[CANCER RESEARCH 56, 2936–2939, July 1, 1996]

Advances in Brief

Evaluation of the *FHIT* Gene in Colorectal Cancers[1]

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Abstract

A variety of studies suggests that tumor suppressor loci on chromosome 3p are important in various forms of human neoplasia. Recently, a chromosome 3p14.2 gene called *FHIT* was discovered and proposed as a candidate tumor suppressor gene in colorectal and other cancers. We evaluated the *FHIT* gene in a panel of colorectal cancer cell lines and xenografts, which allowed a comprehensive mutational analysis. A transcript containing the complete coding sequence was found to be expressed at robust levels in 29 of 31 cancers tested. The complete sequence of the coding region of the gene was determined and found to be normal in all 29 of these cases. These studies suggest either that *FHIT* is inactivated by an unusual mechanism or that it plays a role in relatively few colorectal tumors.

Introduction

A variety of studies have suggested that tumor suppressor loci on chromosome 3p play important roles in human neoplasia. Although several genes have been proposed to represent these suppressors, only in the case of the *VHL* gene at chromosome 3p25 has unequivocal evidence implicating a specific gene been amassed (1). The identification of the *VHL* gene was facilitated considerably by the availability of cancer-prone families in which genetic linkage data and germline mutations could be used as aids to gene discovery and validation. The other loci on chromosome 3p are generally not associated with cancer predisposition, and the search for these genes, and their validation as tumor suppressors, is accordingly more difficult.

Among the chromosome 3 regions thought to harbor tumor suppressor loci, chromosome 3p14.2 has recently become prominent. Several different lines of investigation led to the consideration of this region. A reciprocal t(3;8)(p14.2;q24) chromosome translocation segregating with disease in a family with renal cell carcinomas first highlighted this chromosomal position (2, 3). Subsequently, an aphidicolin-inducible fragile site, *FRA3B,* was found to be indistinguishable from the t(3;8) translocation (4). Numerous studies have demonstrated that chromosome 3p allelic losses occur in many forms of cancers, although the losses appear to implicate several loci rather than point to a single common region of loss (5–11). Additionally, RDA[3] was used to map a relatively frequent site of homozygous deletion in colorectal cancers to 3p14.2 (12). As homozygously deleted sequences are believed generally to lie within or adjacent to tumor suppressor genes (13), the RDA results strongly supported the existence of a tumor suppressor gene in this area (14).

Finally, Ohta *et al.* (15) have recently used a positional cloning approach to identify a novel gene that spanned the t(3;8) breakpoint. They named this gene *FHIT* (fragile histidine triad gene), reflecting its homology to the *Schizosaccharomyces pombe* gene encoding Ap_4A asymmetrical hydrolase (16). In addition to its disruption in the kindred with the t(3;8) translocation, the authors identified aberrant transcripts of the gene in many cancers, including those of the colon, esophagus, stomach, and lung (15, 17). *FHIT* was therefore proposed as a candidate tumor suppressor gene. In the current study, we have used a molecular genetic approach to determine the frequency and extent of alterations of this gene in a panel of colorectal cancer lines particularly well suited for such analyses (37).

Materials and Methods

The generation and maintenance of early-passage xenografts and cell lines derived from colorectal cancers has been described previously (18, 19). Xenografts were used at first passage, and cell lines were used prior to the sixth *in vitro* passage.

RNA was purified from tumor lines and normal tissues, and cDNA was generated from RNA as described previously (20–22). Each cDNA synthesis reaction was paired with a parallel reaction without reverse transcriptase as a control. PCR amplifications were carried out in 96-well plates in 25-μl reaction mixtures containing 6.7 mM Tris-HCl (pH 8.8); 16.6 mM ammonium sulfate; 67 mM magnesium chloride; 10 mM β-mercaptoethanol; 6% DMSO, 300 μM each of dATP, dGTP, dCTP, and dTTP; and 175 ng of each primer. An initial denaturation at 95°C for 90 s was followed by 35 cycles of 95°C for 30 s, 62°C for 60 s, and 70°C for 60 s. Procedures for sequencing of PCR products have been described previously (20–22).

The primers used for amplification of markers on chromosome 3p14 are described in Kastury *et al.* (11) and Lisitsyn *et al.* (12). The primers used for RT-PCR analysis of the *FHIT* gene were 5′-CAT GTC GTT CAG ATT TGG CCA ACA TCT C-3′ (FP1) and 5′-ACA GGA TGG TGA GAT GAA GAA ACT GC-3 ′ (RP2) for exons 5–10, and 5-′CTG TAA AGG TCC GTA GTG C-3′ (MUR5) and RP2 for exons 3–10. These products were sequenced directly using the primers 5′-GAG GAC TCC GAA GAG GTA GC-3′ (SP1), 5′-TCG GCC ACT TCA TCA GGA CG-3′ (RSP1), 5′-CAG GAC ATG TCC TTG TGT GC-3′ (SP2), and 5′-CGG ACA GAC TGT GAA GCA CG-3′ (SP3) and SequiTherm polymerase (Epicentre, Madison, WI). These primers allowed determination of sequences encompassing the entire open reading frame. For verification of the integrity of the RNA samples, control RT-PCR amplifications using primers specific for genes on chromosome 18 were performed (37).

Results and Discussion

Many of the problems limiting unambiguous interpretation of the genetic analyses of tumors stem from contaminating nonneoplastic cells. DNA from such cells can complicate the interpretation of allelic losses, and RNA from such cells can mask the presence of tumor-specific altered transcripts. These problems can be avoided through the analysis of early-passage cell lines derived from colorectal can-

Received 4/30/96; accepted 5/15/96.

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[1] Supported by the Clayton Fund; NIH Grants CA35494, CA43460, CA09243, CA57208, CA57345, and CA62924; and American Cancer Society Grant FRA-451. B. V. is an Investigator of the Howard Hughes Medical Institute.

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[3] The abbreviations used are: RDA, representational difference analysis; Ap_4A, diadenosine 5′,5‴-P^1,P^4-tetraphosphate; RT, reverse transcription.

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cers. The genetic profiles of such lines has been shown to be very similar to those of the primary tumors from which the lines were derived (23–25, 37).

Thirty-one such early-passage lines (20 passaged as xenografts and 11 passaged *in vitro,* each from a different patient) were chosen for analysis. These cases included all lines established in our laboratories in which high-quality RNA and DNA, as well as corresponding normal tissues from the same patients, were available, and in which the microsatellite instability (26–28) characteristic of mismatch repair deficiency (29) was not observed; such instability makes it difficult to interpret genetic analyses (27).

The tumors were first studied at the genomic level using five microsatellite markers. Two of these markers (D3S1300 and D3S1481) were located within the *FHIT* gene, and three (D3S1234, D3S1295 and D3S1313) were slightly telomeric (11, 15). The normal tissues of all 31 samples were heterozygous with at least two of these markers, and allelic losses were observed in 7 of the tumors (22%; examples in Fig. 1).

To search for homozygous deletions, *i.e.,* cases in which both parental alleles were lost from the tumor, we examined the same five microsatellite markers, three nonpolymorphic markers delineated with RDA (12, 14) and shown subsequently to lie within intron 5 of *FHIT* (15), and exons 3 and 5 of *FHIT.* Only one of the 31 cases exhibited a homozygous deletion of any of these sequences. This case was one of the seven tumors with an allelic loss of surrounding 3p sequences, and the homozygous deletion included two RDA markers from within *FHIT* but did not include exons 5–10 of the gene (see below).

Tumor suppressor genes can be inactivated in two ways (13): (*a*) expression can be eliminated or reduced substantially by mutations in regulatory sequences; (*b*) the protein encoded by the gene can be altered qualitatively by missense or nonsense mutations. To determine whether *FHIT* expression was altered substantially, we amplified exons 5–10 of the *FHIT* gene from tumor cDNA. These exons were chosen for analysis because they contained the entire open reading frame of the *FHIT* gene and because all previously documented alterations of *FHIT* involved at least one of these five exons.

To perform the new analyses, we designed primers that allowed robust, single-stage amplification and did not coamplify murine transcripts that might complicate the analysis of the xenografts. *FHIT*-specific products of full-length size were observed readily in 29 of the 31 cases (examples in Fig. 2). In each of these 29 cases, a single, major RT-PCR product was observed. Faint bands of larger or smaller size, presumably representing alternatively spliced transcripts, were seen occasionally, but these were no more prominent in the tumor samples than in normal colon or brain (Fig. 2, *Lanes C* and *B*). In 2 of the 31 cases, no RT-PCR products at all could be generated; the integrity of the RNA and cDNA in these samples was tested as described in "Materials and Methods." A single, major RT-PCR product was observed in the case with the homozygous deletion within *FHIT* noted above as well as in the other six cases with allelic losses (examples in Fig. 2*A*, pairs 3, 4, and 6).

To determine whether subtle alterations in the coding sequence of *FHIT* existed within these transcripts, we determined the sequence of the RT-PCR products as described in "Materials and Methods." Only three sequence variants were detected, and these were likely to represent polymorphisms, as each was silent. Two involved G to A transitions at codon 183 (threonine), and one involved C to T transitions at codon 294 (histidine).

We also used primers very similar to those used by Ohta *et al.* (15) for analysis of the same samples. The results confirmed those noted above, and a single, major RT-PCR product containing exons 3–10 was observed in 27 of the 31 cancers (examples in Fig. 2*B*). In two cases, no RT-PCR product was observed (the same two cases showing no amplification with the exon 5–10 primer set). In two other cases, a smaller product was observed in addition to the product of normal size (data not shown).

Some of the results obtained in the studies described above are in apparent conflict with data published previously. Two improvements in the approach used to evaluate *FHIT* in this study may be responsible for these differences. (*a*) The availability of early-passage lines made it possible to exclude contamination of the samples with nucleic acids derived from nonneoplastic cells of primary tumors, such as those used in the previous studies (11, 15). In such primary tumors, it is often difficult to distinguish true allelic loss from allelic imbalance (*i.e.,* two or more copies of one allele, and one copy of the other), which can lead to errors in estimating the proportion of tumors with such losses. In early-passage lines, such losses are unambiguous (see Fig. 1) and correlate highly with those observed in microdissected primary tumors (23–25). This might explain the lower prevalence of allelic loss observed in our study [7 of 31 (22%)] compared to that of Kastury *et al.* [15 of 32 (47%); Ref. 11], although differences in tumor stage or simple statistical variation might also account for this discrepancy. (*b*) Our PCR conditions were apparently more robust than those of Ohta *et al.* (15), who used a nested PCR strategy (15). These authors described two types of altered transcripts. Although the exons that were lost varied from tumor to tumor, the missing exons in "class I" transcripts always included exon 5, and those in "class II" transcripts always included exon 8 (15). We found little evidence for either of these classes of transcripts. In our experience, nested PCR can result in overrepresentation of shorter, alternatively spliced transcripts in addition to or instead of the normal product. In fact, Ohta *et al.* (15) observed full-length RT-PCR products in most of the cases in which aberrant products were observed but suggested that some of these normal products might have emanated from contaminating nonneoplastic cells. Our studies show clearly that these normal products are actually derived from neoplastic cells, and furthermore, that these were the major products in normal colon as well as in nearly all of the 31 colorectal cancers examined. It is also important that we did not observe any point mutations or other subtle changes of the type that occur characteristically in tumor suppressor genes (13). Finally, it is possible that the differences in the extent of aberrant transcripts

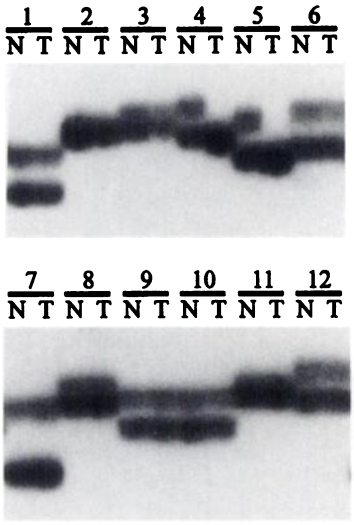

Fig. 1. Allelic losses of the *FHIT* locus. Primers specific for the microsatellite marker D3S1300 were used to amplify genomic DNA from early-passage colorectal cancer cell lines and normal colon of the same patients. Each normal (N) tumor (T) pair is derived from a different patient. Pairs 1 (Co50), 3 (Co79), 6 (Co84), 7 (Mx8), 8 (Mx9), 9 (Mx10), 10 (Mx11), and 12 (Mx13) retained heterozygosity; pairs 4 (Co80) and 5 (Co82) lost one allele; and pairs 2 (Co74) and 11 (Mx12) were uninformative (*i.e.,* the normal cells of the patients were not heterozygous for the marker).

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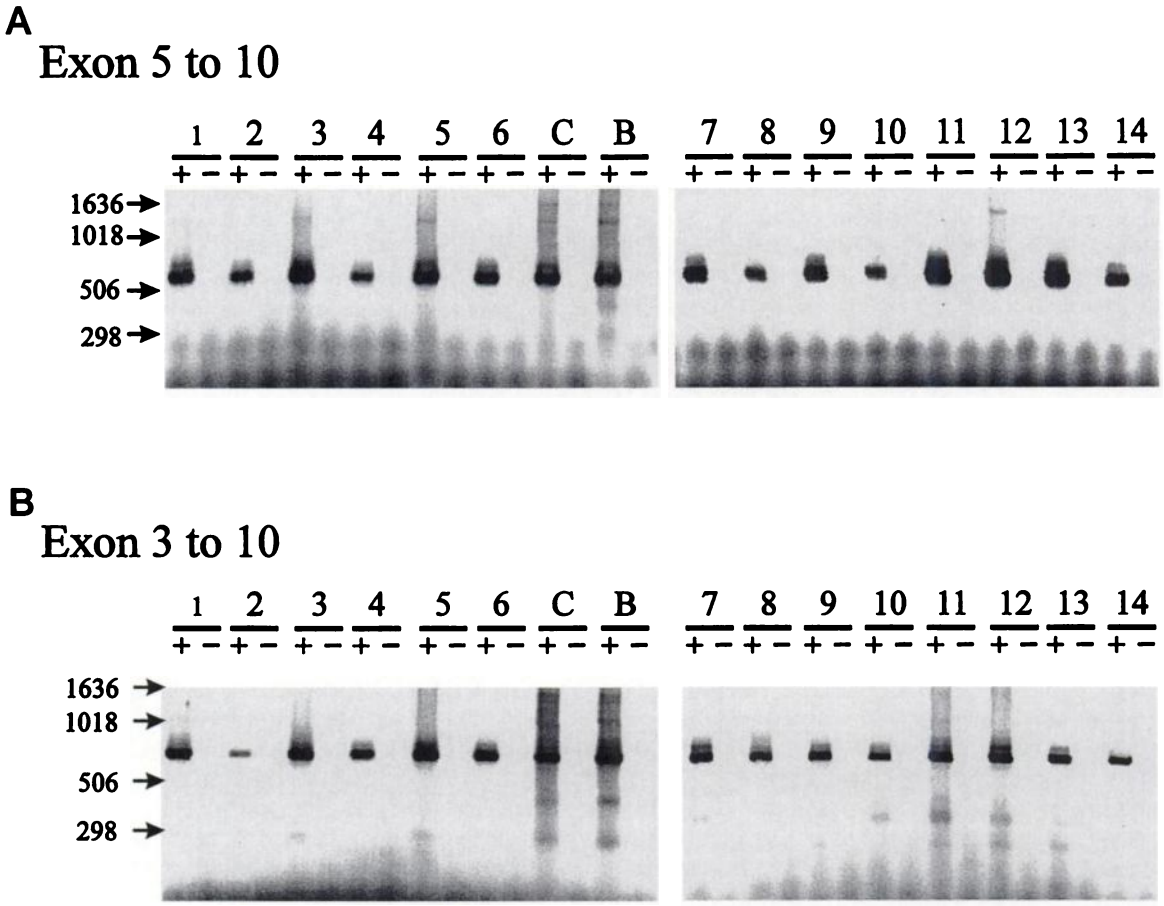

Fig. 2. RT-PCR analysis of the *FHIT* transcript. RT-PCR analysis was performed as described in "Materials and Methods," using RNA from the colorectal cancer cell lines as templates and primers allowing amplification of exons 5–10 (*A*) or exons 3–10 (*B*). *Lanes marked + and −* correspond to RT reactions performed with and without reverse transcriptase, respectively. RNA from different colorectal cancers (Co48, Co50, Co79, Co82, Co89, Co90, Mx3, Mx5, Mx7, Mx9, Mx10, Mx12, Mx15, and Mx16) were the templates for pairs 1–14, respectively, while RNA from normal colon or normal brain was used in *Lanes C* and *B*, respectively. The migration of molecular weight markers is indicated in kb in the *left margin*.

observed in the two studies simply reflect statistical variation. Although Ohta *et al.* (15) and Sozzi *et al.* (17) concluded that *FHIT* was expressed aberrantly in more than half the tumors of several different types that they examined, their data on colorectal tumors was limited to eight cases, three of which appeared to exhibit truncated RT-PCR products (15, 17).

In 29 of the 31 cases examined in this study, a normal-sized RT-PCR product, containing the complete coding region of *FHIT*, was observed. In 27 of these 29 cases, the only major PCR product was that corresponding to the normal, whereas in 2 of the 29 cases, a smaller PCR product was observed in addition to the normal product. Even assuming that the absence of a normal product (two cases) or the additional presence of a smaller product (two cases) is functionally important, the maximal involvement of *FHIT* was confined to the 4 of 31 tumors (13%) in which any abnormality was observed. In these four cases, however, it is possible that *FHIT* was not the only gene in the region that was altered. Likewise, the homology of *FHIT* to Ap_4A hydrolase does not provide any obvious clues as to how the type of aberrances noted would lead to tumor initiation or progression. Finally, although the examination of early-passage colorectal cancers could conceivably overestimate the proportion of tumors exhibiting alterations of a given gene, such a panel is extremely unlikely to underestimate this proportion.

Several interpretations of these data are therefore possible. (*a*) The *FHIT* gene is not involved causally in any colorectal tumor and is simply located adjacent to or surrounding another gene (the true "target") that drives a clonal selection process. Genes within genes have been previously demonstrated to exist, including those that affect tumor suppressor loci (30–34). (*b*) *FHIT* is indeed a true target, but its role is limited to the small number of colorectal tumors (~13%) that exhibit altered transcripts. (*c*) *FHIT* is functionally altered in more tumors than are apparent in our studies, but such alterations affect the extent of its translation rather than its coding sequence. This possibility could not be addressed fully in our studies, but can be analyzed in the future through examination of the *FHIT* protein. (*d*) The losses of genetic material in this region are not related to tumorigenesis at all, and reflect simply the plasticity of the cancer cell genome. Notable in this regard is the inducible fragile site, *FRA3B*, which lies within *FHIT* and could make this region particularly susceptible to some form of cancer-specific genetic instability (35, 36). Additional evaluations of tumors and normal tissues, as well as complete sequencing of the chromosomal region containing *FHIT*, should clarify these issues in the future.

Acknowledgments

We thank Drs. Kay Huebner and Carlo M. Croce for critical reading of the manuscript and Ms. Tina Gwiazda for expert secretarial assistance.

References

1. Linehan, W. M., Lerman, M. I., and Zbar, B. Identification of the von Hippel-Lindau (*VHL*) gene. Its role in renal cancer. JAMA, *273:* 564–570, 1995.
2. Cohen, A. J., Li, F. P., Berg, S., Marchetto, D. J., Tsai, S., Jacobs, S. C., and Brown, R. S. Hereditary renal-cell carcinoma associated with a chromosomal translocation. N. Engl. J. Med., *301:* 592–595, 1979.
3. Wang, N., and Perkins, K. L. Involvement of band 3p14 in t(3;8) hereditary renal carcinoma. Cancer Genet. Cytogenet., *11:* 479–481, 1984.

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4. Paradee, W., Mullins, C., He, Z., Glover, T., Wilke, C., Opalka, B., Schutte, J., and Smith, D. I. Precise localization of aphidicolin-induced breakpoints on the short arm of human chromosome 3. Genomics, *27:* 358–361, 1995.
5. Naylor, S. L., Johnson, B. E., Minna, J. D., and Sakaguchi, A. Y. Loss of heterozygosity of chromosome 3p markers in small-cell lung cancer. Nature (Lond.), *329:* 451–454, 1987.
6. Brauch, H., Johnson, B., Hovis, J., Yano, T., Gazdar, A., Pettengill, O. S., Graziano, S., Sorenson, G. D., Poiesz, B. J., Minna, J., Linehan, M., and Zbar, B. Molecular analysis of the short arm of chromosome 3 in small-cell and non-small-cell carcinoma of the lung. N. Engl. J. Med., *317:* 1109–1113, 1987.
7. Zbar, B., Brauch, H., Talmadge, C., and Linehan, M. Loss of alleles of loci on the short arm of chromosome 3 in renal cell carcinoma. Nature (Lond.), *327:* 721–724, 1987.
8. Zeiger, M. A., Gnarra, J. R., Zbar, B., Linehan, W. M., and Pass, H. I. Loss of heterozygosity on the short arm of chromosome 3 in mesothelioma cell lines and solid tumors. Genes Chromosomes & Cancer, *11:* 15–20, 1994.
9. Zeiger, M. A., Zbar, B., Keiser, H., Linehan, W. M., and Gnarra, J. R. Loss of heterozygosity on the short arm of chromosome 3 in sporadic, von Hippel-Lindau disease-associated, and familial pheochromocytoma. Genes Chromosomes & Cancer, *13:* 151–156, 1995.
10. Mooibroek, H., Osinga, J., Postmus, P. E., Carritt, B., and Buys, C. H. Loss of heterozygosity for a chromosome 3 sequence presumably at 3p21 in small cell lung cancer. Cancer Genet. Cytogenet., *27:* 361–365, 1987.
11. Kastury, K., Baffa, R., Druck, T., Ohta, M., Cotticelli, M. G., Inoue, H., Negrini, M., Rugge, M., Huang, D., Croce, C. M., Palazzo, J., and Huebner, K. Potential gastrointestinal tumor suppressor locus at the 3p14.2 FRA3B site identified by homozygous deletions in tumor cell lines. Cancer Res., *56:* 978–983, 1996.
12. Lisitsyn, N. A., Leach, F. S., Vogelstein, B., and Wigler, M. H. Detection of genetic loss in tumors by representational difference analysis. Cold Spring Harbor Symp. Quant. Biol., *59:* 585–587, 1994.
13. Knudson, A. G. Antioncogenes and human cancer. Proc. Natl. Acad. Sci. USA, *90:* 10914–10921, 1993.
14. Lisitsyn, N. A., Lisitsina, N. M., Dalbagni, G., Barker, P., Sanchez, C. A., Gnarra, J., Linehan, W. M., Reid, B. J., and Wigler, M. H. Comparative genomic analysis of tumors: detection of DNA losses and amplification. Proc. Natl. Acad. Sci. USA, *92:* 151–155, 1995.
15. Ohta, M., Hiroshi, I., Cotticelli, M. G., Kastury, K., Baffa, R., Palazzo, J., Siprashvili, Z., Mori, M., McCue, P., Druck, T., Croce, C. M., and Huebner, K. The *FHIT* gene, spanning the chromosome 3p14.2 fragile site and renal carcinoma-associated t(3;8) breakpoint, is abnormal in digestive tract cancers. Cell, *84:* 587–597, 1996.
16. Huang, Y., Garrison, P. N., and Barnes, L. D. Cloning of the *Schizosaccharomyces pombe* gene encoding diadenosine 5′,5‴-p-1,p-4-tetraphosphate [ap(4)a] asymmetrical hydrolase: sequence similarity with the histidine triad (HIT) protein family. Biochem. J., *312:* 925–932, 1995.
17. Sozzi, G., Veronese, M. L., Negrini, M., Baffa, R., Cotticelli, M. G., Inoue, H., Tornielli, S., Pilotti, S., De Gregorio, L., Pastorino, U., Pierotti, M. A., Ohta, M., Huebner, K., and Croce, C. M. The *FHIT* gene at 3p14.2 is abnormal in lung cancer. Cell, *85:* 17–26, 1996.
18. Markowitz, S., Wang, J., Myeroff, L., Parsons, R., Sun, L., Lutterbaugh, J., Fan, R. S., Zborowska, E., Kinzler, K. W., Vogelstein, B., Brattain, M., and Willcon, J. K. V. Inactivation of the type II TGF-β receptor in colon cancer cells with microsatellite instability. Science (Washington DC), *268:* 1336–1338, 1995.
19. Willson, J. Cell Culture of human colon adenomas and carcinomas. Cancer Res., *47:* 2704–2713, 1987.
20. Leach, F. S., Nicolaides, N. C., Papadopoulos, N., Liu, B., Jen, J., Parsons, R., Peltomäki, P., Sistonen, P., Aaltonen, L. A., Nyström-Lahti, M., Guan, X-Y., Zhang, J., Meltzer, P. S., Yu, J-W., Kao, F-T., Chen, D. J., Cerosaletti, K. M., Fournier, R. E. K., Todd, S., Lewis, T., Leach, R. J., Naylor, S. L., Weissenbach, J., Mecklin, J-P., Järvinen, H., Peterson, G. M., Hamilton, S. R., Green, J., Jass, J., Watson, P., Lynch, H. T., Trent, J. M., de la Chapelle, A., Kinzler, K. W., and Vogelstein, B. Mutations of a mutS homolog in hereditary nonpolyposis colorectal cancer. Cell, *75:* 1215–1225, 1993.
21. Papadopoulos, N., Nicolaides, N. C., Wei, Y-F., Ruben, S. M., Carter, K. C., Rosen, C. A., Haseltine, W. A., Fleischmann, R. D., Fraser, C. M., Adams, M. D., Venter, J. C., Hamilton, S. R., Peterson, G. M., Watson, P., Lynch, H. T., Peltomäki, P., Mecklin, J-P., de la Chapelle, A., Kinzler, K. W., and Vogelstein, B. Mutation of a mutL homolog in hereditary colon cancer. Science (Washington DC), *263:* 1625–1629, 1994.
22. Liu, B., Parsons, R., Papadopoulos, N., Nicolaides, N. C., Lynch, H. T., Watson, P., Jass, J. R., Dunlop, M., Wyllie, A., Peltomaki, P., de la Chapelle, A., Hamilton, S. R., Vogelstein, B., and Kinzler, K. W. Analysis of mismatch repair genes in hereditary non-polyposis colorectal cancer patients. Nat. Med., *2:* 169–174, 1996.
23. Baker, S. J., Preisinger, A. C., Jessup, J. M., Paraskeva, C., Markowitz, S., Willson, J. K., Hamilton, S., and Vogelstein, B. *p53* gene mutations occur in combination with 17p allelic deletions as late events in colorectal tumorigenesis. Cancer Res., *50:* 7717–7722, 1990.
24. Hahn, S. A., Seymour, A. B., Hoque, A. T., Schutte, M., da Costa, L. T., Redston, M. S., Caldas, C., Weinstein, C. L., Fischer, A., Yeo, C. J., Hruban, R. H., and Kern, S. E. Allelotype of pancreatic adenocarcinoma using xenograft enrichment. Cancer Res., *55:* 4670–4675, 1995.
25. McQueen, H. A., Wyllie, A. H., Piris, J., Foster, E., and Bird, C. C. Stability of critical genetic lesions in human colorectal carcinoma xenografts. Br. J. Cancer, *63:* 94–96, 1991.
26. Thibodeau, S. N., Bren, G., and Schaid, D. Microsatellite instability in cancer of the proximal colon. Science (Washington DC), *260:* 816–819, 1993.
27. Ionov, Y., Peinado, M. A., Malkhosyan, S., Shibata, D., and Perucho, M. Ubiquitous somatic mutations in simple repeated sequences reveal a new mechanism for colonic carcinogenesis. Nature (Lond.), *363:* 558–561, 1993.
28. Aaltonen, L. A., Peltomäki, P., Leach, F. S., Sistonen, P., Pylkkanen, L., Mecklin, J. P., Jarvinen, H., Powell, S. M., Jen, J., Hamilton, S. R., Petersen, G. M., Kinzler, K. W., Vogelstein, B., and de la Chapelle, A. Clues to the pathogenesis of familial colorectal cancer. Science (Washington DC), *260:* 812–816, 1993.
29. Marra, G., and Boland, C. R. Hereditary nonpolyposis colorectal cancer: the syndrome, the genes, and historical perspectives. J. Natl. Cancer Inst., *87:* 1114–1125, 1995.
30. White, R., Viskochil, D., and O'Connell, P. Identification and characterization of the gene for neurofibromatosis type 1. Curr. Opin. Neurobiol., *1:* 462–467, 1991.
31. Goldberg, N. S., and Collins, F. S. The hunt for the neurofibromatosis gene. Arch. Dermatol., *127:* 1705–1707, 1991.
32. Cawthon, R. M., Andersen, L. B., Buchberg, A. M., Xu, G. F., O'Connell, P., Viskochil, D., Weiss, R. B., Wallace, M. R., Marchuk, D. A., Culver, M., Stevens, J., Jenkins, N. A., Copeland, N. G., Collins, F. S., and White, R. cDNA sequence and genomic structure of *Ev12b,* a gene lying within an intron of the neurofibromatosis type 1 gene. Genomics, *9:* 446–460, 1991.
33. Viskochil, D., Cawthon, R., O'Connell, P., Xu, G. F., Stevens, J., Culver, M., Carey, J., and White, R. The gene encoding the oligodendrocyte-myelin glycoprotein is embedded within the neurofibromatosis type 1 gene. Mol. Cell. Biol., *11:* 906–912, 1991.
34. O'Connell, P., Viskochil, D., Buchberg, A. M., Fountain, J., Cawthon, R. M., Culver, M., Stevens, J., Rich, D. C., Ledbetter, D. H., Wallace, M., Carey, J. C., Jenkins, N. A., Copeland, N. G., Collins, F. S., and White, R. The human homolog of murine Evi-2 lies between two von Recklinghausen neurofibromatosis translocations. Genomics, *7:* 547–554, 1990.
35. Glover, T. W., Coyle-Morris, J. F., Li, F. P., Brown, R. S., Berger, C. S., Gemmill, R. M., and Hecht, F. Translocation t(3;8)(p14.2;q24.1) in renal cell carcinoma affects expression of the common fragile site at 3p14(FRA3B) in lymphocytes. Cancer Genet. Cytogenet., *31:* 69–73, 1988.
36. Sutherland, G. R. Chromosomal fragile sites. Genet. Anal. Tech. Appl., *8:* 161–166, 1991.
37. Thiagalingam, S., Lengauer, C., Leach, F. S., Schutte, M., Hahn, S., Overhauser, J., Wilson, J. K. V., Markowitz, S., Hamilton, S. R., Kern, S. E., Kinzler, K. W., and Vogelstein, B. Evaluation of candidate tumor suppressor genes on chromosome 18 in colorectal cancers. Nat. Genet., in press, 1996.

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Cancer Res 1996;56:2936-2939.

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