[CANCER RESEARCH 62, 1129–1133, February 15, 2002]

Targeted Inactivation of p53 in Human Cells Does Not Result in Aneuploidy[1]

Fred Bunz, Christine Fauth, Michael R. Speicher, Annie Dutriaux, John M. Sedivy, Kenneth W. Kinzler, Bert Vogelstein, and Christoph Lengauer[2]

The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, [F. B., K. W. K., B. V., C. L.] and the Howard Hughes Medical Institute [B. V.], Johns Hopkins University, Baltimore, Maryland 21231; Institute of Anthropology and Human Genetics, University of Munich, Munich 80333, Germany [C. F., M. R. S.]; and Division of Biology and Medicine, Brown University, Providence, Rhode Island 02912 [A. D., J. M. S.]

ABSTRACT

Because p53 mutation and aneuploidy usually coexist, it has been suggested that p53 inactivation leads to aneuploidy. We have rigorously tested this hypothesis in diploid human cell lines in which p53 was experimentally inactivated by targeted homologous recombination. Cells completely deficient in p53 did not become aneuploid, although a slight tendency toward tetraploidization was observed. No increased rates of numerical or structural chromosomal instabilities were observed in the p53-deficient cells. Rates of sister chromatid exchange and homologous recombination were also unaffected by p53 status. These results show that inactivation of p53 does not, in and of itself, lead to the development of aneuploidy.

INTRODUCTION

Loss of p53 typically occurs in the late stages of tumor formation, and such tumors are almost always aneuploid (1, 2). Aneuploidy denotes a chromosome number different from a multiple of 2n and is often associated with structural alterations of chromosomes. This aneuploidy likely results from a high rate of losses and gains of chromosomes during abnormal mitoses (3). The concurrence of p53 loss and aneuploidy in naturally occurring and experimental neoplasms has led to the hypothesis that p53 functions to actively repress CIN[3] (4, 5). However, such correlations do not prove causality.

To rigorously test whether p53 deficiency caused measurable changes in the structural or numerical stability of chromosomes, we evaluated human cell lines whose *p53* alleles were disrupted by gene targeting. By comparing these lines to parental cells containing wild-type *p53* alleles, unambiguous assessments of the role of endogenous p53 in various forms of genetic instability could be made.

MATERIALS AND METHODS

Cells and Cell Culture. The human colon cancer cell line HCT116 and its derivatives HCTp53HET, which has a single *p53* allele disrupted, and HCTp53KO, which has both *p53* alleles disrupted (6), were cultured in McCoy's 5A supplemented with 10% FCS and penicillin/streptomycin. No selective drug was included during routine culture.

One allele of p53 was disrupted in lung-derived human fetal fibroblasts (FBp53HET) by homologous integration of a targeting vector. The remaining *p53* allele was found to be lost in a subpopulation of cells that had an increased life span (FBp53KO), resulting in a p53 null cell line (6). Fetal fibroblast cell lines were grown in Ham's F-10 medium supplemented with 15% FCS, penicillin/streptomycin, and 2 mM glutamine.

FISH. Methods for FISH analysis with chromosome-specific centromeric probes and quantitative analysis of chromosome loss rates have been described (7). To prepare metaphase spreads, cells were treated with 0.1 μg/ml Colcemid (KaryoMax; Life Technologies, Inc.) and processed by standard methods. Multiplex-FISH analysis was performed as described (8). The full karyotype of wild-type HCT116 cells (9) was confirmed as 45,X,−Y, der(10)dup(10)(q24q26)t(10;16)(q26;q24), der(16)t(8;16)(q13;p13), der(18)t(17;18)(q21;p11.3).

SCE Assay. Assays for SCE were performed essentially as described (10). Cells were grown in bromodeoxyuridine for 40 h, an interval in which chromatin was replicated twice. Differentially stained chromosomes were bleached under UV light and visualized under fluorescence. Individual exchanges between sister chromatids were scored manually.

Assay of Homologous Integration. A targeting vector (pRV6.9 h) with a total of 6.9 kb of sequence homologous to a region encompassing exon 3 of the *HPRT* gene has been described previously (11) and was modified for our experiments. The presence of both neomycin and hygromycin resistance markers in the HCT116 derivatives necessitated the construction of a version of this vector, designated pE3PUR, in which the neomycin transferase gene was replaced with a cassette encoding puromycin resistance.

All cell lines were passaged in medium supplemented with hypoxanthine/aminopterin/thymidine supplement (Life Technologies, Inc.) for 10–15 generations. The targeting vector was introduced into cells using Lipofectamine (Life Technologies, Inc.). Resistant colonies were grown in 2.5 μg/ml puromycin for 5 days, at which time the medium was removed and exchanged with medium containing 6-TG (Sigma Chemical Co.). 6-TG-resistant clones were grown for an additional 10 days. Genomic DNA was purified by a spin column procedure (Qiagen), and homologous integration was assessed by PCR as described (11).

RESULTS

Numerical and Structural Stability of Chromosomes. The colorectal cell line HCT116 has intact, wild-type *p53* genes and is stably diploid (7). The pathways leading to p53-dependent cell cycle arrest and apoptosis appear to be intact in HCT116 cells (12), and the two wild-type *p53* alleles in this line have been successfully targeted by homologous recombination, resulting in cells that do not express detectable p53 protein (6). These lines were examined by M-FISH, a technique that allows the visualization and identification of individual metaphase chromosomes that are "painted" with different colors. Twenty-two metaphases of parental HCT116 cells and of each p53-targeted subclone were examined in this way after growth for >50 generations in the same medium. In each case, the majority of metaphases from each cell line were indistinguishable from one another. Eighteen of 22 of the p53-deficient cells showed no karyotypic abnormalities, a fraction similar to that observed in parental HCT116 cells in the current study as well as in previous analyses of similar parental cells (Fig. 1*a*; Refs. 13, 14). In the remaining four metaphases, the following changes were observed: (*a*) absence of one copy of chromosome 11; (*b*) a derivative chromosome produced by fusion of chromosomes 13 and 16; (*c*) absence of single copies of chromosomes 4 and der(10); and (*d*) absence of chromosomes X and 20 and the absence of a single copy of chromosome 12. A similar degree of numerical aberrations was observed in the parental HCT116 cell line, and the same aberrations were never found in more than one cell, suggesting that these karyotypic changes were not clonal.

One persistent change observed in all HCT116 derivatives and not

Received 6/13/01; accepted 12/14/01.

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.

[1] This work was supported by the Clayton Fund, the V-Foundation, and NIH Grant CA 43460.

[2] To whom requests for reprints should be addressed, at The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, CRB Room 585, 1650 Orleans Street, Baltimore, MD 21231. E-mail: lengauer@jhmi.edu.

[3] The abbreviations used are: CIN, chromosomal instability; FISH, fluorescence *in situ* hybridization; SCE, sister chromatid exchange; HPRT, hypoxanthine phosphoribosyl transferase; 6-TG, 6-thioguanine; HR, homologous recombination.

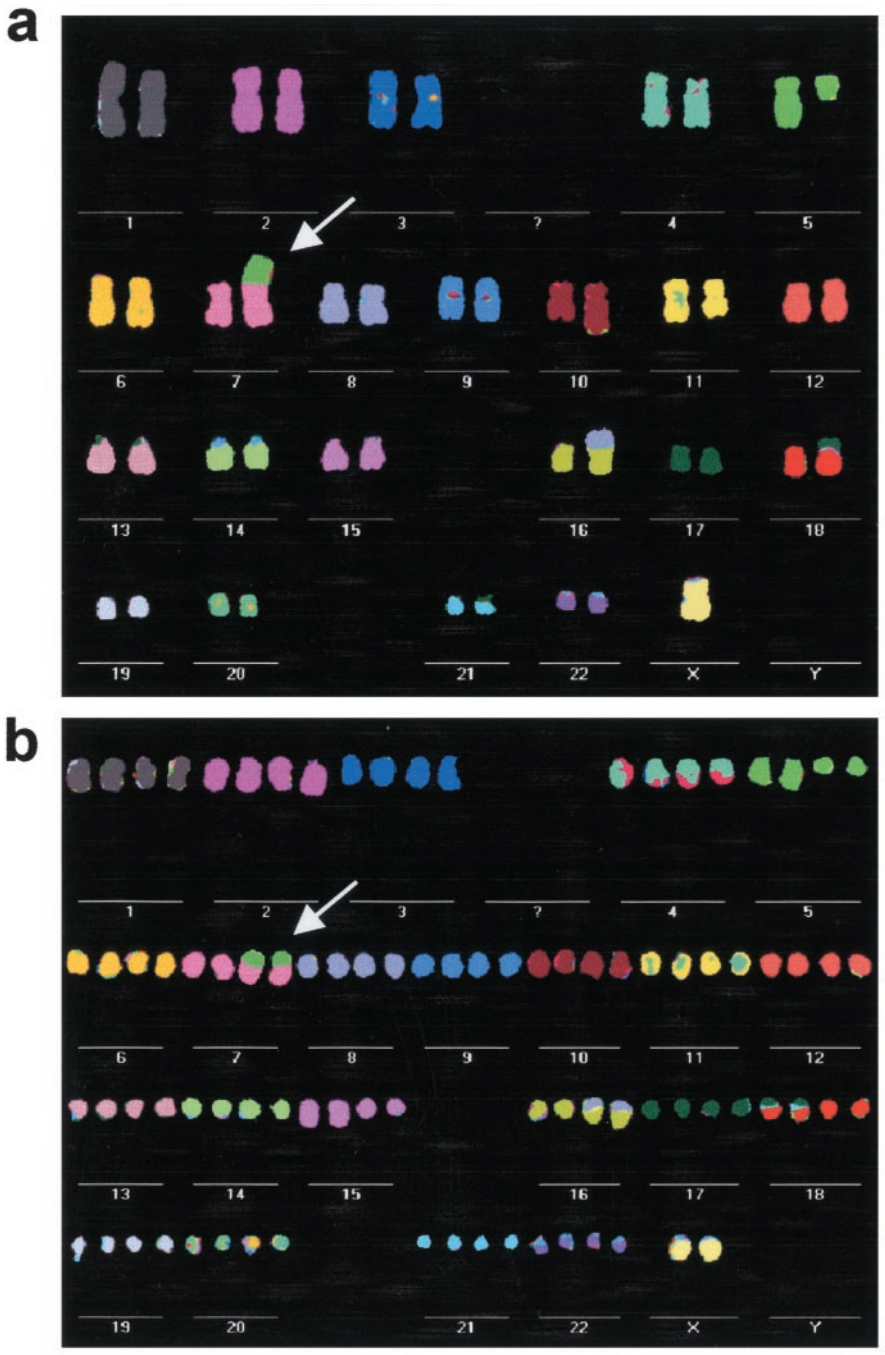

Fig. 1. Multiplex-FISH. *a*, representative metaphase of HCTp53KO, present in 18 of 22 metaphases that were evaluated extensively. The only clonal change between the parental line HCT116 and the p53-targeted derivatives is the translocation between chromosome 5 and 7 (*arrow*). *b*, example of a chromosomally stable, tetraploid cell found in a small fraction of the p53-deficient cells analyzed. *Arrow* indicates the two t(5;7) translocation chromosomes.

in the parental population was a balanced translocation between chromosomes 5 and 7 (Fig. 1*a*). This was observed in both the HCTp53HET (data not shown) and in the HCTp53KO clones. In previous experiments, we have shown that heterozygous disruption of p53 has no measurable effect on several important p53 functions, including those that govern the regulation of cell cycle checkpoints (6). This translocation therefore appears to be a random change that occurred before the homozygous targeting of p53 and therefore was unrelated to the disruption of p53 function.

Losses and Gains of Specific Chromosomes. The M-FISH assays described above show that the state of the chromosome complement was unchanged after p53 disruption. Such states cannot reliably be used to determine rates of chromosome changes, however. We therefore used FISH with probes for specific centromeric sequences to determine the precise rates of chromosome losses and gains in these cells. Immediately after targeting of the second *p53* allele, five subclones were isolated by limiting dilution. These "knockout" cell lines (HCTp53KO.1, KO.2, and others), plus a sibling control line (HCTp53HET) that was heterozygous for p53 disruption, were serially passaged in parallel. Each clone was grown for 25 generations after its establishment, and 100 interphase cells were examined with probes for several different chromosomes. Previous examination of unstable cells with these probes revealed no apparent bias as to which chromosomes are lost or gained (7). No significant differences in the rate of loss or gain of single chromosomes were observed among the lines, whether or not intact *p53* genes were present (Table 1). In particular, the number of cells that contained a chromosome number different from the modal number (two per autosome, one for the single

Table 1 *Losses and gains of chromosomes*

Cell line	Chromosome	*n*	1	2	3	4	>4	% off mode
Colorectal								
Stable (HCT116)	Average of eight different chromosomes	100 each						4
Unstable (HT29)	Average of nine different chromosomes	100 each						51
HCTp53HET	7	100	2	94	4	0	0	6
	12	100	0	93	6	1	0	6
HCTp53KO.1	1	100	0	94	6	0	0	6
	15	100	2	96	0	2	0	2
HCTp53KO.2	18	100	0	100	0	0	0	0
HCTp53KO.3	3	100	2	94	4	0	0	6
	18	100	0	98	2	0	0	2
HCTp53KO.4	3	100	0	100	0	0	0	0
	17	100	0	100	0	0	0	0
	18	100	0	100	0	0	0	0
	X	100	100	0	0	0	0	0
HCTp53KO.5	1	100	3	96	1	0	0	4
	7	100	1	96	3	0	0	4
	12	100	0	99	1	0	0	1
	15	100	4	94	2	0	0	6
	17	100	2	98	0	0	0	2
	X	100	97	3	0	0	0	3
Fibroblast								
FBWT	7	100	0	90	4	6	0	4
	12	100	1	88	4	7	0	5
	17	100	1	91	3	5	0	4
FBp53HET	1	49	0	92	6	2	0	6
	17	50	8	88	4	0	0	12
	18	100	0	94	5	1	0	5
	X	50	2	90	8	0	0	8
FBp53KO	1	100	2	89	3	6	0	5
	3	99	0	85	2	13	0	2
	7	104	7	85	1	7	0	8
	12	105	6	86	2	6	0	8
	17	110	5	76	2	17	0	7
	18	102	2	74	4	18	2	6
	X	110	0	78	0	21	1	0

X chromosome) was never >6%, regardless of *p53* genotype. This high degree of stability is consistent with previous studies of wild-type HCT116 cells (7, 14).

Homologous Recombination. Another type of genetic instability has been associated with aberrant rates of HR (15). A function for p53 in the suppression of HR has been suggested by experiments in murine knockout cell lines (16) as well as in human cells that overexpress a dominant mutant *p53* allele (17). We quantified HR in our line in two different ways. The first involved counting of SCE in the presence of bromodeoxyuridine. SCE has been shown to be associated with the high levels of HR in cells from Bloom's syndrome patients (10, 18). SCE rates in HCT116 cells and its p53-disrupted derivatives were similar (Table 2).

A second means of assaying HR processes involves measurement of the frequency of integration of a gene-targeting vector. An assay was used that takes advantage of the fact that a functional *HPRT* gene product is required for growth in medium containing hypoxanthine/aminopterin/thymidine supplements. Disruption of this gene, which is present as a single copy on the X chromosome, is required for cell growth in medium supplemented with the purine analogue 6-TG. Cells that have undergone homologous recombination at the *HPRT* locus can be grown as colonies after 6-TG selection. The frequency of HR among the population of clones that had stably integrated the vector was approximately 4×10^{-4} (Table 3). The HR frequency did not differ significantly between parental and p53-deficient cell lines (Table 3).

Table 2 *SCE*

Genotype	*n*	SCE/Metaphase	SD
HCT116	10	4.1	±1.0
HCTp53HET	10	3.5	±0.7
HCTp53KO	10	3.9	±0.6

Table 3 *Frequency of targeted plasmid integration*

Genotype	Clones screened	HPRT targeted	Targeting frequency
HCT116	9.7×10^3	4	4.1×10^{-4}
HCTp53KO	1.2×10^4	4	3.3×10^{-4}

The Effects of p53 Knock-Out in Normal Human Fibroblasts. To determine whether p53 disruption affected chromosome loss or gain rates in cells not derived from a cancer, we analyzed analogous primary human fibroblast cells (FBWT). In addition to parental cells, two derivative cell lines, one with a single targeted *p53* allele (FBp53HET), and another that had undergone loss of heterozygosity at the *p53* locus which resulted in deletion of p53 (FBp53KO), were examined. M-FISH analyses of these cells could not be performed, because the fibroblast lines grew relatively slowly and exhibited a low frequency of cells in metaphase. However, we could assess CIN through our standard FISH analysis of interphase cells using centromeric probes (7). The fibroblast-derived lines generally showed a somewhat greater number of nonmodal cells than the HCT116 cancer cells (Table 1). However, in 10 of 11 determinations, the fraction of cells off the mode was ≤8%, and there was no correlation of this number with p53 genotype. In contrast, 25–30% of the cells with a CIN phenotype typically are found to lose or gain any tested chromosome, with a correspondingly higher variation in chromosome number from cell to cell (7).

Interestingly, although the fibroblast lines with disrupted *p53* genes did not show high rates of loss or gain of specific single chromosomes, these cells had a marked tendency to become tetraploid (Table 1). Thus, p53-deficient fibroblasts exhibited a high

percentage of metaphases with four and occasionally eight markers per chromosome. Such effects have been noted in p53-deficient rodent fibroblast cells treated with inhibitors of mitotic spindle formation (19, 20).

No obvious tendency to tetraploidization was evident in our CIN assays of p53-deficient HCT116 cells (Table 1). However, inspired by the fibroblast results, we assessed a larger number of cells to determine whether any differences in tetraploidization occurred. In 400 metaphases from the p53-deficient cells, 35 exhibited tetraploidy (example in Fig. 1*b*), whereas a smaller fraction (10 of 400) of parental HCT116 cells were tetraploid. This 3.5-fold difference in tetraploidization, although small, was statistically significant ($P < 0.001$, χ^2 test).

DISCUSSION

In summary, our studies of isogenic human cell lines provide no evidence that p53 disruption causes aneuploidy, either in a cell line derived from a colon cancer or from one derived from normal human fibroblasts. Over many generations, chromosome number remained stable in both the wild-type and p53-deficient cells. No structural instability was observed by karyotyping and no increased rate in SCE was observed in the HCT116-derived cells. Furthermore, the rate of homologous integration of an exogenous DNA construct did not differ between p53-proficient and p53-deficient cells.

One might legitimately ask whether the mismatch repair deficiency in HCT116 cells (21) may prevent the advent of CIN, even with p53 disrupted. This hypothesis has been excluded in recent experiments wherein it was demonstrated that the targeted disruption of *hSecurin*, a gene involved in the regulation of anaphase initiation, leads to marked chromosomal instability in HCT116 cells (14). The securin-deficient cells were studied with some of the same assays used here to evaluate CIN.

Do these results therefore imply that p53 plays no role in maintaining chromosome stability? A conservative interpretation of our results is that p53 disruption alone does not cause chromosome instability. It remains possible that, in the presence of other genetic or epigenetic alterations, the absence of p53 could exacerbate a preexisting tendency toward such instability. For example, although we observed no CIN in the cell lines studied here, they exhibited a small but consistent tendency to become tetraploid in the two cell types studied. This tendency to polyploidy has been attributed to the loss of the G_1 checkpoint function of p53 (22), which prevents rereplication of the genome, and should be distinguished from CIN, in which individual chromosomes are gained or lost in a haphazard fashion (7). It is unclear whether the tetraploid cells in either the HCTp53KO or the FBp53KO population maintain their proliferative potential, because we have not yet been able to recover tetraploid clones of any of these cells. Regardless, the difference in the frequencies of tetraploidization between the epithelial-derived cancer cells and the fibroblasts demonstrates that loss of p53 can have different consequences in different cell types. Such cell type differences as well as differences between murine and human cells may explain the discrepancies among previous studies (23, 24) and those recorded here.

Previous studies have implicated p53-independent pathways in the development of chromosomal instability. Tetraploidy has been observed previously in human cells transduced with viral oncogenes, although not with those that interact directly with p53 (25). Experiments with murine tumor models have suggested that other mutations in caretaker genes such as *Brca1* (26) and *Brca2* (27) can lead to instability in the context of *p53* mutation.

Most importantly, we believe that our results convincingly demonstrate, in both normal human fibroblasts and human epithelial cancer cells, that p53 loss does not simply lead to aneuploidy. This conclusion is consistent with the previously documented existence of human diploid cancers (2, 5), as well as diploid murine experimental tumors (28) that contain mutant *p53* genes. It is also consistent with observations on colorectal tumors showing that aneuploidy occurs early during the neoplastic process, whereas p53 inactivation occurs only decades later, near the transition from adenoma to carcinoma (29). We conclude that the aneuploidy that is nearly ubiquitous in cancers should not be ascribed primarily to defects in p53, and that a continuing search for the central mediators of chromosomal instability is justified.

ACKNOWLEDGMENTS

We acknowledge the expert assistance of Leslie Meszler of the Johns Hopkins Cell Imaging Core Facility.

REFERENCES

1. 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.
2. Auer, G. U., Heselmeyer, K. M., Steinbeck, R. G., Munck-Wikland, E., and Zetterberg, A. D. The relationship between aneuploidy and p53 overexpression during genesis of colorectal adenocarcinoma. Virchows Arch., *424:* 343–347, 1994.
3. Lengauer, C., Kinzler, K. W., and Vogelstein, B. Genetic instabilities in human cancers. Nature (Lond.), *396:* 643–649, 1998.
4. Harvey, M., Sands, A. T., Weiss, R. S., Hegi, M. E., Wiseman, R. W., Pantazis, P., Giovanella, B. C., Tainsky, M. A., Bradley, A., and Donehower, L. A. *In vitro* growth characteristics of embryo fibroblasts isolated from p53-deficient mice. Oncogene, *8:* 2457–2467, 1993.
5. Campomenosi, P., Assereto, P., Bogliolo, M., Fronza, G., Abbondandolo, A., Capasso, A., Bellomo, P. F., Monaco, R., Rapallo, A., Sciutto, A., Orecchia, R., Geido, E., and Giaretti, W. p53 mutations and DNA ploidy in colorectal adenocarcinomas. Anal. Cell. Pathol., *17:* 1–12, 1998.
6. 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 G_2 arrest after DNA damage. Science (Wash. DC), *282:* 1497–1501, 1998.
7. Lengauer, C., Kinzler, K. W., and Vogelstein, B. Genetic instability in colorectal cancers. Nature (Lond.), *386:* 623–627, 1997.
8. Azofeifa, J., Fauth, C., Kraus, J., Maierhofer, C., Langer, S., Bolzer, A., Reichman, J., Schuffenhauer, S., and Speicher, M. R. An optimized probe set for the detection of small interchromosomal aberrations by use of 24-color FISH. Am. J. Hum. Genet., *66:* 1684–1688, 2000.
9. Abdel-Rahman, W. M., Katsura, K., Rens, W., Gorman, P. A., Sheer, D., Bicknell, D., Bodmer, W. F., Arends, M. J., Wyllie, A. H., and Edwards, P. A. Spectral karyotyping suggests additional subsets of colorectal cancers characterized by pattern of chromosome rearrangement. Proc. Natl. Acad. Sci. USA, *98:* 2538–2543, 2001.
10. 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.
11. Zheng, H., Hasty, P., Brenneman, M. A., Grompe, M., Gibbs, R. A., Wilson, J. H., and Bradley, A. Fidelity of targeted recombination in human fibroblasts and murine embryonic stem cells. Proc. Natl. Acad. Sci. USA, *88:* 8067–8071, 1991.
12. Waldman, T., Kinzler, K. W., and Vogelstein, B. p21 is necessary for the p53-mediated G_1 arrest in human cancer cells. Cancer Res., *55:* 5187–5190, 1995.
13. Masramon, L., Ribas, M., Cifuentes, P., Arribas, R., Garcia, F., Egozcue, J., Peinado, M. A., and Miro, R. Cytogenetic characterization of two colon cell lines by using conventional G-banding, comparative genomic hybridization, and whole chromosome painting. Cancer Genet. Cytogenet., *121:* 17–21, 2000.
14. 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.
15. Jasin, M. Chromosome breaks and genomic instability. Cancer Investig., *18:* 78–86, 2000.
16. Willers, H., McCarthy, E. E., Alberti, W., Dahm-Daphi, J., and Powell, S. N. Loss of wild-type p53 function is responsible for up-regulated homologous recombination in immortal rodent fibroblasts. Int. J. Radiat. Biol., *76:* 1055–1062, 2000.
17. Mekeel, K. L., Tang, W., Kachnic, L. A., Luo, C. M., DeFrank, J. S., and Powell, S. N. Inactivation of p53 results in high rates of homologous recombination. Oncogene, *14:* 1847–1857, 1997.
18. 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.

19. Di Leonardo, A., Khan, S. H., Linke, S. P., Greco, V., Seidita, G., and Wahl, G. M. DNA rereplication in the presence of mitotic spindle inhibitors in human and mouse fibroblasts lacking either p53 or pRb function. Cancer Res., *57:* 1013–1019, 1997.
20. Lanni, J. S., and Jacks, T. Characterization of the p53-dependent postmitotic checkpoint following spindle disruption. Mol. Cell. Biol., *18:* 1055–1064, 1998.
21. Eshleman, J. R., Casey, G., Kochera, M. E., Sedwick, W. D., Swinler, S. E., Veigl, M. L., Willson, J. K., Schwartz, S., and Markowitz, S. D. Chromosome number and structure both are markedly stable in RER colorectal cancers and are not destabilized by mutation of p53. Oncogene, *17:* 719–725, 1998.
22. Khan, S. H., and Wahl, G. M. p53 and pRb prevent rereplication in response to microtubule inhibitors by mediating a reversible G_1 arrest. Cancer Res., *58:* 396–401, 1998.
23. Honma, M., Momose, M., Tanabe, H., Sakamoto, H., Yu, Y., Little, J. B., Sofuni, T., and Hayashi, M. Requirement of wild-type p53 protein for maintenance of chromosomal integrity. Mol. Carcinog., *28:* 203–214, 2000.
24. Ghebranious, N., and Donehower, L. A. Mouse models in tumor suppression. Oncogene, *17:* 3385–3400, 1998.
25. White, A. E., Livanos, E. M., and Tlsty, T. D. Differential disruption of genomic integrity and cell cycle regulation in normal human fibroblasts by the HPV oncoproteins. Genes Dev., *8:* 666–677, 1994.
26. Deng, C. X., and Scott, F. Role of the tumor suppressor gene *Brca1* in genetic stability and mammary gland tumor formation. Oncogene, *19:* 1059–1064, 2000.
27. Venkitaraman, A. R. Chromosome stability, DNA recombination, and the BRCA2 tumour suppressor. Curr. Opin. Cell Biol., *13:* 338–343, 2001.
28. Lu, X., Magrane, G., Yin, C., Louis, D. N., Gray, J., and Van Dyke, T. Selective inactivation of p53 facilitates mouse epithelial tumor progression without chromosomal instability. Mol. Cell. Biol., *21:* 6017–6030, 2001.
29. 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.

Targeted Inactivation of p53 in Human Cells Does Not Result in Aneuploidy

Fred Bunz, Christine Fauth, Michael R. Speicher, et al.

Cancer Res 2002;62:1129-1133.