Hum Genet (1989) 83:45–48

Human Genetics

Fibroblast-specific common fragile sites induced by aphidicolin

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Summary. The distribution and frequency of aphidicolin-induced common fragile sites were studied in chromosomes of cultured skin fibroblasts and PHA-stimulated lymphocytes from five normal individuals; 0.2 μ*M* aphidicolin was added for the last 26 h of culture. Skin fibroblasts from five fra(X)-positive patients were also studied in the same manner. Fragile sites most frequently found in fibroblasts from normal individuals were 3q26.2, 7q11.23, 16q23, 1p31, 10q11.2, 12q23 and 7q31, whereas those in lymphocytes from the same individuals were 3p14, 16q23, Xp22, 7q32 and 14q24. The distribution of fragile sites in fibroblasts from fra(X)-positive patients was essentially identical with that in normal individuals. The average number of gaps and breaks in 100 metaphases was 36.8 in fibroblasts from normal individuals, 113.8 in those from fra(X)-positive patients, and 279 in lymphocytes from normal individuals. Their rates of chromosome-type breaks and gaps were 7.9%, 29.7% and 54.5%, respectively. Thus, the distribution and frequency of aphidicolin-induced fragile sites were different between skin fibroblasts and lymphocytes, possibly reflecting differences in their DNA replication sequence or gene activity.

Introduction

Fragile sites on human chromosomes are now divided into two major groups, i.e., rare and common sites, depending on their frequency in the general population. Common fragile sites have previously been induced by aphidicolin (Glover et al. 1984), enhanced by the addition of ethanol (Kuwano and Kajii 1987), by thymidylate stress and by a number of mutagens (Yunis et al. 1987). Their frequency can be further increased using caffeine and theophyline. Common fragile sites have been mainly studied in chromosomes of PHA-stimulated cultured helper T lymphocytes. There are few studies in which other tissues have been used (Morgan et al. 1988).

This report deals with the difference in the distribution and frequency of aphidicolin-induced fragile sites between skin fibroblasts and lymphocytes from normal individuals. Data on fibroblasts from fra(X)-positive patients are also presented.

Materials and methods

Cultured skin fibroblasts and PHA-stimulated peripheral blood lymphocytes were studied in five healthy Japanese individuals, two men and three women ranging in age from 24 to 35 years (Table 1). We also studied five fragile X-positive individuals, two male and three female subjects ranging in age from 1 to 34 years.

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Table 1. Individuals studied

	Case	Sex	Age (years)	Breaks in lymphocytes under routine culture conditions (%)
Fra(X)-negative	1	F	35	1.5
	2	M	27	0.5
	3	M	24	2.5
	4	F	25	–
	5	F	33	2
Fra(X)-positive	6	M	6	1
	7	M	1	–
	8	F	12	4
	9	F	8	6
	10	F	34	1

Fibroblast culture

Skin fibroblasts were established and maintained in culture with Eagle's minimum essential medium (MEM) and 10% fetal calf serum. Cells were subcultured at two or three passages, and aphidicolin, dissolved in ethanol, was added 22 h after subculturing, with the final concentration of these substances being 0.2 μ*M* and 0.02% respectively. Cultures were further maintained for another 26 h and treated with 0.02 μg/ml Colcemid for the last 2 h. Cells were processed in the usual manner for the preparation of chromosomes. Gaps and breaks were detected and photographed on conventional Giemsa-stained chromosomes; they were then destained and trypsin-G banded, and their band-localization was determined on photographed chromosomes.

Skin fibroblasts were cultured from five fra(X)-positive individuals, frozen in liquid nitrogen, thawed and subcultured: they were treated with 0.2 μ*M* aphidicolin at early passages, and processed in the same manner as were the fibroblasts from the normal individuals.

Culture of lymphocytes from peripheral blood

Short-term cultures of whole blood were set up, maintained and processed as described previously (Kuwano and Kajii 1987). Aphidicolin (0.2 μ*M*) was added 26 h prior to harvesting, as in the fibroblast cultures.

Results

Normal individuals

Gaps and breaks were scored in 200 metaphases in the skin fibroblast cultures and in 100 metaphases in the lymphocyte cultures from each of the five normal individuals, each culture

having been treated with 0.2 μ*M* aphidicolin for the previous 26 h (Table 2). The average number of breaks per 100 metaphases was 36.8 for the fibroblasts and 279 for the lymphocytes. Of the gaps and breaks encountered in the fibroblasts, only 7.9% were of the chromosome type, the rest being of the chromatid type; the corresponding figure for the lymphocytes was 54.5%.

In the fibroblasts, 13 sites exhibited 4% or more of the total breaks in one or more individuals, accounting for 82% of the total fragile sites observed. Of these, 7 sites were observed in two or more individuals (Fig. 1). The most frequent among them was 3q26.2 with 23.6% of the total breaks. This site was seen in all five subjects. Other sites seen in all subjects were 7q11.23, with a frequency of 12.8%, and 16q23, with a 10.3%

Table 2. Analysis of aphidicolin-induced breaks on skin fibroblasts and lymphocytes from normal individuals. Numbers in parentheses represent percentages of total breaks. Underlined sites are those with 4% or more of the total breaks in two or more individuals

Tissue	Skin fibroblasts[a]					
Subject	1	2	3	4	5	Total
Total breaks	63	48	43	113	101	368
Chromosome type breaks	4 (6.3)	4 (8.3)	2 (4.7)	8 (7.1)	11 (10.9)	29 (7.9)
Band						
1p31		2 (4.2)	4 (9.3)	6 (5.3)	8 (7.9)	22 (6.0)
2p32						4 (1.1)
3p14				6 (5.3)		10 (2.7)
3q13		2 (4.2)				2 (0.5)
3q26.2	20 (31.7)	7 (14.6)	14 (32.6)	24 (21.2)	22 (21.8)	87 (23.6)
5q32						2 (0.5)
7q11.23	9 (14.3)	6 (12.5)	5 (11.6)	13 (11.5)	14 (13.9)	47 (12.8)
7q31			2 (4.7)		6 (5.9)	13 (3.5)
7q32						2 (0.5)
10q11.2	4 (6.3)	3 (6.3)		7 (6.2)		17 (4.6)
11q21		2 (4.2)				3 (0.8)
11q22		2 (4.2)				3 (0.8)
12q23				12 (10.6)	5 (5.0)	17 (4.6)
13q32		2 (4.2)				5 (1.4)
14q24						1 (0.3)
16q23	5 (7.9)	5 (10.4)	6 (14.0)	12 (10.6)	10 (9.9)	38 (10.3)
20p12					5 (5.0)	10 (2.7)
Xp22						5 (1.4)
Tissue	Peripheral blood lymphocytes[b]					
Subject	1	2	3	4	5	Total
Total breaks	288	275	434	274	124	1395
Chromosome type breaks	141 (49.0)	157 (57.1)	246 (56.7)	167 (60.9)	49 (39.5)	760 (54.5)
Band						
1p31						12 (0.9)
2q32						40 (2.7)
3p14	39 (13.5)	50 (18.2)	96 (22.1)	40 (14.6)	27 (21.8)	252 (18.1)
3q13						1 (0.1)
3q26.2						9 (0.6)
5q32						0
7q11.2						3 (0.2)
7q31						9 (0.6)
7q32	15 (5.2)	20 (7.3)		19 (6.9)	5 (4.0)	74 (5.3)
10q11.2						2 (0.1)
11q21						3 (0.2)
11q22						1 (0.1)
12q23						0
13q32						1 (0.1)
14q24		13 (4.7)		22 (8.0)		58 (4.2)
16q23	21 (7.3)	45 (16.3)	64 (14.7)	39 (14.2)	13 (10.5)	182 (13.0)
20p12						5 (0.4)
Xp22	43 (14.9)	11 (4.0)		18 (6.6)	13 (10.5)	96 (6.9)

[a] Values based on 200 cells from each subject
[b] Values based on 100 cells from each subject

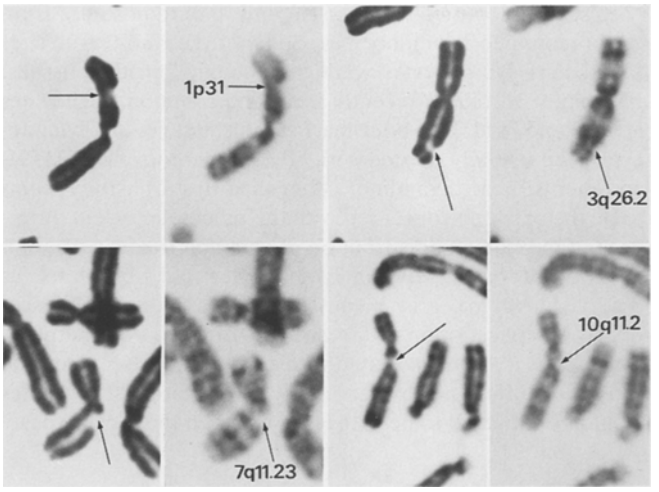

Fig. 1. Common fragile sites in the fibroblasts from normal individuals: 1p31, 3q26.2, 7q11.23, and 10q11.2. Each stained with conventional Giemsa stain (*left*), followed by trypsin-G banding (*right*)

frequency. Other sites of breakage observed in decreasing order of frequency were: 1p31 (6%), 10q11.2 (4.6%), 12q23 (4.6%) and 7q31 (3.5%). All 7 sites were observed at least once in both chromosomal homologues. The remaining 6 sites were observed in only one individual, and so were considered provisional; they were 3p14, 3q13, 11q21, 11q22, 13q32 and 20p12.

Among the chromosomes in lymphocytes from the same individuals, there were 5 sites that occurred in two or more individuals: 3p14 (18.1%), 16q23 (13%), Xp22 (6.9%), 7q32 (5.3%) and 14q24 (4.2%). Thus, both the frequency and distribution of common fragile sites were considerably different between skin fibroblasts and lymphocytes. The sites at 3q26.2, 7q11.23, 1p31, 10q11.2, 12q23, 7q31, all frequent in fibroblasts, accounted for less than 1% of the total breaks in lymphocytes. The site at 12q23 was never encountered in lymphocytes.

Exceptions were those breaks at 16q23 and 3p14. The site at 16q23 was observed in fibroblasts with a 10.3% frequency and at 13% in lymphocytes, whereas the corresponding figures for 3p14 were 2.7% and 18.1%.

Fra(X)-positive individuals

Gaps and breaks in skin fibroblast cultures from five fra(X)-positive individuals, scored in 100 metaphases from each, averaged 113.8 (Table 3). The distribution and frequency of the breaks were essentially identical with those in the fibroblasts from normal individuals. Those with 4% or more of the total breaks in two or more individuals were, in decreasing order, 3q26.2, 7q11.23, 16q23, 1p31, 10q11.2 and 3p14. These data agree with the findings obtained from fibroblasts of the normal individuals.

With regard to fra(X), only two individuals were positive at the site at Xq27.3, one in a single cell and the other in two

Table 3. Analysis of aphidicolin-induced breaks on skin fibroblasts from fragile X positive individuals. FA−, Folic acid deficient medium; FUdR, fluorodeoxyuridine

Tissue	Skin fibroblasts[a]					
Subject	6	7	8	9	10	Total
Total breaks	106	74	186	91	112	569
Chromosome type breaks	23 (21.7)	18 (24.3)	88 (47.3)	18 (19.8)	22 (19.6)	169 (29.7)
Band						
1p31	7 (6.6)			8 (8.8)	6 (5.4)	30 (5.3)
2q32		3 (4.1)				16 (2.8)
3p14	7 (6.6)	3 (4.1)		4 (4.4)	6 (5.4)	24 (4.2)
3q13		3 (4.1)				3 (0.5)
3q26.2	15 (14.2)	11 (14.9)	19 (10.2)	21 (23.1)	23 (20.5)	89 (15.6)
5q32				4 (4.4)		7 (1.2)
7q11.23	13 (12.3)	15 (20.3)	15 (8.1)	12 (13.2)	10 (8.9)	65 (11.4)
7q31						7 (1.2)
7q32						1 (0.2)
10q11.2	7 (6.6)		8 (4.3)	5 (5.5)	8 (7.1)	30 (5.3)
11q21						0
11q22						0
12q23					6 (5.4)	14 (2.5)
13q32		3 (4.1)				8 (1.4)
14q24						3 (0.5)
16q23	15 (14.2)	7 (9.5)	14 (7.5)	8 (8.8)	7 (6.3)	51 (9.0)
20q12						10 (1.8)
Xp22						3 (0.5)
Xq27	0	1 (1.4)	2 (1.1)	0	0	3 (0.5)
Fra(X) in peripheral blood lymphocytes						
Treatment	FA−	FUdR	FA−	FUdR	FUdR	
%	9	44	21	36	22	

[a] Values based on 100 cells from each subject

cells. The rate of fra(X) chromosomes in lymphocytes from the five patients ranged from 9% to 44%. Thus, aphidicolin is apparently not efficient in inducing the fra(X) site in fibroblast cultures.

Discussion

The results of our study indicate that the frequency and distribution of aphidicolin-induced common fragile sites are different between skin fibroblasts and lymphocytes. Of the 7 fragile sites frequently encountered in the skin fibroblasts from the five normal individuals studied, 6 (3q26.2, 7q11.23, 1p31, 10q11.2, 12q23 and 7q31) were either infrequent or never encountered in lymphocytes from the same individuals. Conversely, the sites at Xp22, 7q32 and 14q24, frequent among the lymphocytes, were either infrequent or never encountered in the fibroblasts. Only the sites at 16q23 and 3p14 were found at a rate of 4% or more of the total breaks in one or more individuals in both skin fibroblasts and lymphocytes. The results of the analysis of the skin fibroblasts from five fra(X)-positive individuals support these findings.

Chromosome-vs chromatid-type breaks

The rate of chromosome-type gaps and breaks was 7.9% in the fibroblasts from normal individuals and 54.5% in the lymphocytes from the same individuals, the remainder being chromatid-type breaks. The difference may reflect the number of divisions a cell has passed through after the introduction of aphidicolin into culture. The rate of chromosome-type breaks and gaps in the fibroblasts from fra(X)-positive individuals was 29.7%, i.e., much higher than the 7.9% rate in those from normal individuals. The difference between the total number of gaps and breaks in 100 metaphases was substantial: 36.8 in those from normal individuals and 113.8 in the fibroblasts from fra(X)-positive individuals. These differences may reflect the difference in age distribution between the two groups: 24–35 years in the normal individuals and 1–34 years in the fra(X)-positive patients.

New fragile sites

Of the fragile sites frequently encountered in the skin fibroblasts studied, the one at 3q26.2 was previously encountered in our study on the common fragile sites in lymphocytes induced by folic acid deficiency or BrdU (Kuwano et al. 1988). The site at 7q11.23 was recently reported to be induced in bone marrow cells by sequential treatment with methotrexate and thymidine (Morgan et al. 1988). Aphidicolin-induced sites at 1p31.2 and 7q31.2 were listed as confirmed common fragile sites by the Human Gene Mapping 9 Workshop (Sutherland and Mattei 1987). The sites at 1p31.2, 3p26.2, 7q11.23 were induced in lymphocytes using various mutagens and carcinogens (Yunis et al. 1987). Thus, most of the aphidicolin-induced fragile sites frequent in fibroblasts have also been induced in lymphocytes (and bone marrow) by various means. Exceptions are those at 10q11.2 and 12q23. These two sites, frequent in fibroblasts, were hitherto unknown.

Tissue-specific fragile site expression

Very little is known about the tissue-dependent difference of fragile site expression. Most of the rare and common fragile sites so far identified were first found in chromosomes from cultured helper T lymphocytes. Some of the rare fragile sites found in T lymphocytes were then sought in other tissues. Morgan et al. (1988) recently identified common fragile sites at 4q23 and 7q11.23 in bone marrow cells, but not in T lymphocytes, sequentially treated with 10^{-7} *M* methotrexate (MTX) and then 10^{-5} *M* thymidine. Other than this, our study seems to be the only one concerned with the tissue-dependent difference of the rate and distribution of common fragile sites.

The difference between lymphocytes and fibroblasts, as observed here, may arise because of different rates or sequences of DNA replication in different types of cells. Alternatively, fragile sites may be associated with active genes. Because gene activity is different between cell types, different fragile sites would be expected to be expressed between these cell types.

Aphidicolin vs other inducers of fragile sites

In an effort to induce fragile sites in cultured skin fibroblasts, we tried various conditions, including folic acid deficient medium, or the presence of MTX, FUdR, FUdR plus caffeine, excess thymidine or aphidicolin. Most of these conditions resulted in either a very low level of fragile site expression or suppressed mitoses, both being inadequate for analysis. Excess thymidine, as described by Sutherland and Baker (1986) for the induction of the fragile X and other rare folate-sensitive sites, induced a modest level of common fragile sites. Aphidicolin induced a relatively high number of breaks and gaps without suppressing mitotic activity. Addition of 1% ethanol to 0.2 μM aphidicolin enhanced the frequency of breaks in the skin fibroblasts from one individual by 52%, as compared with those treated with 0.02% ethanol plus aphidicolin. Their mitotic activity, however, was suppressed. Thus, aphidicolin with 0.02% ethanol was selected for the study. Studies of various types of cells for aphidicolin-induced fragile sites are now being carried out in our laboratory.

Acknowledgements. This work was supported by Grants-in-Aid for Cancer and Scientific Research from the Ministry of Education, Science and Culture.

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Received August 28, 1988