[CANCER RESEARCH 63, 8578–8581, December 15, 2003]

Advances in Brief

Hyper-Recombination and Genetic Instability in BLM-Deficient Epithelial Cells

Giovanni Traverso,[1,2] Chetan Bettegowda,[1] Jürgen Kraus,[3] Michael R. Speicher,[3] Kenneth W. Kinzler,[1] Bert Vogelstein,[1] and Christoph Lengauer[1]

[1]Howard Hughes Medical Institute and Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University, Baltimore, Maryland; [2]Trinity College, Cambridge, United Kingdom; and [3]Institut für Humangenetik, Technische Universität München and GSF, München, Germany

Abstract

Genetic instability appears to be required for a normal colorectal epithelial cell to evolve into a cancerous one. Bloom syndrome patients have a strong predisposition to cancer that affects a variety of tissues. The mechanism of disease is attributed to genomic instability, but many questions about the nature of this instability have not yet been answered. To investigate these issues, we used gene-targeting techniques to disrupt the *BLM* gene in karyotypically stable colorectal cancer epithelial cells. *BLM* knockout cells showed an increased tendency of sister chromatids to exchange DNA strands and were substantially more likely to undergo homologous recombination at chromosomal loci than parental cells. Surprisingly, BLM-deficient colorectal cancer epithelial cells did not display gross chromosomal rearrangements nor a change in the rates of chromosome gains and losses. However, the enhanced homologous recombination was associated with losses of heterozygosity. These observations define a type of genetic instability that has significant implications for the evolution of cancer.

Introduction

The human Bloom syndrome gene (*BLM*) encodes a homologue of the *Escherichia coli* RecQ DNA helicase (1, 2). DNA helicase unwinds double-stranded DNA molecules, a process required for various aspects of DNA metabolism, including transcription, DNA repair, and replication (3). Mutations in the *BLM* homologues of mouse, *Drosophila*, yeast, *E. coli*, and *Caenorhabditis elegans* all cause a pronounced genomic instability phenotype manifest by gross chromosomal rearrangements, chromosomal nondisjunction, elevated levels of somatic recombination, and losses of heterozygosity (LOH). In *Drosophila*, mutations at the mus309 locus (DmBLM) result in 10-fold increased levels of nondisjunction as well as in whole chromosome loss (4). In Sgs1 mutant *Saccharomyces cerevisiae*, 20-fold increases in gross chromosomal rearrangements as well as elevated rates of homologous recombination have been observed (5, 6). *Saccharomyces pombe* mutants for Rqh1 are endowed with a hyper-recombinogenic and chromosome nondisjunction phenotype (7). Data from mouse models display all of the above mentioned forms of instability (8, 9). Gross chromosomal rearrangements have been reported in a few hematological neoplasms of Bloom syndrome patients who carry a defective BLM protein (10). Moreover, the incidence of exfoliated epithelial cells containing micronuclei is elevated in Bloom syndrome patients compared with individuals with a heterozygous BLM gene mutation status (11). BLM heterozygosity has been found to increase the risk for colorectal cancer in the Ashkenazi population (12). Colorectal adenomas from a Bloom syndrome patient have previously been analyzed cytogenetically (13). Interestingly, the great majority of cells displayed a diploid karyotype. This is in stark contrast to non-Bloom's adenomas, which generally tend to show chromosomal gains/losses even at an early stage (14). This unexpected finding stimulated us to rigorously evaluate chromosomal instability in human epithelial cells in a well-defined experimental system.

Materials and Methods

Cell Culture, Transfection, and Screening for Recombinants. HCT116 cells (American Type Culture Collection, Manassas, VA) were used to generate Blm−/− cell lines. Targeting was performed using the pFred two-vector targeting system described previously in Jallepalli *et al.* (15). Briefly, the left homology arm was amplified using primers L1 and L2, and cloned into *Sal*I and *Eco*RI sites of the pFred-B construct. The right homology arm was amplified with R1 and R2, and cloned into *Bam*HI and *Not*I sites of the pFred-A construct. HCT116 cells were cultured in McCoy's 5A Medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. Using LipofectAmine (Invitrogen, Carlsbad, CA) HCT116 cells were transfected with the two plasmids described above. Clones were selected after 3 weeks of growth under 0.4 mg/ml geneticin (Invitrogen) selection. Recombinant clones were identified by PCR screening. Primer sequences for the left and right homology arms (lowercase sequences contain restriction sites for cloning): L1: 5′-aataattagtcgacTTTTAGCAAATTGGTGACATGA-3′; L2: 5′-attatgaattcGGTCCTCAAAGTTGTCCAGAAA-3′; R1: 5′-attaattggatccCAGATAAGTTTACAGCAGCAGC-3′; and R2: 5′-ataagaattatgcggccgcTTGAAATTGGGGTGGAAGGAC-3′.

Sister Chromatid Exchange (SCE). Cells were grown in 50 μM bromodeoxyuridine for ~35 h (16). After bromodeoxyuridine labeling, cells were incubated with 0.1 μg/ml Colcemid for 5 h. Cells were subjected to standard 0.075 M KCl hypotonic treatment and fixation with cold methanol/glacial acetic acid (3:1). Cells were dropped onto slides and stained with 0.5 μg/ml Hoechst 33258 in PBS for 5 min. Slides were mounted in MacIlvaine's buffer, and SCEs were assessed through standard fluorescent microscopy.

Chromosomal Instability and Micronuclei Formation. The quantitative analysis of chromosomal instability using fluorescence *in situ* hybridization (FISH) analysis with chromosome-specific centromeric probes was performed as described previously by Lengauer *et al.* (17). Multiplex-FISH was performed as described previously by Speicher *et al.* (18). Metaphase spreads were prepared by treating cells with 0.1 μg/ml colcemid (KaryoMax; Invitrogen). Micronuclei were determined after staining cells with Hoechst 33258 as described above.

Homologous Recombination. Site-directed homologous recombination was measured using two different constructs, which vary by an order of magnitude in their recombination frequency. The constructs tested were p53-ATG-neo (19) and pDnmt-hygro (20). Transfections and culturing conditions were performed as described above with the exception of selection for pDnmt1-hygro clones with 0.1 mg/ml hygromycin. Recombinants were identified through PCR screening.

LOH. LOH was determined through the typing of 16 diallelic markers identified previously by Weber *et al.* (21). Typing of single clones was performed using fluorescently labeled primers for each marker in a PCR

Received 9/17/03; accepted 10/27/03.

Grant Support: Translational Research Award from the Virginia and D. K. Ludwig Fund for Cancer Research (C. L.), Bundesministerium für Bildung und Forschung (NGFN KB P06T5 and P06T6; J. K., M. R. S.), and NIH Grant CA 43460. G. T. is the recipient of a Junior Research Fellowship from Trinity College, University of Cambridge, United Kingdom.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Christoph Lengauer, Department of Oncology, 1650 Orleans Street, Johns Hopkins University, Baltimore, MD 21231. E-mail: lengauer@jhmi.edu.

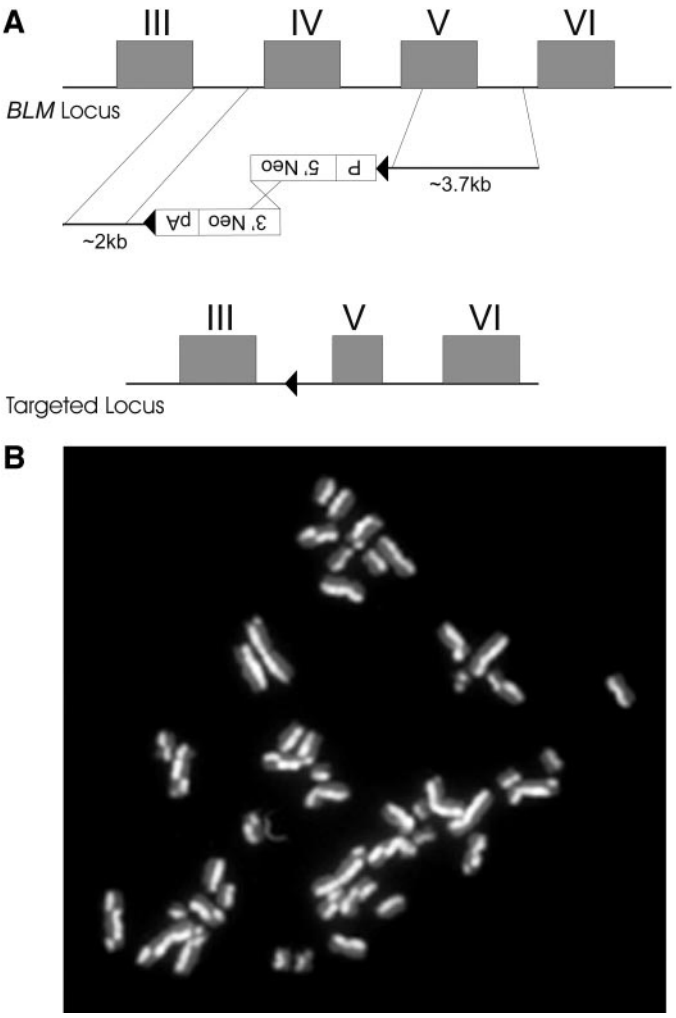

Fig. 1. *BLM*−/− colorectal cancer cells. *A*, cell line HCT116 (American Type Culture Collection, Manassas, VA) was used to generate *BLM*−/− cells. Targeting was performed using the pFred two-vector targeting system described previously (15). The targeted allele results in a truncated *BLM* protein closely resembling those described in Bloom syndrome kindreds (1). Recombinant clones were identified by multiple PCR-based screens. *P*, SV40 promoter. *PA*, *tk* polyadenylation signal. *Neo*, neomycin resistance marker. *B*, metaphase spread of a *BLM*−/− cell showing a high number of SCEs.

reaction. PCR products were resolved on a SpectruMedix SCE-9610 (SpectruMedix, State College, PA).

Results

To evaluate genomic instability in a major site of neoplasia in Bloom syndrome (12, 22), we genetically inactivated the *BLM* locus in the human colorectal cancer cell line HCT116 (Fig. 1*A*). These epithelial cells have been shown previously to have a near-diploid karyotype and no measurable chromosomal instability (17). Knockout cells (*BLM*−/−) from three different clones had growth characteristics indistinguishable from parental cells (data not shown). Two of the *BLM*−/− clones were characterized in detail with respect to genomic instability.

It has been reported that mouse cells with a deficient *BLM* gene frequently show gross chromosomal rearrangements (9). To evaluate such changes in epithelial and fibroblast cells deficient in *BLM*, we performed multiplex-FISH karyotyping (Ref. 18; Fig. 2). Twenty-one of 24 studied metaphases of parental HCT116 cells displayed a clonal population with the characteristic karyotype of 45,X,-Y, der(10)dup(10)q24q26)t(10;16)(q26;q24), der(16)t(8;16)(q13;p13), and der(18)t(17;18)(q21;p11.3; Ref. 23). The heterozygote clone had a similarly stable karyotype (38 of 40 metaphases with characteristic karyotype) as did the two homozygote knockout clones (40 of 45 and 30 of 37 metaphases with the characteristic karyotype, respectively). These results were markedly different from those obtained after disruption of the *hSecurin* gene in the same cells, where only 10 of 40 cells retained the characteristic karyotype ($P < 0.005$; Refs. 15, 17; Fig. 2). We conclude that the prevalence of gross chromosomal rearrangements is not appreciably changed by disruption of the *BLM* gene in colorectal epithelial cells.

To evaluate the rate of chromosomal gains and losses in these cells, we analyzed interphase nuclei from parental, heterozygote, and homozygote cells after passage for 50 generations (17). Karyotypic losses and gains were assessed by counting the number of centromeres per nucleus for chromosomes 7, 12, 17, and X. The numbers of losses and gains were equally low for wild-type and *BLM*−/− homozygous knockout cells; the fraction of cells with centromere numbers different from the modal number was <5% for all of the chromosomes tested in HCT116 parental cells and for 7 of 8 chromosomes tested in two different *BLM*−/− knockouts clones (Table 1*A*). As a positive control, we again evaluated HCT116 cells with an inactivated *hSecurin* gene. Almost half of the *hSecurin*-deficient cells (49%) exhibited centromere numbers different from the modal number ($P < 10^{-6}$; Refs. 15, 17). In addition, we examined Bloom syndrome patient fibroblasts (GM08505C) together with matching control fibroblasts (GM00637H, both from the Coriell Cell Repository) for whole chromosome gains and losses. Interestingly, the percentage of cells with centromere numbers different from the mode in BLM fibroblasts was significantly higher (9%) than in control fibroblasts (2%; $P < 10^{-6}$) but not as high as in cancer cells with chromosomal instability (17).

Cells were also assessed for the presence of micronuclei, implicated previously in genomic instability (11). Micronuclei levels were elevated in the *BLM*−/− cells compared with *BLM*+/+ controls. The *BLM*−/− clones demonstrated 5.8% and 6.5% micronuclei *versus* 1.2% in parental controls ($P < 10^{-8}$). BLM patient fibroblasts demonstrated 11.5% micronuclei *versus* 4.0% control fibroblasts ($P < 0.005$). *hSecurin*−/− cells conversely displayed 13.5% and 16.5% micronuclei in two independently derived clones compared with 1.2% in parental HCT116 cells (15).

We then evaluated SCEs, the prototypical feature found in Bloom syndrome mesenchymal cells (Ref. 24; Fig. 1*B*). All of the HCT116

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y	# of metaphases analyzed
HCT116, parental																			1			1		1	24
Blm+/-			1					1																	40
Blm-/-, clone 1								5																	45
Blm-/-, clone 2				1									1	2								3			37
hSecurin-/-	1	5	6	7	4	6	4	7	4	3	3	5	4	3	5	1	5	1	4	3	4	11*	1		20

Fig. 2. Multiplex-FISH analysis of *BLM*−/− cells. Pictographic representation of Multiplex-FISH (*M-FISH*) data. Each cell line (HCT116 and derivatives including a chromosomally unstable *hSecurin*−/− cell line) was painted by M-FISH and analyzed for alterations of chromosome structure and number. Partial as well as whole chromosomal gains and losses are depicted by *green* and *red boxes*, respectively. Numbers within boxes denote in how many metaphases such chromosomal abnormalities occurred. * indicates that in addition to a loss of chromosome 22 in 11 metaphases, 1 metaphase showed a gain of this chromosome.

Table 1 *Analysis of BLM−/− cells*

A. Centromere losses and gains

Cells	Chromosome[a] 7	12	17	X	Average % cells off the mode[b]
Blm+/+	99	98	96	98	2
Blm−/−	96	94	96	98	4
Blm−/−	98	95	96	98	3
BLM fibroblasts	91	92	93	86	9
Control fibroblasts	99	98	99	98	2
HT29[c]	49	53	45	58	49
Securin−/−[d]	76	70	74	68	28

B. Sister chromatid exchange

Genotype	Events	Metaphases analyzed	Range (per metaphase)	Average number of events per metaphase
Blm+/+	145	34	0–10	4.26
Blm+/+	129	30	0–8	4.30
Blm+/−	156	30	0–10	5.20
Blm−/−	347	34	2–22	10.21
Blm−/−	379	34	5–25	11.15

C. Targeted homologous recombination

Construct	Blm+/+	Blm−/−	Fold increase
Dnmt1-hyg	1/190	12/106	21.5
p53-ATG-neo	1/1141	8/527	17.3

[a] Percentage of cells showing the number of signals that correspond to the modal number of the specific chromosome.
[b] 200 cells were analyzed per chromosome specific centromeric probe.
[c] Ref. 17.
[d] Ref. 15.

BLM−/− clones displayed a significantly elevated number of SCEs. The knockout cells contained an average of 11 SCEs events per metaphase, compared with 5 and 4 SCEs/metaphase in *BLM*+/− and wild-type *BLM*+/+ cells, respectively (Table 1*B*).

Increased levels of SCEs have been directly correlated with increased rates of targeted homologous recombination (9, 25). To test and compare rates of homologous recombination in the *BLM*−/− cells, we transfected them with targeting constructs for two different human genes (19, 20). The two genes chosen for these experiments had been shown previously to have targeting efficiencies that varied by 1 order of magnitude in parental HCT116 cells. Transfection of $\sim 2 \times 10^7$ parental cells with a DNMT1 targeting construct yielded $\sim$1 targeted clone per 100 geneticin-resistant clones, whereas transfection with a p53 targeting construct gave rise to $\sim$1 positive clone per 1100 clones tested. These frequencies were in line with earlier studies (19, 20). However, homologous recombination in the *BLM*−/− background was elevated for both genes by >15 fold ($P < 10^{-5}$ and $P < 0.0002$; Table 1*C*).

Increased rates of homologous recombination have been associated with LOH events in mouse tumor models (9). Also, prior work has demonstrated an elevation in LOH in single lymphoblastoid clones derived from a Bloom's patient (26). Therefore, we sought to measure the rate of background LOH in BLM-deficient cells. The analysis was performed using 16 heterozygous diallelic markers distributed along chromosomes 2 and 3 (Table 2). These markers were typed in 95 individual BLM−/− and 190 BLM+/+ clones. Eight (8 of 95) noncontiguous LOH events were observed in the BLM−/− clones and 1 (1 of 190) in the BLM+/+ clones ($P < 0.0003$).

Discussion

It was surprising that the major manifestations of instability we found in *BLM*−/− colorectal cancer epithelial cells involved only those known to be associated with increased homologous recombination. The rates of whole chromosome gains and losses were essentially unaffected, and gross cytogenetic structural abnormalities could only rarely be identified. In contrast, there was a marked increase in LOH that did not involve whole chromosomes in SCE and in targeted homologous recombination. Hyper-recombinogenic states may prove to be a major mechanism for chromosomal instability in other hereditary and sporadic cancer types. However, our data show that such increased levels of recombination do not necessarily result in elevated levels of structural and numerical chromosome changes. Data supporting the idea that hyper-recombination is responsible for promoting tumorigenesis in *BLM*−/− cells has been obtained in *BLM* mouse models (9).

Apart from the implications for the pathogenesis of Bloom syndrome, the observations reported above have practical applications. The higher rate of recombination found in human *BLM*−/− HCT116 cells should prove useful for creating human somatic knockouts, an otherwise time-consuming, expensive, and labor-intensive task (27). Reversible knockout of the *BLM* gene, using cre-lox technologies, could be applied to any suitable cell type, such as human stem cells. Our results show that *BLM*-deficient human cells can be karyotypi-

Table 2 *Loss of heterozygosity (LOH) analysis in BLM−/− cells*

Ninety five BLM−/− and 190 BLM+/+ single clones were isolated through limiting dilution. Sixteen heterozygous diallelic markers were identified from the panel established by Weber *et al.* (21). Allelic status was determined through fluorescent-primer based PCR. LOH events are defined as events that result in a reduction to homozygosity. A total of eight noncontiguous LOH events were observed in 95 BLM−/− cells as compared with only 1 event out of 190 BLM+/+ clones. Note: clone 59 displayed LOH at seven contiguous loci on chromosome 3. Clone 87 displayed two LOH events on chromosome 2 with an intervening marker which was heterozygote, suggesting two independent nonhomologous recombination events.

Diallelic marker (MID)	Chromosome	Position (mb)	BLM−/− (95) Number of LOH events	BLM−/− (95) Clone ID	BLM+/+ (190) Number of LOH events	BLM+/+ (190) Clone ID
15	2	43.3				
499	2	69.9	1	80		
16	2	86.3				
1450	2	106.7				
1469	2	148.4	3	53, 78, 87		
2014	2	196.9	1	61		
296	2	201.1	1	87	1	22
1559	2	238.1				
1402	3	76.8				
2127	3	77.8	2	59, 62		
42	3	97.1	1	59		
1687	3	123.0	1	59		
1563	3	127.5	1	59		
1100	3	131.6	1	59		
2063	3	204.9	1	59		
1448	3	210.2	1	59		

cally stable, thereby facilitating subsequent evaluations and applications of the engineered clones.

Acknowledgments

We thank Leslie Meszler from the Cell Imaging Core Facility for help with imaging. We also thank Kurt Bachman and Harith Rajagopalan for helpful discussion and technical assistance.

References

1. Ellis, N. A., Groden, J., Ye, T. Z., Straughen, J., Lennon, D. J., Ciocci, S., Proytcheva, M., and German, J. The Bloom's syndrome gene product is homologous to RecQ helicases. Cell, *83:* 655–666, 1995.
2. Harmon, F. G., and Kowalczykowski, S. C. RecQ helicase, in concert with RecA and SSB proteins, initiates and disrupts DNA recombination. Genes Dev., *12:* 1134–1144, 1998.
3. Hickson, I. D. RecQ helicases: caretakers of the genome. Nat. Rev. Cancer, *3:* 169–178, 2003.
4. Kusano, K., Johnson-Schlitz, D. M., and Engels, W. R. Sterility of Drosophila with mutations in the Bloom syndrome gene–complementation by Ku70. Science (Wash. DC), *291:* 2600–2602, 2001.
5. Myung, K., Datta, A., Chen, C., and Kolodner, R. D. SGS1, the Saccharomyces cerevisiae homologue of BLM and WRN, suppresses genome instability and homeologous recombination. Nat. Genet., *27:* 113–116, 2001.
6. Gangloff, S., McDonald, J. P., Bendixen, C., Arthur, L., and Rothstein, R. The yeast type I topoisomerase Top3 interacts with Sgs1, a DNA helicase homolog: a potential eukaryotic reverse gyrase. Mol. Cell Biol., *14:* 8391–8398, 1994.
7. Stewart, E., Chapman, C. R., Al-Khodairy, F., Carr, A. M., and Enoch, T. rqh1+, a fission yeast gene related to the Bloom's and Werner's syndrome genes, is required for reversible S phase arrest. EMBO J., *16:* 2682–2692, 1997.
8. Chester, N., Kuo, F., Kozak, C., O'Hara, C. D., and Leder, P. Stage-specific apoptosis, developmental delay, and embryonic lethality in mice homozygous for a targeted disruption in the murine Bloom's syndrome gene. Genes Dev., *12:* 3382–3393, 1998.
9. Luo, G., Santoro, I. M., McDaniel, L. D., Nishijima, I., Mills, M., Youssoufian, H., Vogel, H., Schultz, R. A., and Bradley, A. Cancer predisposition caused by elevated mitotic recombination in Bloom mice. Nat. Genet., *26:* 424–429, 2000.
10. Poppe, B., Van Limbergen, H., Van Roy, N., Vandecruys, E., De Paepe, A., Benoit, Y., and Speleman, F. Chromosomal aberrations in Bloom syndrome patients with myeloid malignancies. Cancer Genet. Cytogenet., *128:* 39–42, 2001.
11. Rosin, M. P., and German, J. Evidence for chromosome instability *in vivo* in Bloom syndrome: increased numbers of micronuclei in exfoliated cells. Hum. Genet., *71:* 187–191, 1985.
12. Gruber, S. B., Ellis, N. A., Scott, K. K., Almog, R., Kolachana, P., Bonner, J. D., Kirchhoff, T., Tomsho, L. P., Nafa, K., Pierce, H., Low, M., Satagopan, J., Rennert, H., Huang, H., Greenson, J. K., Groden, J., Rapaport, B., Shia, J., Johnson, S., Gregersen, P. K., Harris, C. C., Boyd, J., and Offit, K. BLM heterozygosity and the risk of colorectal cancer. Science (Wash. DC), *297:* 2013, 2002.
13. Lowy, A. M., Kordich, J. J., Gismondi, V., Varesco, L., Blough, R. I., and Groden, J. Numerous colonic adenomas in an individual with Bloom's syndrome. Gastroenterology, *121:* 435–439, 2001.
14. Shih, I. M., Zhou, W., Goodman, S. N., Lengauer, C., Kinzler, K. W., and Vogelstein, B. Evidence that genetic instability occurs at an early stage of colorectal tumorigenesis. Cancer Res., *61:* 818–822, 2001.
15. Jallepalli, P. V., Waizenegger, I. C., Bunz, F., Langer, S., Speicher, M. R., Peters, J., Kinzler, K. W., Vogelstein, B., and Lengauer, C. Securin is required for chromosomal stability in human cells. Cell, *105:* 445–457, 2001.
16. German, J. A., B. Sister chromatid echange(SCE) analysis., pp. 8.6.1–8.6.10. New York: John Wiley & Sons.
17. Lengauer, C., Kinzler, K. W., and Vogelstein, B. Genetic instability in colorectal cancers. Nature (Lond.), *386:* 623–627, 1997.
18. Speicher, M. R., Gwyn Ballard, S., and Ward, D. C. Karyotyping human chromosomes by combinatorial multi-fluor FISH. Nat. Genet., *12:* 368–375, 1996.
19. Bunz, F., Dutriaux, A., Lengauer, C., Waldman, T., Zhou, S., Brown, J. P., Sedivy, J. M., Kinzler, K. W., and Vogelstein, B. Requirement for p53 and p21 to sustain G2 arrest after DNA damage. Science (Wash. DC), *282:* 1497–1501, 1998.
20. Rhee, I., Jair, K. W., Yen, R. W., Lengauer, C., Herman, J. G., Kinzler, K. W., Vogelstein, B., Baylin, S. B., and Schuebel, K. E. CpG methylation is maintained in human cancer cells lacking DNMT1. Nature (Lond.), *404:* 1003–1007, 2000.
21. Weber, J. L., David, D., Heil, J., Fan, Y., Zhao, C., and Marth, G. Human diallelic insertion/deletion polymorphisms. Am. J. Hum. Genet., *71:* 854–862, 2002.
22. Goss, K. H., Risinger, M. A., Kordich, J. J., Sanz, M. M., Straughen, J. E., Slovek, L. E., Capobianco, A. J., German, J., Boivin, G. P., and Groden, J. Enhanced tumor formation in mice heterozygous for Blm mutation. Science (Wash. DC), *297:* 2051–2053, 2002.
23. Bunz, F., Fauth, C., Speicher, M. R., Dutriaux, A., Sedivy, J. M., Kinzler, K. W., Vogelstein, B., and Lengauer, C. Targeted inactivation of p53 in human cells does not result in aneuploidy. Cancer Res., *62:* 1129–1133, 2002.
24. Honma, M., Tadokoro, S., Sakamoto, H., Tanabe, H., Sugimoto, M., Furuichi, Y., Satoh, T., Sofuni, T., Goto, M., and Hayashi, M. Chromosomal instability in B-lymphoblasotoid cell lines from Werner and Bloom syndrome patients. Mutat. Res., *520:* 15–24, 2002.
25. Imamura, O., Fujita, K., Itoh, C., Takeda, S., Furuichi, Y., and Matsumoto, T. Werner and Bloom helicases are involved in DNA repair in a complementary fashion. Oncogene, *21:* 954–963, 2002.
26. Groden, J., Nakamura, Y., and German, J. Molecular evidence that homologous recombination occurs in proliferating human somatic cells. Proc. Natl. Acad. Sci. USA, *87:* 4315–4319, 1990.
27. Sedivy, J. M., Vogelstein, B., Liber, H. L., Hendrickson, E. A., and Rosmarin, A. Gene targeting in human cells without isogenic DNA. Science (Wash. DC), *283:* 9, 1999.