

CYTOGENETIC STUDIES OF LEUKEMIA INDUCED BY 6,8,12- AND 7,8,12-TRIMETHYLBENZ(A)ANTHRACENE*

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Specific chromosomal abnormalities found in rat leukemia induced with 7,12-dimethylbenz(a)anthracene(7,12-DMBA)(1,2) are important in research on chemical carcinogenesis since they were shown to affect cell differentiation (3).

Recently, other aromatic hydrocarbons such as 7,8,12-trimethylbenz(a)-anthracene(7,8,12-TMBA) were shown capable of inducing leukemia and some other cancers in the rodent (4-6). They were also proved to elicit adrenal necrosis in the rat (7,8). Therefore, they seem to act on an animal cell in the same way as their prototype 7,12-DMBA does. Thus, it seems worthwhile to study chromosomes of rat leukemia induced with these compounds.

Materials and Methods

Male and female rats of noninbred Long-Evans strain maintained and raised in this laboratory were used. They were fed a commercial ration (Rockland diet), received water *ad libitum*, and were kept in an air-conditioned room at $25 \pm 2^\circ\text{C}$.

A lipid emulsion of 7,8,12-TMBA¹ (0.5%, w/w) or 6,8,12-TMBA² (0.5%, w/v) was injected into the caudal vein. The first pulse dose was given 21-27 days after birth. A dose of 35 mg/kg body weight of each compound was given 4-6 times at intervals of 10-14 days (6). Leukemia rats were killed under ether anesthesia at the terminal stage of the disease. All the leukemia examined showed the macroscopic appearance of *hepatic type* which was described in the previous papers (6,8).

Since there was no striking difference of karyotype of the leukemia cells between bone marrow and liver or spleen in 7,12-DMBA-induced leukemia (2), mostly chromosome analysis of bone marrow cells was performed in the present experiment. Approximately 40 cells were analyzed in each case and at least five cells in a stemline were examined for karyotype. The nomenclature of chromosomes in the present study has been described in previous papers (1,2).

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¹ Lipid emulsion of 7,8,12-TMBA was prepared by P. E. Schurr, The Upjohn Company, Kalamazoo, Mich.

² Lipid emulsion of 6,8,12-TMBA was prepared in this laboratory using the technique described in reference 7.

RESULTS

Among the 62 leukemias induced with 7,8,12-TMBA and the 2 induced with 6,8,12-TMBA, 15 leukemias (23.4%) were shown to have the stemline(s) with chromosomal abnormalities involving the largest telocentric chromosome (C-1 chromosome). An additional eight leukemias revealed various other chromosomal abnormalities. 41 leukemias (64.1%) had a predominant stemline with normal karyotype.

Leukemias with C-1 Trisomic Anomaly (Table I)

Leukemia T-1.—Modal chromosome number was 42. Karyotype analysis of the modal cells revealed the presence of an extra long telocentric marker chromosome and monosomy of one member of C-2–C-9 chromosomes (Fig. 2). The length of the large marker chromosome was almost equivalent to the sum of C-1 and C-8-size chromosome, and this leukemia cell was considered to have C-1 trisomy of translocation type, as was described and named *translocation type 1* in one leukemia induced with 7,12-DMBA (2). 16 other cells with different chromosome number showed the same chromosomal abnormality although 12 cells with 43 chromosomes had an additional presence of one of A-group chromosomes.

Leukemias T-5 and T-6.—The predominant stemline cell had typical C-1 trisomy (Fig. 1). T-5 had another stemline with normal karyotype.

Leukemias T-16 and T-18.—Both leukemias consisted of two stemlines: one with normal karyotype and the other with C-1 trisomy of *translocation type 2* (Fig. 3) already described in many leukemias induced with 7,12-DMBA (1, 2).

Leukemia T-24.—An almost pure stemline with typical C-1 trisomy was found.

Leukemia T-27.—39 cells had a large chromosome which appeared like the large metacentric marker chromosome in *translocation type 2* C-1 trisomy (Fig. 4). However, considering that the B-1 chromosome in these cells was monosomic, it is probable that this marker was formed by translocation of an extra C-1 to the long arm of B-1 chromosome. This new translocation type has been found in one of the 7,12-DMBA-induced leukemias³ and was designated *translocation type 5*. This leukemia was accompanied with other chromosomal abnormalities: each cell had trisomy of B-2 and 23 cells with 41 and 42 chromosomes had monosomy of A-7 chromosome.

Leukemia T-32.—This leukemia was characterized by having typical trisomy of C-1 chromosome, and an accompanied monosomy of B-3 was found in 5 cells with 42 chromosomes. Another modal cell with 42 chromosomes had normal karyotype.

Leukemia T-42.—7 cells with chromosome number 41 and 42 had *translocation type 2* C-1 trisomy and 3 cells with 43 chromosomes had typical C-1 trisomy. 19 cells with 42 chromosomes had normal karyotype.

Leukemia T-50.—All the cells examined had typical trisomy of C-1 chromosome. 17 cells with 41, 42, and 43 chromosomes had monosomic B-5 chromosome and a small metacentric marker which from its appearance and the abnormal characteristics of the short arms of one B-3 was thought to be formed by arm exchange between B-3 and B-5 chromosomes. Three other cells with 40 and 42 chromosomes had small subtelocentric

³ Sugiyama, T. Unpublished data.

TABLE I
*Leukemias with Abnormal C-1 Chromosome Constitution**

Leukemia No.	Sex	Total cells analyzed	Chromosome No.								Main stemline (s)		
			40	41	42	43	44	45	46	3N	4N	%	
T-1	F	38	2a	2a	17a, 3	12a					1	1	C-1 trisomy: 86.8
T-5	F	40	1a, 1	2	1a, 14	17a, 1	2a		1a				C-1 trisomy: 55.0 2N: 35.0
T-6	F	40		2a	3a, 8	25a	1a		1a				C-1 trisomy: 80.0
T-12	F	40	5b	5b	27b, 1	2b							Long C-1: 97.5
T-16	F	40	1a	1a	20a, 18							1	C-1 trisomy: 55.0 2N: 45.0
T-18	F	40		1a	26a, 13								C-1 trisomy: 67.5 2N: 32.5
T-24	F	40		1a	4a, 2	31a		1a				1	C-1 trisomy: 92.5
T-27	F	40		3a	20a, 1	14a		2a					C-1 trisomy: 97.5
T-28	F	40	2b	2b, 1	29b, 3	1					1	1	Long C-1: 82.5
T-32	F	40		1a, 1	5a, 22	9a						2	Long C-1: 37.5 2N: 55.0
T-36	M	40		1b	34b, 5								C-1 trisomy: 87.5
T-42	F	40	1b, 3	2a, 5	5a, 19	3a	1a	1					2N: 47.5 C-1 trisomy: 27.5
T-49	F	40		1b	1b	38b							Long C-1: 100.0
T-50	F	40	1a	1a	14a	24a							C-1 trisomy: 100.0
T-61	F	40		1b	35b	1a, 2c			1c				Long C-1: 90.0

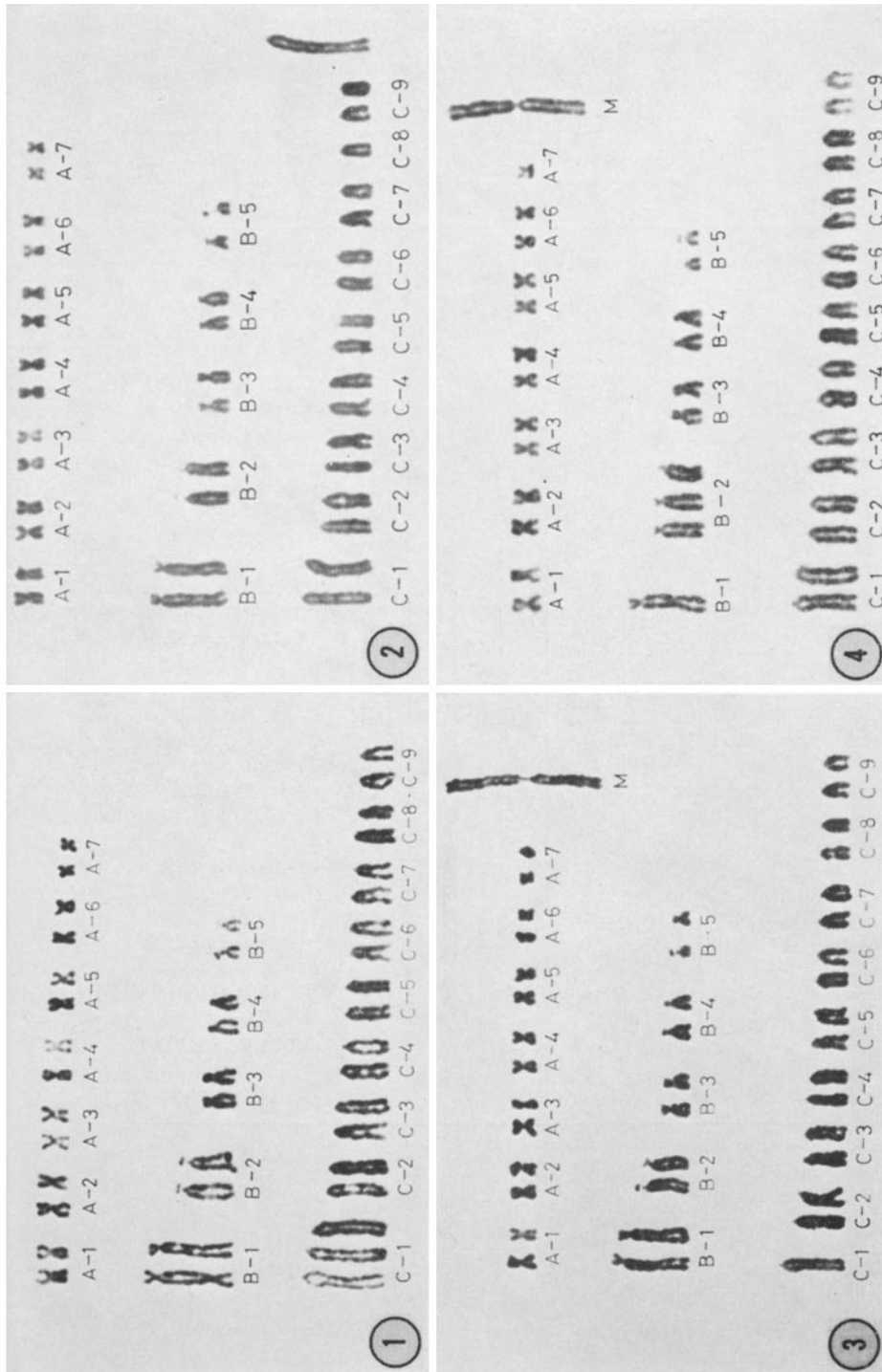
* a, cells with C-1 trisomy.

b, cells with long C-1.

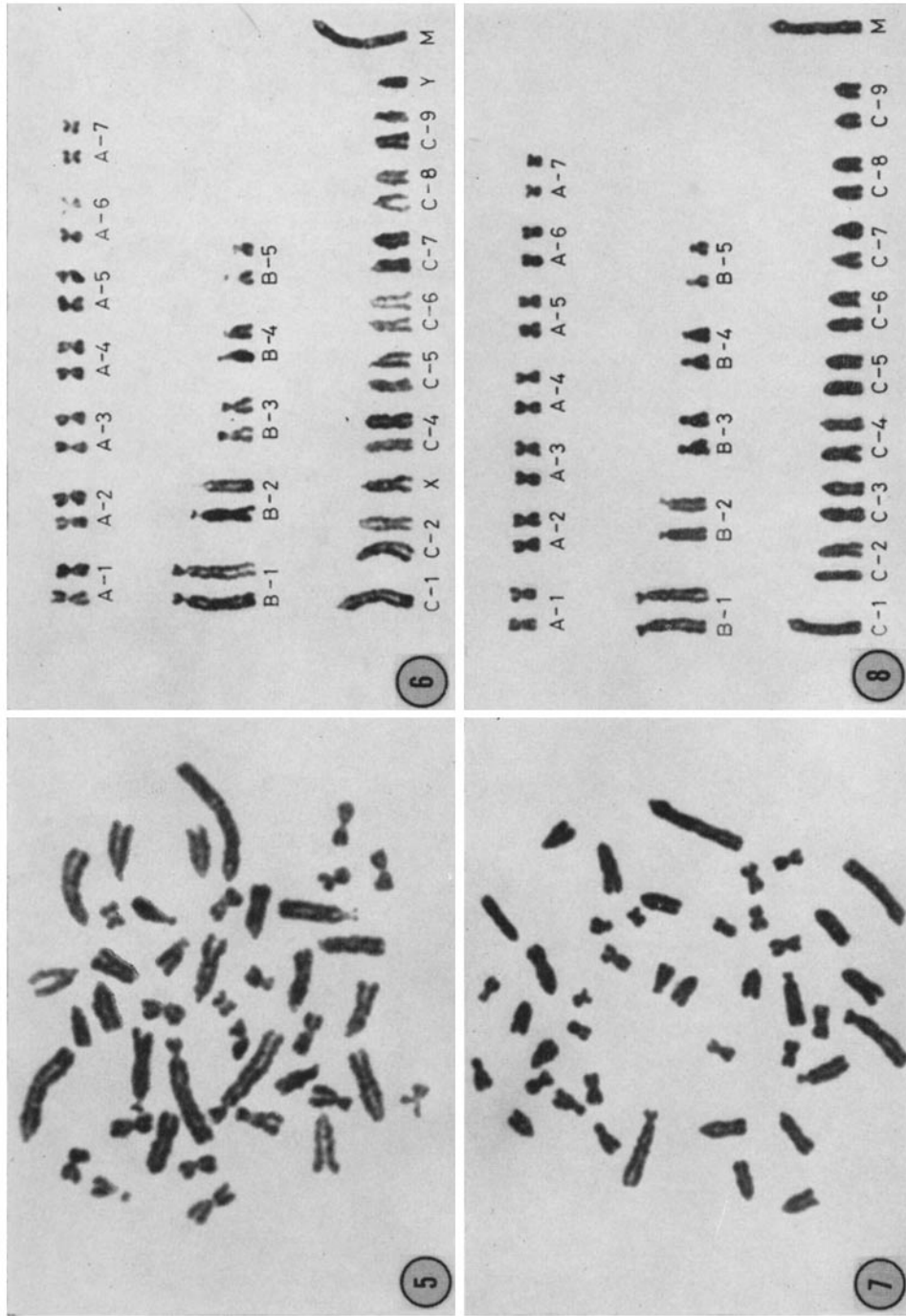
c, cells with C-1 tetrasomy.

2N, the stemline with normal karyotype.

Small Cell Populations.—As is seen in Tables I, III, and IV, a small number of C-1 trisomic cells were found in leukemias T-23, T-29, T-54, T-56, and T-61, and a few C-1 tetrasomic cells were detected in leukemia T-61. All of them were typical trisomy or tetrasomy.



Figs. 1-4. Various types of C-1 trisomy found in leukemia cells induced by 7,8,12-TMBA.
 Fig. 1. Karyotype of a leukemia cell in leukemia T-5 with a typical trisomy of C-1 chromosome. Fig. 2. Karyotype of a leukemia cell in leukemia T-1, with 42 chromosomes containing C-1 trisomy of *translocation type 1*. The large marker is considered to have been formed by a fusion of additional C-1 with a C-group chromosome possibly of C-8 size. Fig. 3. Karyotype of a leukemia cell with 42 chromosomes containing C-1 trisomy of *translocation type 2*, leukemia T-18. Fig. 4. Karyotype of a cell with 42 chromosomes having C-1 trisomy of *translocation type 5* in which an additional C-1 chromosome is possibly fused with a missing B-1 chromosome. Note other additional chromosomal abnormalities such as B-2 trisomy and A-7 monosomy, leukemia T-27.



FIGS. 5 and 6. Metaphase spread and karyotype of a leukemia cell with long C-1 chromosome (M). Chromosome number is 42. Leukemia T-36 induced with 7,8,12-TMBA.
 FIGS. 7 and 8. Metaphase spread and karyotype of a leukemia cell with long C-1 chromosome (M). Leukemia T-49 induced with 6,8,12-TMBA.

marker chromosomes which were possibly formed by translocations among B-3, B-4, B-5, and one C-group chromosome.

Leukemias with a Long C-1 Chromosome (Tables I and II)

Four leukemias T-12, T-28, T-36, and T-61 induced with 7,8,12-TMBA and one induced with 6,8,12-TMBA, T-49, were shown to have a characteristic chromosomal abnormality which could be referred to as *long C-1* (Figs. 5-8). This anomaly consisted of a striking elongation of one C-1 chromosome. Four long C-1 chromosomes found in T-12, T-28, T-36, and T-49 had no structural abnormality apart from the length, while the one found in T-61 had satellite chromosomes at the distal end. By comparison with the long arms of B-1 chromosomes, the corresponding C-1 chromosomes revealed the original length. As is clear from Table II, the degree of elonga-

TABLE II
*Degree of Chromosome Elongation in Long C-1 Chromosome Abnormality**

Leukemia No.	Carcinogen used	Elongation rate (= long C-1/C-1)
		Mean \pm SD
T-12	7,8,12-TMBA	1.349 \pm 0.087
T-28	7,8,12-TMBA	1.322 \pm 0.078‡
T-36	7,8,12-TMBA	1.271 \pm 0.040§
T-49	6,8,12-TMBA	1.263 \pm 0.067§
T-61	7,8,12-TMBA	1.590 \pm 0.125§

* The values represent the ratio of long C-1 to short (normal size) C-1, measured in eight well-spread metaphase cells.

‡ Difference from the value in T-12 not significant.

§ Difference from the value in T-12 significant in the range of $0.05 > p > 0.02$.

tion was different from case to case. By statistical study, at least three classes of elongation rate were identified: 1.26, 1.35, and 1.59. In every case, the remaining chromosomal members seemed perfect. The above five leukemias had the long C-1 cells as the predominant stemline, although there were also cases in which they remained in a small population. The elongation rate of C-1 chromosome in these cells was: 1.15 (T-7), 1.23 (T-38), 1.47 (T-48), 1.19 (T-54), 1.12 (T-56), 1.18 and 1.19 (T-58), and 1.19 and 1.11 (T-60). They were classified as a "long C-1" cell because one C-1 chromosome was apparently longer than another C-1 chromosome and at the same time it was longer than the length of C-1 expected from the mean length of two B-1 chromosomes. However, considering the fact that each corresponding chromosome complement often shows a different size as a preparation artifact, it was not clear whether all the cells listed above were cells with long C-1 abnormality, and it was also impossible to presume the elongation rate of C-1 chromosome from a measurement in one cell.

Leukemias with Other Chromosomal Abnormalities (Table III)

Leukemia T-3.—This leukemia which had 41 modal chromosome number showed the deletion of one member of A-group chromosome and one or a pair of B-3, B-4, and B-5 chromosomes, and the presence of three or four markers. One marker was a telo-

TABLE III
Leukemias with Various Other Chromosomal Abnormalities*

Leukemia No.	Sex	Total Cells Analyzed	Chromosome number								Stemline with chromosome abnormalities (%)	
			41	42	43	44	45	46	47	48		
T-3	M	25	14d, 4	1d, 6								Small subtelo- and sub-metacentric markers: 60.0
T-26	F	9		1	8d							Small metacentric marker: 88.9
T-45	M	40	3d	20d, 15		1d, 1						Large submetacentric marker: 100.0
T-63	F	40				16d	16d	6d	1d	1d	1d	Medium metacentric marker: 100.0
T-64	F	40	$\frac{51}{4d}$	$\frac{52}{7d}$	$\frac{53}{24d}$	$\frac{54}{5d}$						Two subtelocentric markers: 100.0
T-19	F	26	$\frac{2N}{1}$	$\frac{56}{1d}$	$\frac{60}{1d}$	$\frac{61}{3d}$	$\frac{62}{20d}$					Triploid with a minute: 96.2
T-55	M	40	$\frac{2N}{26}$	$\frac{3N}{1}$	$\frac{72}{1}$	$\frac{73}{1}$	$\frac{74}{3}$	$\frac{75}{7}$	$\frac{4N}{1}$			Hypotetraploid: 30.0
T-56	F	40	$\frac{42}{1b, 4}$	$\frac{43}{1a}$	$\frac{49}{2}$	$\frac{57}{1d}$	$\frac{58}{1d}$	$\frac{59}{3d}$	$\frac{60}{5d}$	$\frac{61}{15d}$	$\frac{62}{7d}$	Triploid with a minute and two submetacentric markers: 80.0

a, cells with C-1 trisomy. b, cells with long C-1. d, cells with other marker(s).

centric smaller than Y chromosome. The other three markers, from their size, seemed to be formed by translocations among the missing chromosome complements. The modal cells with 41 chromosomes consisted of cells with various combinations of the above chromosome abnormalities.

Leukemia T-26.—This leukemia was granulocytic leukemia with peroxidase-positive granules. Almost 5% of the cells had eosinophilic granules. Only nine metaphases were obtained in the chromosome specimens and eight cells had a metacentric chromosome

of a size of A-1-2 chromosomes. A detailed analysis showed that this was an extra chromosome having a slightly different arm structure from those of A-1 or A-2.

Leukemia T-45.—The presence of a large submetacentric marker and monosomy of B-1 chromosome characterized this leukemia. The long arm of this marker was almost equivalent to the long arm of B-1 chromosome. Therefore, this marker is related with monosomy of B-1 chromosome. The same abnormality was already described in one leukemia induced with 7,12-DMBA (Leukemia No. 23 in reference 2).

Leukemia T-63.—This leukemia had two marker chromosomes: one medium metacentric chromosome and one B-1-like subtelocentric marker with a slightly shorter long arm. The cells with 45 chromosomes had an additional subtelocentric chromosome as large as B-5 chromosome with a little different structure.

Leukemia T-64.—Two subtelocentric markers were present in this leukemia: one similar with B-1 but with a shorter long arm and the other a large subtelocentric marker with a prominent satellite structure. B-5 monosomy was found. Trisomies of B-3 and B-4, the increase of 4 chromosomes in both A- and C-groups made the modal chromosome number of this leukemia 53.

Leukemia T-19.—Karyotype analysis of this leukemia showed the triploid character and the presence of a minute chromosome. One member from both the A and C groups was lost constituting the chromosome number of 62.

Leukemia T-55.—This leukemia had two modal cells: one was of normal karyotype and the other was hypotetraploid with 75 chromosome number.

Leukemia T-56.—This leukemia revealed also a triploid nature with an occasional presence of a small and a medium submetacentric marker and a minute chromosome. One-third of the cells had disomic character regarding the B-1 chromosome. Because of various combinations of the above abnormalities, the modal cells with 61 chromosomes did not show a uniform chromosome pattern.

Small Cell Populations with Various Chromosomal Abnormalities.—Some leukemias contained a small cell population with various chromosomal abnormalities. Among them were B-3 trisomy in 4 cells with 43 chromosomes in T-10, 7 cells with 43 chromosomes in T-31 in which some member of C-2-9 chromosomes were triplicated. 6 cells in T-11 and T-14, 7 cells in T-4, T-9, and T-15, 8 cells in T-30, with 41 chromosomes and 5 cells with 43 chromosomes in T-57 did not show any consistent chromosome change. Cells with various markers were found in leukemias T-10, T-21, T-39, and T-43 (Table IV).

Leukemias with Normal Karyotype (Table IV)

40 leukemias induced with 7,8,12-TMBA and 1 leukemia (T-44) induced with 6,8,12-TMBA had the predominant stemline with normal karyotype.

DISCUSSION

The chromosomal abnormalities in rat leukemia induced with 7,8,12- and 6,8,12-TMBA were essentially identical with those in the leukemias induced with 7,12-DMBA. The incidence of cell populations with C-1 trisomy was considerably lower in 7,8,12-TMBA-induced leukemia. Especially interesting in the present study is a rather high occurrence of another characteristic chro-

TABLE IV
Leukemias with Normal Karyotype

Leukemia No.	Sex	Total cells Analyzed	Chromosome No.								Cells with normal karyotype (%)		
			40	41	42	43	44	45	46	3N		4N	
T-2	F	29	2	4	20	1					1	1	69.0
T-4	M	40	3	7	28	1						1	70.0
T-7	M	40	2	4	1b, 33								82.5
T-8	M	40	1	4	35								87.5
T-9	M	40	4	7	28							1	70.0
T-10	F	40	5	3	23	5d, 4							57.5
T-11	M	40	3	6	30							1	75.0
T-13	F	40	1		38							1	95.0
T-14	F	40	1	6	33								82.5
T-15	M	40	2	7	28	1	1					1	70.0
T-17	F	40		1	38			1					95.0
T-20	M	40			40								100.0
T-21	F	25	2	1	20	1d	1d						80.0
T-22	F	40		2	38								95.0
T-23	F	40			38	1a						1	95.0
T-25	M	40		4	36								90.0
T-29	F	40	2	1	31	2a, 3			1				77.5
T-30	F	37	2	8	25					1	1	1	67.6
T-31	M	37	3	3	21	7				1	1	2	56.8
T-33	F	40	2	3	35								87.5
T-34	M	40		2	38								95.0
T-35	F	40		1	39								97.5
T-37	M	40		2	38								95.0
T-38	F	40		1	1b, 36							2	90.0
T-39	F	28	1	3	2d, 20							2	71.4
T-40	F	40	1	1	36							2	90.0
T-41	F	40	2	2	33	2						1	82.5
T-43	F	40	1	1d, 4	34								85.0
T-44	M	40	6	4	30								75.0
T-46	F	36	3	4	29								80.6
T-47	F	40		1	39								97.5
T-48	M	31		3	1b, 26	1							83.9
T-51	F	40	2	1	36							1	90.0
T-52	M	40			40								100.0
T-53	M	40		2	37							1	92.5
T-54	F	32	3	3	1b, 23					1a		1	71.9
T-57	F	40		2	33	5	1						82.5
T-58	F	40	1	1	2b, 36								90.0
T-59	F	40	1	1	38								95.0
T-60	F	40	1		2b, 36	1							90.0
T-62	F	40	2	1	36							1	90.0

a, cells with C-1 trisomy. b, cells with long C-1. d, cells with other marker chromosome(s).

mosome abnormality involving the same chromosome complement, *long C-1*, in 7,8,12- and 6,8,12-TMBA-induced rat leukemia. This chromosome abnormality was also found in 7,12-DMBA-induced rat leukemias, although the cell populations having this abnormality usually did not constitute the predominant stemline (9). By measurement of chromosomes of cells from five leukemias with long C-1, at least three long C-1's of a different length were identified. Since there was no evidence of interchromosomal chromatid exchange in these leukemia karyograms, and several chromatid sites of a normal C-1 chromosome have been shown extremely susceptible to the breaking action of carcinogens⁴, possibly intrachromosomal chromatid exchanges at these target sites may be responsible for the induction of this new chromosome abnormality. Therefore, just as C-1 trisomy is a gene-redundant condition, long C-1 may be another type of chromatid duplication. From this point of view, this chromosome abnormality would be another important tool for clarifying the role of gene imbalance in malignant cell transformation suggested in the previous report (3).

Difference in the incidence of each specific chromosomal abnormality does not necessarily mean a different mechanism of chemical carcinogenesis. The difference in toxicity and the different speed of carcinogen action and possibly of metabolism might have produced a host condition in which the selective growth of C-1 trisomy cells was difficult. Although there still remains a possibility that the three hydrocarbons acted on cells in the same metabolite form, the results obtained in the present work again suggest a very specific and simple mechanism leading to the specific chromosomal abnormalities involving C-1 chromosome.

SUMMARY

Cytogenetic studies on 64 rat leukemias induced with 7,8,12- and 6,8,12-trimethylbenz(a)anthracene were performed. Highly distinctive changes were found repeatedly in one special pair of chromosomes. 10 leukemias (15.6%) showed the presence of stemline(s) with trisomy of the largest telocentric chromosome (C-1 trisomy). Another chromosome abnormality, elongation of one of the pairs of the same chromosome (*long C-1*), was found in 5 leukemias (7.8%) as the predominant stemline and in 7 other cases as a small cell population. This chromosome abnormality, despite slight differences in their elongation rate, was defined as a new specific chromosome abnormality. Other chromosome abnormalities not related with C-1 chromosome were found in 8 cases (12.5%). The remaining 41 leukemias (64.1%) had the predominant stemline with normal karyotype. From this study it appears that three structurally different hydrocarbon carcinogens, 7,12-dimethylbenz(a)anthracene and 7,8,12- and 6,8,12-trimethylbenz(a)anthracene act on blood-forming cells by a common specific mechanism.

⁴ Sugiyama, T. Unpublished data.

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