Jpn. J. Cancer Res. (Gann), 79, 1024–1030 ; September, 1988

Chromosome Fragility of Lymphocytes from Breast Cancer Patients in Relation to Epidemiologic Data

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The chromosomal fragility of peripheral blood lymphocytes from 50 women, undergoing operations for breast tumors (47 carcinomas, 2 intraductal papillomatoses and 1 malignant lymphoma) was studied to ascertain the association between chromosome fragility and epidemiologic data, such as a family or personal history of cancer, hormonal status, etc. Under conditions of folic acid and thymidine depletion, the average number of gaps and breaks on the patients' lymphocyte chromosome was 6.02 ± 5.28 and that in the control medium was 2.0 ± 2.0 while those of healthy controls were 5.8 ± 5.5 and 1.36 ± 1.22. These gaps and breaks were mostly seen in group A chromosomes (4.1 ± 2.6) in 24 patients, including the 2 with benign tumors and the 1 with the lymphoma as well as 11 healthy controls. They were frequent in group B (3.0 ± 0) in 3 patients, in group C (4.3 ± 2.9) in 11 patients, and in groups D (2.0 ± 1.0) and E (3.0 ± 1.0) in 3 patients from each. This different distribution of gaps and breaks correlated neither with the patients' age nor with their tumor's histology, but patients having a late menarche were distributed in non-A groups. There was low inducibility of breaks in patients with a family history of breast cancer and/or relatively rare cancers. The availability of common fragile sites for studying an individual's susceptibility to cancer is discussed. One patient showed a bromodeoxyuridine-requiring heritable 10q25 fragile site. Another, with triple primary cancers, showed a constitutional translocation of t(5;19)(q15;q13).

Key words: Breast cancer — Chromosome fragility — Epidemiology

The presence of familial breast cancer aggregations and Lynch's cancer family syndrome indicates that at least some patients with breast cancer should have a predisposition to breast cancer.[1,2] Although in the cancer-family syndrome, breast cancer is considered to show a dominant inheritance pattern, the presence of a recessive gene for breast cancer has not yet been determined. If the presence of these cancer-related genes could be detected using somatic cells, there could be a genetic prediction for individuals with a high risk of breast cancer.

Detailed work on how to find some useful markers for evaluating susceptibility to cancer has been done in the field of genetic epidemiology. Evaluation of sister chromatid exchange (SCE) is one approach, and a higher occurrence of cancer in Bloom's syndrome suggested its usefulness for studying genetic susceptibility. Later work, however, has revealed the frequency of SCE to be more influenced by environmental exposures, such as smoking and radiation, than by an individual's predisposition[3]; however, the fragile sites on the predisposed individuals' chromosomes seemed present to a larger extent. Fragile sites are specific sites on chromosomes, at which gaps and breaks are induced when cells are cultured in a medium deprived of folic acid and thymidine or in other media containing distamycin A, bromodeoxyuridine (BrdU), aphidicholine, etc.[4–7] Among a large recognized number of such sites, 17 have been confirmed as being heritable and over 50, constitutive.

Recent studies by Yunis and Soreng[8] showed 20 of the 51 fragile sites to be recorded in human complement maps close to one of the two breakpoints found in 26 out of the 31 specific chromosome abnormalities noted in leukemias, lymphomas and solid tumors. This suggests that errors at these sites lead to tumor-associated rearrangements, so that individuals with a higher fragile site frequency have a higher risk of producing cancer

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cells.[9] Namely, if constitutive fragile site expression is indeed genetically determined, then the inheritance of some of these sites may possibly indicate a genetic predisposition to cancer.

In the present paper, we report the frequency of gaps and breaks, with special reference to both heritable and constitutive (common fragile) fragile sites, in patients with breast tumors, analyzed in relation to other epidemiologic information on the patients.

Materials and Methods

Patients All patients were admitted consecutively to the National Cancer Center Hospital for breast cancer resection, from May to September, 1986. These patients had no history of previous radiotherapy or chemotherapy, but had undergone a routine chest X-ray a few weeks before admission. The histologies of the resected breast tumors were confirmed by surgical pathology to be, 47 carcinomas, 2 benign breast tumors and one malignant lymphoma. The patients were all women, and age distribution and histologic tumor type are listed in Table I. Twenty-one patients were premenopausal, and 29 postmenopausal. Radioimmunoassay was used to detect estrogen and progesterone receptors on the neoplastic cells in 26 patients by courtesy of Dr. Adachi, Endocrinology Division, National Cancer Center Research Institute.

Controls Eleven healthy female volunteers who had no family history of cancer were studied as controls by the same methods as used for the patients.

Cell Culture Whole heparinized venous blood (0.5 ml) was cultured in RPMI 1640 medium (Nissui, Tokyo) supplemented with 10% fetal bovine serum and 2% phytohemagglutinin M (Difco, Tokyo) and incubated at 37° in 5% CO_2 (Forma, Los Angeles) for 72 hr before harvesting. This specimen was used as a baseline control for the number of gaps and breaks on chromosomes. For detecting distamycin A-inducible fragile sites, distamycin A (Sigma, Tokyo) was added 24 hr before harvest to a final concentration of 50 μg/ml.[10] For detecting BrdU-requiring fragile sites, BrdU (Sigma) was added 24 hr before harvest to a final concentration of 7 μg/ml.[11] For detecting folate-sensitive fragile sites, blood (0.5 ml) was cultured for 72 hr in Ham's F-10 medium, free of hypoxanthine, thymidine and folic acid (Formula No. 78-5227, Gibco, Tokyo), supplemented with 5% fetal bovine serum and 2% PHA-M.[11]

Slide Preparation and Scoring Colcemid was added to the culture medium 2 hr prior to harvest, and cells were treated with 0.075*M* KCl, fixed in Carnoy's fixative (methanol:acetic acid=3:1) and dropped on to vapor-moistened slides. These were dried and stained with Giemsa (Merck, West Germany). At least 50 metaphase cells were scored in each culture tube in order to determine the number of gaps and breaks on chromosomes, and their locations were separately determined by chromosomal grouping: A to G. A standard trypsin-Giemsa banding technique was employed for detailed analyses in selected cases.

Epidemiologic Information Information on the patients' past illnesses, menarche history, menstruation cycle, pregnancies, deliveries, experiences during lactation, use of hormones, family history of cancer (especially breast cancer) and cigarette smoking habit was extracted from their hospital records.

Statistical Evaluation Multivariate analyses of various factors involved were carried out to determine whether or not they were related to the frequencies and locations of the gaps and breaks. The calculations were done by the SAS program package on a HITAC N240-H computer at the National Cancer Center.

Table I. Age Distribution and Histologic Type of Breast Tumor

Age range	No. of cases	Histologic type	No. of cases
30–34	1	Invasive ductal carcinoma	41
35–39	5	Invasive ductal + lobular carcinoma	1
40–44	4	Invasive lobular carcinoma	1
45–49	12	Apocrine carcinoma	2
50–54	8	Mucinous carcinoma	1
55–59	8	Medullary carcinoma with lymphoid stroma	1
60–64	7	Intraductal papillomatosis	2
65–69	4	Malignant lymphoma	1
70–74	1		
Total	50		50

RESULTS

Frequency of Fragile Sites on Chromosomes and Other Abnormalities The number of gaps and breaks on chromosomes in the control culture varied from 0 to 9, with a mean value of 2.0±2.0. The induction treatment by depletion of thymidine and folic acid increased the mean number of gaps and breaks to 6.0±5.3, the number increasing in all but one case (Fig. 1). The number of gaps and breaks in the healthy controls was 5.8±5.5 after the treatment and 1.36±1.22 without treatment.

The gaps and breaks on chromosomes were not evenly distributed among chromosome groups. The greatest number of sites was usually found in group A chromosomes (nos. 1 to 3) mostly due to the frequent site 3p14, and 24 patients (48%) showed this pattern. Control healthy persons and patients with benign lesions and lymphoma also belonged to this category. The mean number of breaks and gaps on group A chromosomes in this group was 4.1±2.6. Eleven patients (22%) had frequent sites in group C chromosomes (nos. 6 to 12), with a mean number of 4.2±2.9, and 3 patients (6%) in each of groups B (nos. 4, 5), with a mean of 3.0 ±1.0, D (nos. 13 to 15), with a mean of 2.0±1.0, and E (nos. 16 to 18), with a mean of 3.0±1.0. Five patients had no gaps or breaks on their chromosomes, at least none which the present induction conditions could reveal.

Only one patient was found to be a carrier of BrdU-requiring heritable fragile sites with a frequency of 15% (Fig. 2). One patient had a constitutional translocation, t(5;19)(q15; q13)(Fig. 3).

Relationship between Chromosome Fragility and Other Epidimiologic Information There was a family history of cancer in first and second degree relatives in 32 patients. Twenty-nine cancers were recognized in the first degree relatives (Table II). Rare cancers, such as laryngeal and thyroid, were noticed in the fathers, and breast and uterine cancers were much more frequent in patients' mothers than in the general population. Four of 5 cancers in patients' sisters were breast cancer, and 2 of 7 cancers in patients' brothers were leukemia. Such a trend occurred to a lesser extent in the second degree relatives, but the proportion of breast cancer in aunts was still

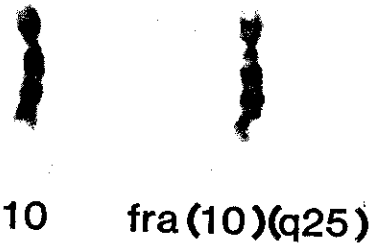

Fig. 2. BrdU-induced heritable fragile site, fra(10)(q25).

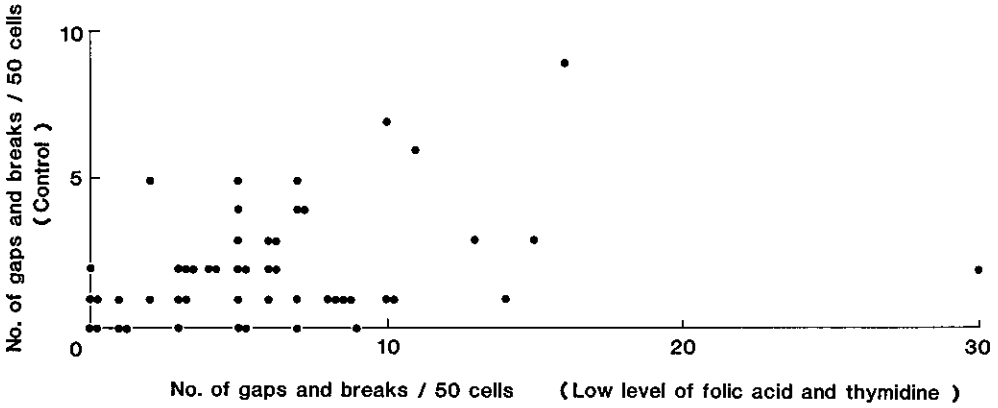

Fig. 1. Number of gaps and breaks on chromosomes in control medium (vertical line) and in folic acid and thymidine-depleted medium (horizontal line). Each dot represents one patient.

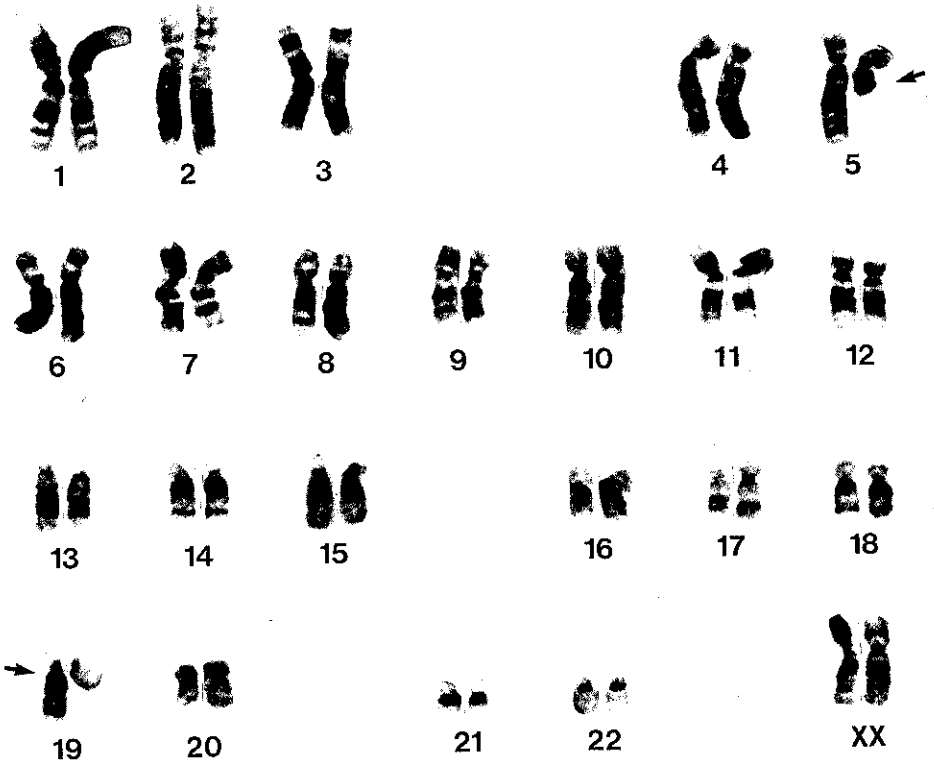

Fig. 3. Translocation of lymphocytes t(5;19)(q15;q13). This patient had triple primary cancer.

high compared to that in the general population. Family history of breast cancer among second degree relatives was present in 7 patients: 2 in mothers, 2 in sisters, 2 in a sister and an aunt and 1 in an aunt. One patient had a mother with uterine cancer and a sister with breast cancer, and in her case, the number of breaks was low (3/50); the distribution pattern was group D. Generally, patients with a family history of breast cancer of other rare cancers showed a higher number of gaps and breaks even in the control medium, so that the difference in the numbers of breaks between the control and the treatment media was small or negative.

A patient with fra(10)(q25) was 69 years old and had no family history of cancer except for her father's hepatoma. On the other hand, a patient with t(5;19)(q15;q13) had been operated on for an ovarian cyst at age 28 and for genital Paget's disease at 48, and was found to have bilateral primary breast cancer at 54. Her sister and aunt had also been treated for breast cancer, and her grandfather had died from laryngeal cancer. The numbers of breaks in this patient were 4 in the control medium and 7 upon induction.

Other factors, such as age at diagnosis, histologic type, state of menstruation, pregnancy, etc., were not related to the frequencies and distribution patterns of the gaps and breaks on the chromosomes (Table III). However, the patients who had had a previous operation, or menarche at an age of more than 15 years and those with rheumatoid arthritis, or hormone receptors, were more often categorized into a non-A group than their counterparts (Table III). There were only 7 cigarette smokers in the present study; 2 had no breaks and the others showed moderately increased values (average difference 7.4), mostly in group C chromosomes.

Table II. Numbers and Sites of Cancer with Family History

	First degree relatives				Second degree relatives	
	Father	Mother	Sister	Brother	Uncle	Aunt
Tongue	1					
Salivary gland					1	
Esophagus					2	
Stomach	3	1	1	2	6	3
Rectum				1	4	
Gall bladder						1
Larynx	4					
Lung				1	2	
Thyroid	2					
Prostate				1		
Osteosarcoma		1				
Leukemia				2		
Breast		2	4			3
Uterus		2				
Total	10	6	5	7	15	7

Table III. Fragile Site Distribution between Groups and Epidemiology

Group	Smoking	Past history						Menarche (age)		Menstrual cycle		Lactation		Hormone receptor	
		Operation +	Operation −	Myoma	Appe	RA	Basedow	≤14	15<	28–30 days	Irregular	Good	Poor	+	−
A	2	9	16	1	4	—	2	18*	4*	15	7	8	12	2	9
B	—	1	1	—	1	—	—	2	—	2	—	1	1	1	—
C	3	5	6	2	2	2	—	6	5	7	3	4	4	3	4
D	—	1	2	1	1	1	—	2	1	2	1	2	1	1	2
E	—	2	1	1	1	—	—	1	1	1	2	—	3	—	2
—	2	4	2	1	1	—	1	2	4	4	1	2	1	—	2
Total	7	22	28	6	10	3	3	31	15	31	14	17	22	7	19

Myoma, operated leiomyoma of the uterus; Appe, operated appendicitis; RA, rheumatoid arthritis.
* $P<0.05$ by χ^2 test.

Discussion

Breast carcinoma is considered to be a disease covering several etiologically heterogeneous cancers, even when it is hereditary.[12] The clarification of the individual genetic polymorphisms which could be related to susceptibility to cancer is important to determine a high risk group for breast cancer. A recently developed method for examining allelic restriction fragment-length polymorphism (RFLP) revealed RFLP in the c-Ha-*ras*-1 locus to show rare alleles in 41% of breast cancer patients, compared with only 9% in controls.[13] In a chromosome study, C-band heteromorphism, polymorphism of AG-stained nucleolus-organizer regions, and others, were applied to clarify individual differences in patients.[14, 15]

The number of gaps and breaks on chromosomes was considered in the present study to be consistent in the main with that of common fragile sites, since G-banding for seven healthy subjects showed a good correlation between the numbers of gaps and breaks and those of common fragile sites ascertained by G-banding (in preparation). In normal indi-

viduals the pattern of gaps and breaks on chromosomes is distributed mainly in group A chromosomes. On the other hand, almost half our patients had a pattern different from A. Abnormalities in chromosomes nos. 1, 9, and 16 have often been found in breast carcinoma,[16, 17] so that the fragility in these regions may be related to the cancer susceptibility. The exact significance of this different distribution of breaks in patients with breast cancer is not yet known, but it reflected some host factors, because the patients with late menarche belonged to non-A groups with statistical significance. Unlike sister chromatid exchange, the effects of cigarette smoking were not clear, although 3 of 5 smokers belonged to group C.

It was noteworthy that the patients with a family history of breast cancer or other rare cancers tended to have low or negative differences in numbers of breaks between control medium and folate depletion medium, and relatively high background values. The proportion of the various cancers in the family history was quite different from that in the general population, i.e. low incidences of stomach, lung and liver cancers and high incidences of laryngeal and thyroid cancers among fathers.

The biological role of heritable fragile sites in oncogenesis is known only to a very small extent; fra(16)(q22) has been found in two sisters from a cancer-susceptible family who had malignant or premalignant conditions.[18] Although no similar distamycin A-induced rare fragile site was found in the present study, one patient had a BrdU-requiring heritable fragile site at 10q25. Sutherland reported[19] that there was an incidence of this fragile site of about 2.5% in the Australian Caucasian population, while Sanfilippo *et al.*[20] found it to be 0.55% in 1,444 Italians who had been referred to a cytogenetic clinic. Takahashi *et al.*[21] found 3 cases (0.29%) of fra(10)(q25) in 1,022 healthy Japanese blood donors. The fragile site at 10q25 does not correspond to the cancer breakpoint, and no proto-oncogene has been assigned to this point. Dubé *et al.*,[22] however, found a new translocation, t(10;14)(q24–25;q11), in acute lymphoblastic leukemia and in a non-Hodgkin's lymphoma of T cell origin which Le Beau[7] considered to be t(10;14)(q24;q11).

Constitutional chromosome abnormalities are often associated wih cancer. The increased incidence of acute leukemia in children with Down's syndrome[23–25] is a good example. An association between Klinefelter's syndrome and breast cancer has also been reported.[26, 27] One patient in the present study showed *de novo* reciprocal translocation t(5;19)(q15;q13), and she had bilateral primary breast cancers in addition to Paget's disease, and three of her relatives also had cancer. Familial translocation t(13;18)(q13;12) with a high incidence of neoplasia[28] and the case[29] reported of a breast cancer patient with t(Cq-;Dq+), a sister and maternal aunt of whom had breast carcinomas, may indicate a higher risk of breast or other cancers in individuals with chromosomal abnormalities.

ACKNOWLEDGMENTS

A part of this work was supported by Grants-in-Aid for Cancer Research from the Ministry of Health and Welfare, and the Ministry of Education, Science and Culture.

(Received March 10, 1988/Accepted Aug. 8, 1988)

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