Translocation t(3;8)(p14.2;q24.1) in Renal Cell Carcinoma Affects Expression of the Common Fragile Site at 3p14(FRA3B) in Lymphocytes

Thomas W. Glover, Jane F. Coyle-Morris, Frederick P. Li, Robert S. Brown, Carol S. Berger, Robert M. Gemmill, and Frederick Hecht

ABSTRACT: *The common fragile site at 3p14(FRA3B) is cytogenetically close to the positions of translocation and deletion breakpoints frequently observed in renal cell carcinoma (RCC) and small cell carcinoma of the lung. Possible involvement of this fragile site in the familial RCC* t(3;8)(p14.2;q24.1) *was investigated. Expression of FRA3B, induced by treatment of lymphocytes with aphidicolin, is altered by the translocation. These results suggest that the fragile site is very close to, if not coincident with, the translocation breakpoint.*

INTRODUCTION

Common fragile sites have been observed in peripheral blood lymphocytes cultured either under conditions causing thymidylate stress or with drugs that inhibit DNA replication or repair [1–6]. Although the rare fragile site at Xq27 has been associated with X-linked mental retardation, the biological significance of common fragile sites has yet to be determined [7]. The suggestion has been made that these are sites that predispose to chromosomal breakage and rearrangements characteristic of certain neoplasias [6–12]. The most readily induced common fragile site occurs in band 3p14(FRA3B), a region that has been associated with deletions in small cell carcinoma (SCC) of the lung and both translocations and deletions in renal cell carcinoma (RCC) [13–17].

The apparent coincidence of FRA3B and these translocation or deletion breakpoints led us to examine fragile site expression in cells from two members of a family in which the constitutional t(3;8)(p14.2;q24.1) segregates with a greatly increased risk for RCC [16]. Aphidicolin, an inhibitor of DNA polymerase α, was used

From The Genetics and Cancer Centers of Southwest Biomedical Research Institute, Scottsdale, AZ (J. F. C. -M., C. S. B., R. M. G., F. H.), the Departments of Pediatrics and Human Genetics, University of Michigan, Ann Arbor, MI (T. W. G.), Sidney Farber Cancer Institute, Boston, MA (F. P. L.), Beth Israel Hospital, Boston, MA (R. S. B.)

Address requests for reprints to Dr. Thomas W. Glover, University of Michigan Medical Center, Department of Pediatrics, Section of Pediatric Genetics, D1225MPB, Box 0718, Ann Arbor, MI 48109-0010.

Received July 10, 1987; accepted September 4, 1987.

Cancer Genet Cytogenet 31:69–73 (1988)
0165-4608/88/$03.50

to induce fragile site expression in PHA-stimulated peripheral blood lymphocytes. These studies demonstrated fragile site expression at the translocation breakpoint in both the derivative chromosome #3 and derivative #8, despite the fact that there is not normally a highly expressing common fragile site at 8q24. This observation suggests that FRA3B expression has been affected in some way by the translocation event.

METHODS

Heparinized venous blood was obtained from two members (designated 9542 and 9944) of the RCC family, both of whom carried the t(3;8)(p14.2;q24.1) [16]. Whole blood was cultured at 37°C for 96 hours in RPMI 1640 medium containing folic acid (FA+) (Irvine Scientific) plus 10% fetal bovine serum (Hy-Clone, Sterile Systems, Inc.), or RPMI 1640 without folic acid (FA−) with 5% fetal bovine serum. All media were supplemented with 2 mM glutamine, phytohemagglutinin, and penicillin/streptomycin. Aphidicolin (dissolved in dimethylsulfoxide) was added directly to the cultures to a final concentration of 0.2 μM, 26 hours before harvest.

Cells were scored blindly for chromosome aberrations without knowledge of treatment. Air-dried slides were banded with CMA_2S [N,N *bis*-(6-chloro-2-methoxyacridin-9-yl) spermine], which has a very slow quenching rate and allows ample time for specific location of breakpoints [18]. Analysis was performed by two independent observers and results were totaled.

RESULTS

Cells were cultured in medium with or without folic acid and with or without 0.2 μM aphidicolin. Fragile site expression was scored for normal chromosomes #3 and #8, together with the reciprocal derivative chromosomes generated by the translocation. The results presented in Table 1 demonstrate the expression of FRA3B. The fragile site was observed on the normal #3, as expected. It was observed not only on the derivative #3 chromosome, but also on the derivative #8 at or near the translocation breakpoints. Figure 1 shows an example of a metaphase in which fragile sites on the normal #3, derivative #3, and derivative #8 are simultaneously expressed. Table 1 shows that there is less expression of the fragile site in each of the derivative chromosomes relative to the normal #3. When the data for the derivative chromosomes are combined, however, the numbers approximate that seen in the normal #3, except in one case (9542, FA+, 0.2 μM aphidicolin), where the sum of the breaks in the derivative chromosomes was only half of that seen in the normal #3.

Table 1 FRA3B Expression in t(3;8)(p14.2;q24.1) lymphocytes

			Breaks or gaps/cells			
Subject	Medium	Aphidicolin (μM)	Normal, 3p14	Normal, 8q24	der3p14	der8q24
9542	FA+	0	1/25	0/25	0/25	0/25
	FA+	0.2	20/75	1/75	2/75	7/75
	FA−	0	3/25	0/25	0/25	0/25
	FA−	0.2	19/50	0/50	5/50	7/50
9944	FA+	0	0/50	0/50	0/50	0/50
	FA+	0.2	18/73	0/73	5/73	15/73

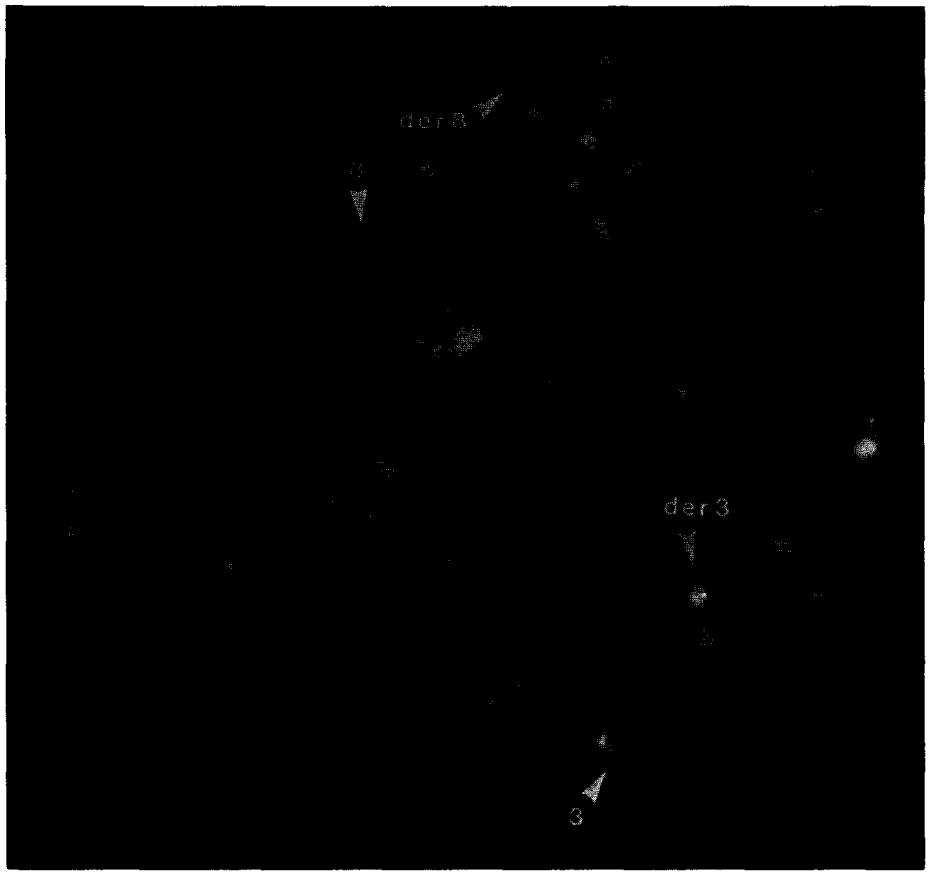

Figure 1 A representative Q-banded metaphase from 9542 after treatment with 0.2 μM aphidicolin for 26 hours. Arrows mark the expressed fragile site in the normal #3, der(3), and der(8). The normal #8, with no visible fragile site, is also marked with an arrow. In the case of the derivative chromosomes, the fragile site is located at or near the translocation breakpoints.

Culturing the cells in folic acid-free medium (FA−) creates a condition of thymidylate stress resulting in a low-level expression of FRA3B and of fragile sites at other locations (data not shown). When aphidicolin is added to cells grown in FA− media, this expression is greatly enhanced.

DISCUSSION

FRA3B is the most readily inducible of the aphidicolin-sensitive fragile sites in lymphocytes [5]. The existence of a constitutional translocation with one breakpoint at 3p14.2 provided an opportunity to study the effect of this rearrangement on FRA3B expression. The aberrations that can be seen at the translocation breakpoints suggest that the translocation has altered the expression of FRA3B. There are at least six events that could have led to this observation. FRA3B could have been split by the translocation or perhaps duplicated. It is also possible that there are two fragile sites in band 3p14.2 and the translocation broke between the sites. Alternatively, the translocation could have generated an altogether new fragile site or,

by coincidence, a previously undescribed rare fragile site could be present at 8q24 in this family. Finally, we could have observed a greatly enhanced expression of a constitutive fragile site reported to be present at 8q24.1 [6]. This is unlikely, however, because this fragile site has not been observed, except in an occasional cell, in our laboratories using the culture conditions described here. The numbers in Table 1 favor the hypothesis that FRA3B has been split by the translocation and make it unlikely that it has been duplicated. If the fragile site is an amplified sequence, as has been suggested for the fragile X [19, 20], this would be possible. Regardless of the event that caused the appearance of weakly expressing sites on the derivative #3 and derivative #8 chromosomes, the results strongly suggest that the translocation breakpoint and FRA3B are very close to one another and possibly coincident.

The oncogene *myc* has been mapped to 8q24.1, the location of the chromosome #8 breakpoint in these cells. This fact suggests that *myc* could be involved in the development of cancer in this family. It has been shown by somatic cell hybridization that *myc* has been moved to the derivative #3 chromosome; however, Southern blot hybridization has failed to reveal any molecular rearrangement of *myc* in this instance [21]. Furthermore, we have used pulsed field gel electrophoresis to construct a map that covers a 1500-kb region surrounding *myc* and have been unable to detect the translocation breakpoint [22]. Of the cytogenetic rearrangements seen in RCC, 3p14 is more consistently involved than 8q24 [17]. These observations do not support a primary role for the *myc* oncogene in RCC.

Although the role of fragile sites in the development of cancer is unclear, the observation has been made that the breakpoints of certain cancer rearrangements are cytogenetically located at or near fragile sites [6–12]. Some have suggested that this is merely a coincidence [23], whereas, others suggest the possibility of a direct causal relationship [6, 8–11]. The observation that FRA3B is in close proximity to the t(3;8)(p14.2;q24.1) breakpoint makes it tempting to speculate that this particular fragile site may have had a causal role in the generation of this translocation or at least by coincidence is right at the translocation breakpoint. Further experimentation is necessary before this speculation can be confirmed.

Supported by NIH Grants RO1-CA-43222 (T. W. G.), CA-41546 (R. M. G.), and program project CA-41124 (F. H.) from the National Cancer Institute.

REFERENCES

1. Sutherland GR (1979): Heritable fragile sites on human chromosomes. I. Factors affecting expression in lymphocyte culture. Am J Hum Genet 31:125–135.
2. Glover TW (1981): FUdR induction of the X chromosome fragile site: Evidence for the mechanism of folic acid and thymidine inhibition. Am J Hum Genet 33:232–242.
3. Tommerup N, Poulsen H, Brøndum-Nielson K (1981): 5-fluoro-2′ deoxyuridine induction of the fragile site on Xq28 associated with X-linked mental retardation. J Med Genet 18:374–376.
4. Mattei MG, Mattei JF, Giraud F (1981): Expression in lymphocyte and fibroblast culture of the fragile X chromosome: a new technical approach. Hum Genet 59:166–169.
5. Glover TW, Berger C, Coyle J, Echo B (1984): DNA polymerase alpha inhibition by aphidicolin induces gaps and breaks at common fragile sites in human chromosomes. Hum Genet 67:136–142.
6. Yunis JJ, Soreng AL (1984): Constitutive fragile sites and cancer. Science 226:1199–1204.
7. Sutherland GR, Hecht F (1985): Fragile Sites on Human Chromosomes. Oxford University Press, NY.

8. Yunis JJ (1983): The chromosomal basis of human neoplasia. Science 221:227–236.
9. Hecht F, Sutherland G (1984): Fragile sites and cancer breakpoints. Cancer Genet Cytogenet 12:179–181.
10. Hecht F, Glover T (1984): Cancer chromosome breakpoints and common fragile sites induced by aphidicolin. Cancer Genet Cytogenet 13:185–188.
11. Le Beau MM, Rowley JD (1984): Heritable fragile sites in cancer. Nature 308:607–608.
12. Le Beau MM (1986): Chromosomal fragile sites and cancer-specific rearrangements. Blood 67:849–858.
13. Whang-Peng J, Benn PA, Kao-Shen CS, Lee EC, Carney DN, Gazdar A, Minna JD (1982): A nonrandom chromosomal abnormality, del3p(14–23), in human small cell lung cancer. Cancer Genet Cytogenet 6:119–134.
14. Falor WH, Ward-Skinner R, Wegryn S (1985): A 3p deletion in small cell lung carcinoma. Cancer Genet Cytogenet 16:175–177.
15. Sozzi G, Bertoglio MG, Borello MG, Giani S, Pilotti S, Pierotti M, Della Porta G (1987): Chromosomal abnormalities in a primary small cell lung cancer. Cancer Genet Cytogenet 27:45–50.
16. Cohen AJ, Li FP, Berg S, Marchetti DJ, Tasi S, Jacobs SC, Brown RS (1979): Hereditary renal-cell carcinoma associated with a chromosomal translocation. N Engl J Med 301:592–595.
17. Berger CS, Sandberg AA, Todd IA, Pennington RD, Haddad FS, Hecht BK, and Hecht F (1986): Chromosomes in kidney, ureter, and bladder cancer. Cancer Genet Cytogenet 23:1–24.
18. Deugau KV, van de Sande JH, Linn CC (1978): $(CMA)_2S$: A double intercalating analogue of quinacrine for chromosomes banding. Am J Hum Genet 30:78A.
19. Nussbaum RL, Airhart SD, Ledbetter DH (1986): Recombination and amplification of pyrimidine-rich sequences may be responsible for initiation and progression of the Xq27 fragile site: An hypothesis. Am J Med Genet 23:715–721.
20. Ledbetter DH, Ledbetter SA, Nussbaum RL (1986): Fragile X expression in normal males: Implications for the nature of the mutation. Nature 324:161–163.
21. Drabkin H, Bradley C, Li F, Patterson D (1985): Analysis of the hereditary renal cell carcinoma 3;8 translocation: Evidence that c-*myc* is translocated. Clin Res 33:1:41A.
22. Gemmill RM, Coyle-Morris J, Ware-Uribe L, Drabkin HA, Brown RS, and Hecht F (1987): 1,500 kb pulsed field gel map surrounding MYC does not include the translocation breakpoint in familial renal cell carcinoma. Am J Hum Genet 41suppl:A215.
23. Simmers RN, Sutherland GR, West A, and Richards RI (1987): Fragile sites at 16q22 are not at the breakpoint of the chromosomal rearrangement in AMMoL. Science 236:92–94.