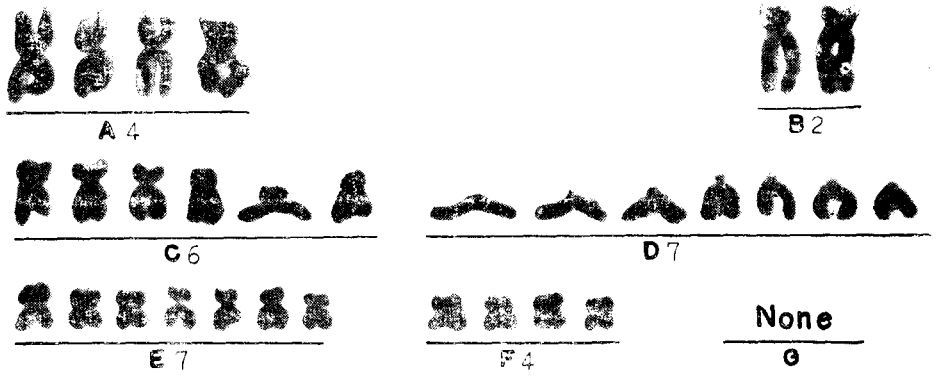

4

Fig. 3. Hypodiploid metaphase and karyotype containing 28 chromosomes from case 2. Whole chromosomes are missing in group D and G.

Fig. 4. Metaphase and karyotype containing 30 chromosomes from cell of case 2. Note the lack of chromosomes in group G.

SUMMARY

The chromosome constitutions in two cases of uterine carcinoma were studied *in vitro* with particular interest to the non-random distribution. The results obtained are as follows:

1. The chromosome numbers of one case were found within a normal diploid range with mode at 46, while those of the other were within hypotriploid range with mode at 60.

2. The data from the latter case indicated possible existence of non-random distribution of chromosome.

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