Fragile Sites at 16q22 Are Not at the Breakpoint of the Chromosomal Rearrangement in AMMoL

R. N. SIMMERS, G. R. SUTHERLAND, A. WEST, R. I. RICHARDS

There is much speculation about fragile sites on human chromosomes predisposing to specific chromosome rearrangements seen in cancer. Acute myelomonocytic leukemia is characterized by neoplastic chromosome rearrangements involving band 16q22 in patients who carry the rare fragile site at 16q22. This specific leukemic breakpoint is within the metallothionein gene cluster, which is here shown to be proximal to the rare fragile site (*FRA16B*) and to a common fragile site (*FRA16C*) in this region. Hence neither of these fragile sites are at the breakpoint in this leukemic chromosomal rearrangement.

FRAGILE SITES ARE NONSTAINING gaps or breaks at specific points on chromosomes (*1*). The common, or constitutive, fragile sites occur in frequencies approaching homozygosity (*2, 3*), whereas rare fragile sites occur only in some members of the population; for example, carriers of the autosomal folate-sensitive fragile sites have an estimated total frequency of one in 260 individuals (*4*). The folate-sensitive fragile X is the only fragile site associated with a known phenotypic abnormality—fragile (X)-linked mental retardation—which is the commonest cause of inherited mental retardation (*2*).

There have been reports of cancer patients with rare fragile sites in their normal cells in one of the chromosome bands involved in chromosomal rearrangements in the malignant cells (*5–9*). While two locations within a chromosomal band are not necessarily genetically close, this correspondence between bands containing cancer breakpoints and those containing fragile sites has suggested a possible functional relationship and coincidence at the DNA level. The bone marrows of patients with acute myelomonocytic leukemia (AMMoL) with abnormal eosinophils have cells in which there is a rearrangement at 16q22. Such rearrangements include inv(16)(p13q22) (*10, 11*), del(16)(q22) (*11–13*), t(16;16)(p13;q22) (*14*), and t(5;16)(q33;q22) (*15*). A strong association of the distamycin A–inducible rare fragile site at 16q22.1. (*FRA16B*) in normal cells with the occurrence of a rearrangement involving band 16q22 in neoplastic cells from bone marrow has been reported in patients with AMMoL (*5, 7–9, 16*). A total of 17 *FRA16B* carriers was detected in these 24 reported AMMoL cases. Compared to the frequency of about one in 90 *FRA16B* carriers in the general population (*4*), this could suggest a predisposition of *FRA16B* carriers to AMMoL.

Le Beau *et al.* (*17*), using in situ hybridization, found that the metallothionein gene (*MT*) cluster is at 16q22, and that this cluster was split by the chromosomal rearrangement at 16q22 in the four AMMoL patients studied, who were later found to be carriers of *FRA16B* (*8*). Thus, if these rearrangements do coincide with the *FRA16B* locus at the DNA level, it would be expected that the gap or break seen when the fragile site is expressed would interrupt the *MT* cluster.

We first used a probe that cross-hybridizes to most or all of the members of the *MT* gene family, of which most are in the *MT* cluster localized at 16q22 (*17*). The probe was hybridized in situ to chromosomes expressing *FRA16B* (Table 1). The finding of 70 grains proximal and 16 grains distal to the fragile site indicated that the *MT* cluster was proximal to the fragile site. The distal grains in the case of the *MT* probe and *FRA16B* may be accounted for by scatter from the proximal site of hybridization and random background. However, it remained possible that while most of the genes were proximal to the fragile site, a small part of the gene cluster homologous to the probe could have been located distal to it. The results of Le Beau *et al.* show a more central interruption of the cluster by the rearrangements in the four patients they studied (*17*). One possibility is that the fragile sites are not at exactly the same place in all individuals, just as specific chromosome breakpoints are not all at exactly the same place but occur within a region known as a breakpoint cluster region (*18*).

The specific chromosome rearrangement in the bone marrow of AMMoL patients occurs between the *MT2A* gene (*19*) and the *MT1B* gene (*20*) in band 16q22 since probes from each of these regions localize to different sides of the breakpoint at 16q22 (*21*). To test further the coincidence of this breakpoint and *FRA16B*, we independently hybridized probes specific for the 5′ non-

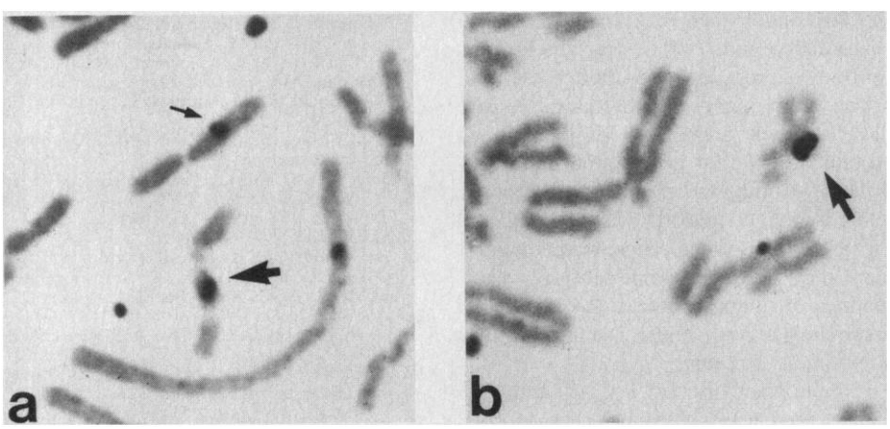

Fig. 1. (**a**) A partial metaphase with *FRA16B* probed for *MT1B*, showing label proximal to the fragile site (large arrow) and on the other chromosome 16 (small arrow). (**b**) A partial metaphase with *FRA16C* probed for *MT2A*, showing label proximal to the fragile site. The probe that hybridizes to most or all of the members of the *MT* gene family consists of a plasmid containing a 150–base pair (bp) insert from the coding region of *MT2A* (*29*). The probe specific for *MT1B* contains 800 bp from the 5′ flanking region in pUC13 (*30*). The probe specific for *MT2A* (*19*) contains 770 bp from the 5′ flanking region in pUC13. The probes were labeled to a specific activity of 2×10^7 to 3×10^7 count/min per microgram of DNA with three tritiated nucleotides and hybridized at 37°C in situ to denatured metaphase chromosomes as described in Simmers *et al.* (*22*). The autoradiographic exposure period was 23 to 33 days. Chromosomes expressing *FRA16B* and *FRA16C* were prepared as described (*3, 31*). All probes were hybridized in situ to metaphases expressing *FRA16B*, and the two specific probes to metaphases expressing *FRA16C* from a *FRA16B*-negative individual. In 50 metaphases not expressing *FRA16B*, after hybridization with the first probe and 29 days of exposure, 57 (11%) of 541 silver grains were scored in 16q, which represents 1.7% of the total chromosome length (*32*) ($P << 10^{-5}$ by cumulative Poisson probabilities). Four weeks of exposure to *MT2A*-probed metaphases resulted in 26 grains (20%) on 16q out of 129 total grains over 50 metaphases ($P << 10^{-5}$). Thirty-three days' exposure to metaphases hybridized with the *MT1B*-specific probe (0.4 μg/ml) resulted in 44 grains (21%) on 16q out of 213 total grains over 50 metaphases ($P << 10^{-5}$).

R. N. Simmers and G. R. Sutherland, Cytogenetics Unit, Department of Histopathology, Adelaide Children's Hospital, North Adelaide, S.A. 5006, Australia.
A. West and R. I. Richards, Howard Florey Institute of Experimental Physiology and Medicine, University of Melbourne, Parkville, Vic. 3052, Australia.

coding regions of the *MT2A* and *MT1B* genes in situ to metaphases from 13 unrelated *FRA16B* individuals expressing the fragile site. In none of these 13 individuals was there evidence of specific hybridization distal to the fragile site. Each showed an excess of label proximal to the fragile site, for both the *MT2A* and *MT1B* probes (Table 1).

The finding of more silver grains than would be expected on the basis of background alone, on the opposite side of the fragile site to that where the majority of silver grains are seen, is in accord with observations made with other probes adjacent to fragile sites. It is probably accounted for by scatter from the site of hybridization, in addition to background. For example, this was seen for the haptoglobin locus, about 10 centimorgans from *FRA16B* (*22*), and for the adenine phosphoribosyltransferase locus, in the chromosomal band adjacent to *FRA16D* (*23*). Such labeling was usually present distal to *FRA16B* when either the *MT2A* or *MT1B* probe was used on the same individual (Table 1) and is not regarded as resulting from specific hybridization. The fragile site in a single individual cannot interrupt two separate loci. The results indicate that *FRA16B*, even if it can vary in position at the molecular level, is not between these two loci that flank the breakpoint of the chromosomal rearrangement associated with AMMoL. Thus the breakpoint and the fragile sites cannot coincide.

Yunis and Soreng described a common fragile site at 16q22.1 (*3*), *FRA16C*, in the same band as the rare fragile site *FRA16B*. In malignant cells from an AMMoL patient with inv(16)(p13.11,q22.1), a higher than normal frequency of expression of *FRA16C* was observed. Three patients with other leukemias had an increased frequency of expression of a common fragile site in the same band as one of the breakpoints of their neoplastic chromosome rearrangements (*3*). It was therefore suggested that individuals with a high frequency of expression of specific common fragile sites are predisposed to certain types of malignancies.

To test whether *FRA16C*, rather than *FRA16B*, interrupts the *MT* cluster, metaphase spreads from one individual in which *FRA16C* had been induced were hybridized in situ with the probes specific for the *MT2A* and *MT1B* genes. Both genes were shown to be proximal to *FRA16C* (Table 2). Our results show that *MT2A* and *MT1B* are proximal to the two fragile sites, *FRA16B* and *FRA16C* (Fig. 1), which are at the interface of bands 16q21 and 16q22 (*3*, *24*), termed 16q22.1. This in turn shows that the specific neoplastic chromosomal rearrangements at 16q22 associated with AMMoL do not occur at the same location

Table 1. Grains scored with respect to *FRA16B* in metaphases probed for the metallothionein gene cluster (*MT*) and the *MT2A* and *MT1B* genes in 13 individuals.

Individual	Probe (μg/ml)	Number of *FRA16B*	Grains proximal on 16q	Grains distal on 16q	χ^{2*}
41A	*MT* (0.05)	273	70	16	33.9 ($P << 0.001$)
4F	*MT2A* (0.4)	59	5	2	
	MT1B (0.2)	51	4	0	
18B	*MT2A* (0.4)	118	26	5	14.2 ($P < 0.001$)
	MT1B (0.2)	67	12	2	7.1 ($P < 0.01$)
22A	*MT2A* (0.4)	47	4	0	
	MT1B (0.2)	17	6	2	
25A	*MT2A* (0.4)	371	59	12	31.1 ($P << 0.001$)
	MT1B (0.02)	158	36	5	23.4 ($P << 0.001$)
36A	*MT2A* (0.4)	299	48	24	8.0 ($P < 0.005$)
	MT1B (0.2)	144	26	0	26.0 ($P << 0.001$)
41A	*MT2A* (0.4)	448	73	19	31.7 ($P << 0.001$)
	MT1B (0.2)	45	6	2	
42E	*MT2A* (0.4)	112	24	4	14.3 ($P < 0.001$)
	MT1B (0.2)	70	13	1	10.3 ($P < 0.005$)
42G	*MT2A* (0.4)	86	23	4	13.4 ($P < 0.001$)
	MT1B (0.2)	62	12	2	7.1 ($P < 0.01$)
50C	*MT2A* (0.4)	370	58	11	32.0 ($P << 0.001$)
	MT1B (0.2)	54	7	1	
52A	*MT2A* (0.4)	248	57	6	41.3 ($P << 0.001$)
	MT1B (0.2)	70	5	2	
53C	*MT2A* (0.4)	528	110	24	55.2 ($P << 0.001$)
	MT1B (0.2)	31	4	0	
56B	*MT2A* (0.4)	129	19	2	13.8 ($P < 0.001$)
	MT1B (0.2)	6	2	0	
63A	*MT2A* (0.4)	136	14	3	7.1 ($P < 0.01$)
	MT1B (0.2)	7	4	0	

*The null hypothesis was that grains proximal = grains distal; $\chi^2(1) = 3.84$, $P \leq 0.05$.

Table 2. Silver grains scored with respect to *FRA16C* after in situ hybridization with probes specific for *MT2A* and *MT1B* at 0.4 μg/ml.

Probe	Number of *FRA16C*	Grains proximal	Grains distal	χ^{2*}
MT2A	92	13	3	6.25 ($P < 0.025$)
MT1B	84	17	4	8.05 ($P < 0.005$)

*The null hypothesis was that grains proximal = grains distal; $\chi^2(1) = 3.84$, $P \leq 0.05$.

as either fragile site and therefore that these rearrangements do not occur as a result of breakage at *FRA16B* or *FRA16C*. While *FRA16B* occurs in about 1% of the population, this does not explain the high frequency of this fragile site in the AMMoL patients with an abnormal chromosome 16. It is possible that in some instances the common fragile site *FRA16C*, and not *FRA16B*, was seen in this patient group.

The breakpoints in the specific chromosome 16 rearrangements in AMMoL have been noted to be difficult to define precisely (*24, 25*). If the conclusions of Le Beau *et al.* are correct and one of the breakpoints splits the *MT* cluster, which we have mapped proximal to the fragile sites at the junction of bands 16q21 and 16q22, that breakpoint and the *MT* cluster are most likely in band 16q21.

There has been increasing conjecture about the relationship between fragile sites and cancer (*3, 5–8, 26–28*). The associations between *FRA16B* and the breakpoint in the long arm of chromosome 16 associated with AMMoL with abnormal bone marrow eosinophils, have provided part of the evidence consisting of patients with a cancer breakpoint and the corresponding fragile site. The results presented here remove some of the support for causal relationship between fragile sites and cancer.

REFERENCES AND NOTES

1. G. R. Sutherland, *Am. J. Hum. Genet.* **31**, 125 (1979).
2. ——— and F. Hecht, *Fragile Sites on Human Chromosomes* (Oxford Univ. Press, New York, 1985).
3. J. J. Yunis and A. L. Soreng, *Science* **226**, 1199 (1984).
4. G. R. Sutherland, *Ann. Hum. Genet.* **49**, 153 (1985).
5. J. J. Yunis, *Cancer Genet. Cytogenet.* **11**, 125 (1984).
6. ———, *ibid.* **12**, 85 (1984).
7. M. M. Le Beau and J. D. Rowley, *Nature (London)* **308**, 607 (1984).
8. M. M. Le Beau, *Blood* **67**, 849 (1986).
9. D. C. Arthur, S. M. Aaseng, C. D. Bloomfield, *Cytogenet. Cell Genet.* **40**, 572 (1985).
10. M. M. Le Beau *et al.*, *N. Engl. J. Med.* **309**, 630 (1983).
11. Fourth International Workshop on Chromosomes in Leukemia (1982); *Cancer Genet. Cytogenet.* **11**, 310 (1984).
12. D. C. Arthur and C. D. Bloomfield, *Blood* **61**, 994 (1983).
13. ———, *ibid.* **62**, 931 (1983).
14. J. R. Testa, D. E. Hogge, S. Misawa, N. Zandparsa, *N. Engl. J. Med.* **310**, 468 (1984).
15. K. Bhambhani, S. Inoue, M. Tyrkus, N. Gohle, *Cancer Genet. Cytogenet.* **20**, 187 (1986).
16. T. W. Glover, J. Coyle-Morris, R. Morgan, *ibid.* **19**, 141 (1986).
17. M. M. Le Beau, M. O. Diaz, M. Karin, J. D. Rowley, *Nature (London)* **313**, 709 (1985).
18. J. Groffen *et al.*, *Cell* **36**, 93 (1984).
19. M. Karin and R. I. Richards, *Nature (London)* **299**, 797 (1982).
20. R. I. Richards, A. Heguy, M. Karin, *Cell* **37**, 263 (1984).
21. M. O. Diaz, M. M. Le Beau, M. Karin, personal communication.
22. R. N. Simmers, I. Stupans, G. R. Sutherland, *Cytogenet. Cell Genet.* **41**, 38 (1986).
23. A. Fratini *et al.*, *ibid.* **43**, 10 (1986).
24. R. Berger *et al.*, *Leuk. Res.* **9**, 279 (1985).
25. D. C. Arthur and C. D. Bloomfield, *Blood* **63**, 242 (1984).
26. J. J. Yunis, *Science* **221**, 227 (1983).
27. F. Hecht and G. R. Sutherland, *Cancer Genet. Cytogenet.* **12**, 179 (1984).
28. F. Hecht and T. W. Glover, *ibid.* **13**, 185 (1984).
29. M. Karin and R. I. Richards, *Nucleic Acids Res.* **10**, 3165 (1982).
30. A. Heguy, A. West, R. I. Richards, M. Karin, *Mol. Cell. Biol.* **6**, 2149 (1986).
31. G. R. Sutherland, P. B. Jacky, E. G. Baker, *Am. J. Hum. Genet.* **36**, 110 (1984).
32. D. G. Harnden and H. P. Klinger, Eds., *An International System for Human Cytogenetic Nomenclature*, published in collaboration with *Cytogenet. Cell Genet.* (Karger, Basel, 1985).

33. We wish to thank E. Baker for technical assistance and the Anti-Cancer Foundation of the Universities of South Australia for grant support.

30 September 1986; accepted 4 February 1987