Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 1755–1759, March 1992
Medical Sciences

Human chromosome 11 contains two different growth suppressor genes for embryonal rhabdomyosarcoma

(microcell hybridization/loss of heterozygosity/recessive cancer genes)

WELLINGTON E. LOH, JR.*†, HEIDI J. SCRABLE‡, ELIZABETH LIVANOS§, MARIE J. ARBOLEDA§, WEBSTER K. CAVENEE‡, MITSUO OSHIMURA¶, AND BERNARD E. WEISSMAN§‖

*Division of Hematology/Oncology, Childrens Hospital of Los Angeles, Los Angeles, CA 90027; §Lineberger Cancer Research Center, University of North Carolina, Chapel Hill, NC 27599; ‡Ludwig Institute for Cancer Research, University of California at San Diego, CA 92093; and ¶Department of Molecular and Cell Genetics, School of Life Sciences, Tottori University, Yonago-city, Japan 684

Communicated by Alfred G. Knudson, November 13, 1991 (received for review April 12, 1991)

ABSTRACT **The identification of acquired homozygosity in human cancers implies locations of tumor suppressor genes without providing functional evidence. The localization of a defect in embryonal rhabdomyosarcomas to chromosomal region 11p15 provides one such example. In this report, we show that transfer of a normal human chromosome 11 into an embryonal rhabdomyosarcoma cell line elicited a dramatic loss of the proliferative capacity of the transferrants. Indeed, the majority of the viable microcell hybrids had either eliminated genetic information on the short arm of the transferred chromosome 11 or increased the copy number of the rhabdomyosarcoma-derived chromosomes 11. Cells that possessed only the long arm of chromosome 11 also demonstrated a decreased growth rate. In contrast, all microcell hybrids retained the ability to form tumors upon inoculation into animals. These functional data support molecular studies indicating loss of genetic information on chromosome 11p15 during the development of embryonal rhabdomyosarcoma. In addition, our studies demonstrate the existence of a second gene on the long arm, previously unrecognized by molecular analyses, which negatively regulates the growth of embryonal rhabdomyosarcoma cell lines.**

The development of human neoplasia appears to entail a sequential series of genetic alterations followed by clonal expansion (1). Some of these steps can consist of the activation of oncogenes or the loss of tumor suppressor gene function. While more than 40 oncogenes have been identified, fewer than 10 tumor suppressor genes have been characterized (2–12). Knowledge of the existence of the latter class of genes relies mainly upon indirect or circumstantial evidence derived from somatic cell hybridization, cytogenetic analyses, and tumor-specific loss of nearby molecular markers (13, 14). With little functional evidence to support data showing their physical locations in the human genome, isolation of tumor suppressor genes remains an arduous task.

One approach to this problem uses the technique of microcell hybridization to transfer a single chromosome from a normal somatic cell into a human tumor cell line (15, 16). We have previously shown a loss of tumorigenic potential after introduction of a normal human chromosome 11 into a Wilms tumor-derived cell line consonant with cytogenetic studies and restriction fragment length polymorphism (RFLP) mapping demonstrating deletions or homozygosis of chromosome 11 in several Wilms tumors (17–19). Recently, cytogenetic evidence of deletions in the long arm of chromosome 6 in melanomas led to a demonstration of suppression of tumorigenicity in human melanoma cells by the introduction of that chromosome (20). Similar studies showed suppression of tumorigenicity in a renal cell carcinoma line after introduction of chromosome 3, consistent with cytogenetic abnormalities in the corresponding tumors (21).

Each of these microcell hybridization studies on suppression of tumorigenicity depended on visible chromosome deletions to identify which chromosome might carry a tumor suppressor gene. In many cases, location of putative tumor suppressor genes relies on molecular evidence alone. For example, embryonal rhabdomyosarcoma, a pediatric tumor of striated muscle, carries no consistent visible cytogenetic abnormality. However, molecular analysis of such tumors has implicated a loss of genetic elements at chromosome 11p15.5 as a crucial event in its etiology (22, 23). To determine whether these genetic events also entailed loss of a tumor suppressor function, we introduced a normal human chromosome 11 into an embryonal rhabdomyosarcoma cell line. The results suggest that loss of two different growth suppressor genes, present on either arm of chromosome 11, contributes to the development of embryonal rhabdomyosarcoma.

MATERIAL AND METHODS

Cell Lines. The RD cell line is a well-characterized embryonal rhabdomyosarcoma cell line derived from the tumor of a 7-year-old patient (24). HDM-18 is a 3T6 mouse cell containing a single human chromosome 11 with an integrated copy of the neomycin resistance (neo^R) gene at 11q14–q22 (25). The neo11/A9neo line retains a different human chromosome 11 with an integrated copy of the neo^R gene at 11p12, while the neo2/A9 donor line contains a single human chromosome 2 with an integrated copy of the same gene (26, 27). All cell lines were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum and penicillin at 100 units/ml. Each cell line was routinely tested for mycoplasma infection and found to be negative (28).

Microcell Hybridization and Gene Transfer. Microcell hybridization was performed as previously described (17) with the following exception. The flasks were coated with poly(D-lysine) at 5 μg/ml for the HDM-18 cell lines to improve the number of microcells. Introduction of the pMAMneo-1 vector (Clontech) into the RD cells was accomplished by the use of Lipofectin reagent (GIBCO/BRL). Selection of neo^R microcell hybrids and transfectants was carried out in growth medium containing G418 (GIBCO/BRL) at 600 μg/ml. Individual colonies were selected by the use of glass cloning cylinders and expanded for further characterization.

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

Abbreviations: RFLP, restriction fragment length polymorphism; neo^R, neomycin resistance or resistant.

†Present address: Department of Pediatrics, H. L. Moffitt Cancer Center, University of South Florida, Tampa, FL 33682.
‖To whom reprint requests should be addressed.

Growth Curves. The population doubling time of each cell line was determined by measuring the growth rate over a 7- to 10-day period. Each cell line was plated into 60-mm Petri dishes at 5×10^4 cells per dish in growth medium and allowed to incubate overnight. Cell number was measured in 3 dishes the next day (day 0) and on days 1, 3, 5, 7, and 10 by using a Coulter Counter. All cells were fed every 3 days with growth medium. Population doubling times represent the average of at least two experiments. The population doubling time was calculated by using the formula population doubling time = $(t_2 - t_1)/3.32 \log(X_2/X_1)$, where X_n represents the cell number at time t_n. The t values used were day 3 and day 5, covering the logarithmic phase of growth for each cell line.

Cytogenetic Analyses. The karyotypes of the RD cell line and the microcell hybrids were determined by Giemsa–trypsin banding as previously described (17). At least 10 metaphase spreads were examined for each cell line.

Analyses of RFLP Markers. The presence of the alleles for three chromosome 11 molecular probes was determined by Southern blotting as previously described (23). Probes were labeled by random priming methods using isolated inserts (23).

RESULTS

Isolation of RD Microcell Hybrids Containing Human Chromosome 11. We initially tried to transfer chromosome 11 from the HDM-18 cell line into the RD cells. In five separate experiments, we isolated few viable microcell hybrids. Most colonies that arose after fusion became quiescent after approximately 10 population doublings. These findings suggested that a suppressor gene for cellular immortality might reside on chromosome 11. However, transfer of chromosome 2 or 6 into the RD cells yielded similar results; most of the colonies failed to proliferate beyond a 1-cm diameter. In many cases, the microcell hybrids contained a near-tetraploid chromosome number, while the RD cell line displayed a near-diploid karyotype (Fig. 1). Because of the well-documented gene-dosage effects on the control of tumorigenicity in somatic cell hybrids, we chose to characterize only near-diploid microcell hybrids for potential alterations in growth properties (21, 29, 30).

Cytogenetic Analysis of the HDM-18/RD Microcell Hybrids. The generation of microcell hybrids frequently causes rearrangements and translocations of individual chromosomes, including the transferred chromosome. This raised the possibility that viable near-diploid microcell hybrids might arise subsequent to the loss of a gene in the introduced chromosome capable of inhibiting growth. To test the validity of this scenario, we performed a detailed cytogenetic analysis of the RD parent and pseudodiploid microcell hybrids containing chromosome 2 or 11. The RD cell line displayed a pseudodiploid karyotype with a mode of 47 chromosomes, including two normal-appearing chromosomes 11 (Fig. 1).

A microcell hybrid containing chromosome 2 showed an additional copy of this chromosome (data not shown). In contrast, all three of the HDM-18/RD microcell hybrids possessed a similar karyotype without an additional copy of the chromosome 11 indicative of the neo^R-selectable transferred material. Each of the clones did carry new chromosome 11 rearrangements. The HDM-18/RD.1 cell line's karyotype included a new marker chromosome containing the long arm of chromosome 11, while the other two cell lines possessed an iso(11q) chromosome (Fig. 1). Thus, the rare appearance of viable pseudodiploid microcell hybrids after introduction of a normal chromosome 11 appeared to require the loss of the short arm.

Detection of the Normal Chromosome 11 in the HDM-18/RD Cells by RFLP Markers. While these data suggested that the rearranged chromosomes 11 in the microcell hybrids were derived from the transferred chromosome 11, cytogenetic analysis could not establish their origins. We, therefore, carried out a molecular analysis of the RD cell line and the microcell hybrids, using RFLP markers on both arms of chromosome 11. Analysis with the probe pE46-TGH2, which hybridizes to the locus *D11S24* on the long arm, confirmed

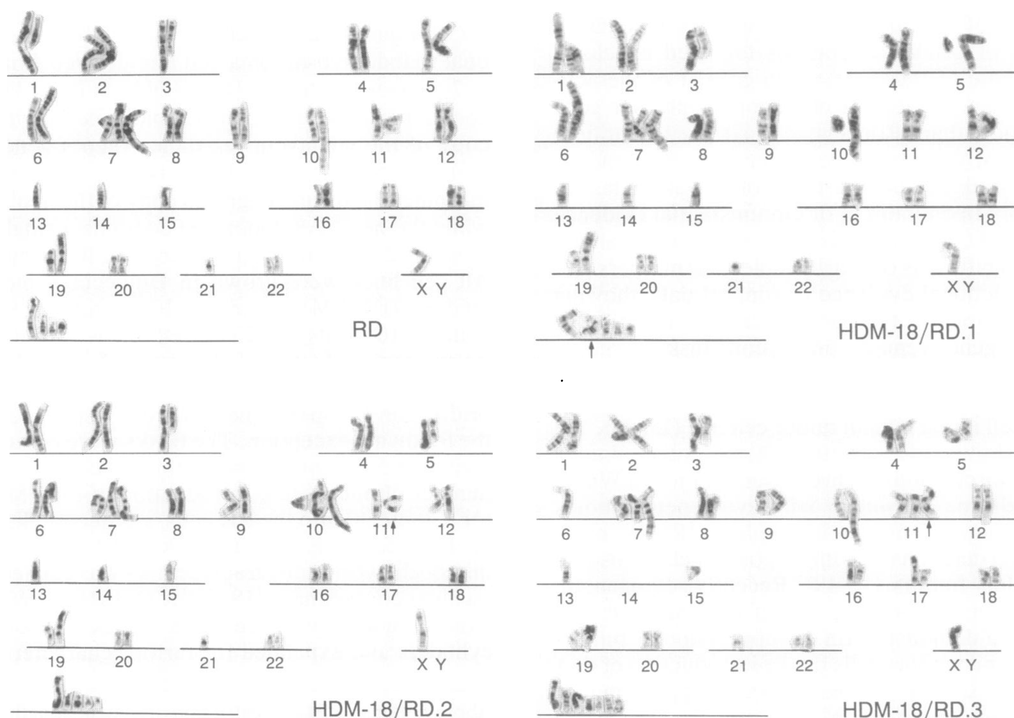

FIG. 1. Cytogenetic analysis of the RD cell line and the microcell hybrids. The karyotypes of the RD cell line and the microcell hybrids were determined by Giemsa–trypsin banding (17). At least 10 metaphase spreads were examined for each cell line. The presumed rearranged chromosome 11 that was introduced into the RD cells is denoted with an arrow.

the presence of the introduced chromosome in the majority of cells of each microcell hybrid (Fig. 2). Furthermore, the stoichiometry of alleles at *D11S24* appears to resemble that in normal diploid cells. On the short arm, however, analysis at the β-globin *HBBC* and *HRAS1* loci in 11p15.5 revealed that the alleles contributed specifically by the introduced chromosome 11 failed to appear in each of the hybrids. Thus, at *HBBC*, a majority of cells lost the 3.5-kb allele at the γ^A locus contributed by the normal chromosome 11, and at *HRAS1* virtually all cells were missing the 0.9-kb allele from the same chromosome. These data support the notion that the marker chromosomes arose from a rearrangement of the introduced chromosome 11.

***In Vitro* and *in Vivo* Growth Properties of the HDM-18/RD Cell Lines.** Despite the loss of the genetic information on the short arm of the normal chromosome 11, it appeared that the microcell hybrids differed from the parental RD cells in both morphology and growth rate. We, therefore, characterized the *in vitro* and *in vivo* growth characteristics of the RD cells and the microcell hybrids. The presence of the extra copy of the long arm of chromosome 11 resulted in a more elongated morphology and an increased number of spindle-shaped cells (data not shown). The microcell hybrids also demonstrated a decreased growth rate compared with the RD parental cells, ranging from slight (HDM-18/RD.2) to substantial (HDM-18/RD.3) (Table 1). In contrast, transfer of a plasmid carrying the neo^R gene (neo/RD.1) or a normal human chromosome 2 (neo2/RD.1 and neo2/RD.2) into the RD cells had no effect on their growth (Table 1) or their morphology (data not shown). However, the decrease in the growth of the microcell hybrids *in vitro* did not correspond to a reduced tumorigenic potential in *nu/nu* (nude) mice (Table 1). Although a range of tumorigenic potentials existed among the various microcell hybrids, no correlation was observed between retarded growth in culture and reduced tumorigenicity *in vivo* (Table 1).

An alternative explanation for the tumorigenicity results above might be that the introduced chromosome was lost from the cells after inoculation into the animals. We examined that possibility by reestablishing cell lines in culture from the tumors formed by the HDM-18/RD.2 cell line, the cell line showing the least reduction in growth rate (Table 1). Cytogenetic analysis of this cell line, designated RD.2 TR1, showed the presence of the iso (11q) chromosome in the majority of the cells, yet they grew with the same population doubling time as the parent cells (data not shown). All of these data together support the contention that the addition of the long arm of chromosome 11 to the RD cells caused a decrease in growth potential in culture but not in animals.

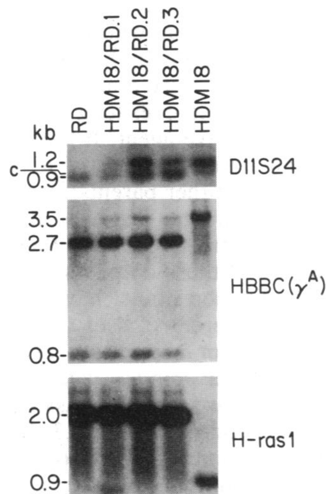

FIG. 2. RFLP analyses of chromosome 11 markers for the RD cell line and the microcell hybrids. The presence of the alleles for three chromosome 11 molecular probes was determined by Southern blotting. Probe pE4b-TGH2 is homologous to the *D11S24* locus mapped to 11q22–23 and reveals alleles of 1.2 and 0.9 kilobases (kb) as well as a constant band (c) at 0.95 kb with *Rsa* I (22, 31). This band is obscured when the smaller allele is present. The probe JW151 is homologous to the β-globin gene cluster and reveals alleles of 3.5 and 2.7 + 0.8 kb at the γ^A locus with *Hin*dIII (32). Probe pEJ 6.6 is homologous to the *HRAS1* gene and detects various-sized *Msp* I fragments depending on the size of the variable number of terminal repeats region of the gene (33).

Table 1. *In vitro* and *in vivo* growth potentials of the RD cells and the microcell hybrids

Cell line	Chromosome introduced	Population doubling time, days	No. of cells injected	Tumorigenic potential
RD	—	0.97 ± 0.08	1×10^7	6/7 (1 wk)
			1×10^6	6/10 (2 wk)
			1×10^5	7/10 (5 wk)
neo/RD.1	—	1.00 ± 0.05		NT
Near-diploid				
HDM-18/RD.1	11	1.54 ± 0.08	1×10^7	9/10 (2 wk)
			1×10^6	8/8 (3 wk)
			1×10^5	4/8 (5 wk)
HDM-18/RD.2	11	1.14 ± 0.01	1×10^7	10/11 (2 wk)
			1×10^6	6/6 (3 wk)
			1×10^5	6/12 (6 wk)
HDM-18/RD.3	11	1.80 ± 0.07	1×10^7	7/8 (3 wk)
			1×10^6	12/15 (4 wk)
			1×10^5	1/15 (12 wk)
neo11/RD.1	11	2.09 ± 0.10	1×10^7	5/6 (6 wk)
			1×10^6	0/6
neo2/RD.1	2	0.88 ± 0.03	1×10^7	5/6 (1 wk)
			1×10^6	5/6 (12 wk)
			1×10^5	0/6
Near-tetraploid				
HDM-18/RD.4	11	1.41 ± 0.05		NT
neo11/RD.2	11	1.42 ± 0.06	1×10^7	NT
			1×10^6	5/6 (3 wk)
			1×10^5	1/6 (6 wk)
neo2/RD.2	2	0.80 ± 0.02		NT

The population doubling time was derived from growth curves; the data represent the averages from three experiments for each cell line and the ranges of observed values. Tumorigenic potential was assayed by subcutaneous inoculation into two sites on BALB/c *nu/nu* (nude) mice with inocula sizes of 1×10^5, 1×10^6, and 1×10^7 cells. Animals were examined weekly for tumor growth and distress. When tumors reached a size of 1 cm, they were excised, reestablished into tissue culture, and characterized for cytogenetic changes. The results are expressed as the number of animals positive for tumor growth/the number of animals injected with cells. The value in parentheses represents the average time for the appearance of tumor nodules on the animals. Tumor growth was considered negative after 6 months. NT, not tested.

The Short Arm of Chromosome 11 Contains a Potent Growth-Suppressor Gene That Operates in a Dose-Dependent Fashion. The fact the neo^R marker was stably integrated into the long arm of the chromosome 11 in the HDM-18 donor cell might have facilitated the loss of the short arm. We, therefore, introduced a different chromosome 11 into the RD cell line with the neo^R plasmid integrated at 11p12 (26). Again, the majority of microcell hybrids arising after monochromosome transfer contained a near-tetraploid chromosome number. However, one cell line, designated neo11/RD.1, showed three copies of chromosome 11 by cytogenetic analysis (Fig. 3*A*). This cell line grew very poorly in culture, more slowly than any of the original set of RD microcell hybrids, and was less tumorigenic (Table 1). RFLP analysis has confirmed the presence of the introduced chromosome 11, including the *HRAS* locus (Fig. 3*B*). Again, cytogenetic analysis of the

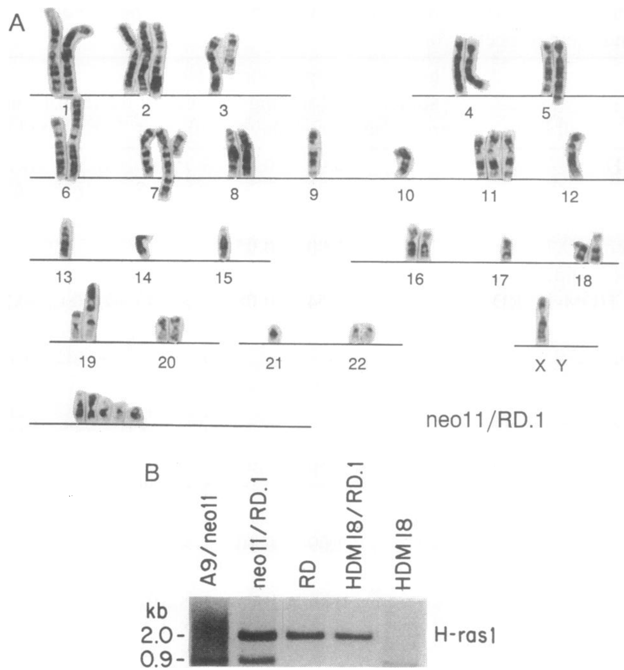

FIG. 3. Cytogenetic and RFLP analysis of the neo11/RD.1 cell line. (*A*) The karyotype of the neo11/RD.1 cell line was determined by Giemsa-trypsin banding (14). At least 10 metaphase spreads were examined. (*B*) The presence of the *HRAS1* allele for chromosome 11 was determined by Southern blotting. The probe pEJ 6.6 is homologous to the *HRAS1* gene and detects various-sized *Msp* I fragments, depending on the size of the variable number of terminal repeats region of the gene (33).

tumor formed by these cells demonstrated the retention of the extra chromosome 11 (data not shown). We interpret these results as cytogenetic support for the molecular evidence for the presence of a potent growth-suppressor gene on the short arm of chromosome 11. These data also support the concept that a distinct growth suppressor gene for embryonal rhabdomyosarcomas maps to the long arm of human chromosome 11.

We initially characterized only those microcell hybrids that retained the near-diploid karyotype to avoid the possibility of gene-dosage effects. We have also examined two near-tetraploid microcell hybrids with chromosome 11 and a near-tetraploid hybrid carrying a transferred chromosome 2 (Table 1). The cell lines with a 4:1 ratio of the RD chromosomes 11 to the normal one grew as poorly as one of the near-diploid microcell hybrids (Table 1). In contrast, a near-tetraploid RD cell line containing a normal chromosome 2 grew slightly faster than the RD cell line (neo2/RD.2). Therefore, the growth suppressor genes on chromosome 11 could also exert their effects on cell lines with additional copies of the parental chromosome 11.

DISCUSSION

Several previous studies have demonstrated a functional correspondence between a particular chromosome deletion and the tumor suppressing activity of that region by microcell hybridization (17, 20, 21). Here, we have shown that loss of heterozygosity can also provide signposts for monochromosome transfer studies in the absence of visible cytogenetic alterations. Such analyses of embryonal rhabdomyosarcoma tumors suggested that loss of genetic material at chromosome 11p15 was central in their development (22, 23). This report provides functional evidence that this region contains a gene that affects cellular proliferation in this lineage. Furthermore, we unexpectedly detected a second gene on chromosome 11 that retarded the growth of tumor cells derived from this type of cancer. Interestingly, this chromosome carried genes that inhibited the proliferation of the embryonal rhabdomyosarcoma cell line without completely suppressing tumorigenicity. For the purposes of this discussion, we will use "tumor suppressor gene" as a general term for recessive cancer genes and "growth suppressor gene" to denote the functions we have mapped to chromosome 11.

Several different studies have suggested that the development of embryonal rhabdomyosarcomas and Wilms tumors may share common steps. Patients with Beckwith–Weidemann syndrome have an increased propensity to develop embryonal cancers, including rhabdomyosarcomas and Wilms tumors (34). Loss of heterozygosity for chromosome 11 occurs in both types of these tumors (19, 35). Furthermore, mitotic recombination mapping studies have implicated the presence of a suppressor gene in the region of 11p15 in both Wilms tumor and rhabdomyosarcomas (22, 36, 37). Our previous studies showed that introduction of a normal human chromosome 11 into a Wilms tumor cell line resulted in complete suppression of tumorigenicity without any apparent effects on *in vitro* growth properties (17). In contrast, gene(s) present on the same chromosome altered the growth of an embryonal rhabdomyosarcoma cell line in culture but not in animals. Thus, the same gene that causes suppression of tumorigenicity in one cancer (Wilms tumor) may function differently in a related cancer (embryonal rhabdomyosarcoma). Alternatively, these diverse effects may result from the action of other tumor suppressor genes on chromosome 11. Three other tumor suppressor genes have been mapped to this chromosome—two Wilms tumor suppressor genes at 11p13 and the HeLa tumor suppressor gene on the long arm (7, 8, 38, 39). Until the isolation of all these recessive cancer genes, their functions in the development of different tumors will remain elusive.

The effect on the growth of RD cells due to the presence of the long arm of chromosome 11 was unexpected, as previous molecular and cytogenetic studies did not implicate a recessive cancer gene at this location. However, alternative explanations exist, including the presence of a normal negative growth regulatory element whose extra copy decreases the rate of cellular proliferation in the RD cells. We feel that this is unlikely, as the decreased doubling time of the microcell hybrids remained stable over long periods in culture in the presence of the selective agent. If the parental chromosomes 11 contained normal copies of a negative growth regulatory gene, loss of either parental chromosome would restore the normal growth rate. However, this event failed to occur over long periods in culture even though no selective pressure for retention of these chromosomes existed.

Somatic cell hybridization studies have provided evidence for two functionally different classes of tumor suppressor genes. The original tumor suppressor genes regulate the growth of tumor cells in animals without affecting their behavior in culture, while a second class consists of genes that control immortality or senescence (13, 40). The microcell hybrids that retained the long arm of chromosome 11 showed an increased doubling time in culture without consistent effects on their tumorigenic potential in nude mice. The microcell hybrid that possessed the entire chromosome 11 showed a 2-fold increase in cell doubling time in culture and an increased latency period for tumor formation upon inoculation in animals. These data suggest that suppressor genes exist that negatively regulate the proliferation of cells both in animals and in culture or perhaps accentuate the activity of the other classes of tumor suppressor genes. As more recessive cancer genes become available for analysis, functional differences will likely fall into sharper focus.

The apparent effects of the genetic information at 11p15 resemble those of p53 in several fashions. Transfer of the

wild-type p53 gene into human cell lines with an abnormal gene causes a profound inhibition of growth *in vitro* (41, 42) and *in vivo* (43). Second, the phenotype of the p53 profile depends on the balance between normal and mutant copies of the gene—i.e., a gene-dosage effect. A wide range of human tumors show loss of heterozygosity at the *HRAS* locus, suggesting that a p53-like gene may reside near the *HRAS* gene (44). Recently, several groups have correlated germ-line alterations in the p53 gene with the Li-Fraumeni syndrome (45, 46). If the growth suppressor gene on chromosome 11p functions in a similar manner, it may participate in an early step in rhabdomyosarcoma development, accounting for its potent growth suppressing activity.

While the microcell hybridization technique maps functions to whole chromosomes, this approach may provide an advantage over single gene transfers in cases where two genes are required for tumor suppression. Thus, if the two suppressor genes for rhabdomyosarcoma function in tandem to cause suppression of growth, transfer of the gene on the short arm alone might have little effect. This hypothesis can be tested and the region on the long arm that contains the other suppressor gene can be identified by developing chromosome 11 fragments for transfer into the RD cells. Use of these smaller segments of chromosome 11 should lead to the precise location of these genes, a necessary step for their isolation by reverse genetics.

We thank Ande West for excellent technical assistance, Dr. Keith Fournier for the gift of the HDM-18 cell line, and Dr. Edison Liu for helpful discussions. This work was supported in part by Grant 44470 from the National Cancer Institute to B.E.W.

1. Nowell, P. C. (1986) *Cancer Res.* **46**, 2203–2207.
2. Friend, S. H., Bernards, R., Rojelj, S., Weinberg, R. A., Rapaport, J. M., Albert, D. M. & Dryja, T. P. (1986) *Nature (London)* **323**, 643–646.
3. Lee, W.-H., Bookstein, R., Hong, F., Young, L.-J., Shew, J.-Y. & Lee, E. Y.-H. P. (1987) *Science* **235**, 1394–1399.
4. Fung, Y.-K., Murphree, A. L., T'ang, A., Qian, J., Hinrichs, S. H. & Benedict, W. F. (1987) *Science* **236**, 1657–1661.
5. Finley, C. A., Hinds, P. W. & Levine, A. J. (1989) *Cell* **57**, 1083–1093.
6. Fearon, E. R., Cho, E. R., Nigro, J. M., Kern, S. E., Simons, J. W., Ruppert, J. M., Hamilton, S. R., Presinger, A. C., Thomas, G., Kinzler, K. W. & Vogelstein, B. (1990) *Science* **247**, 49–56.
7. Call, K. M., Glaser, T., Ito, C. Y., Buckler, A. J., Pelletier, J., Haber, D. A., Rose, E. A., Kral, A., Yeger, H., Lewis, W. H., Jones, C. & Housman, D. (1990) *Cell* **60**, 509–520.
8. Gessler, M., Poustka, A., Cavenee, W., Neve, R. L., Orkin, S. L. & Bruns, G. A. P. (1990) *Nature (London)* **343**, 774–778.
9. Noda, M., Kitayama, H., Matsuzaki, T., Sugimoto, Y., Okayama, H., Bassin, R. H. & Ikawa, Y. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 162–166.
10. Viskochil, D., Buchberg, A. M., Xu, G., Cawthon, R. M., Stevens, J., Wolff, R. K., Culver, M., Carey, J. C., Copeland, N. G., Jenkins, N. A., White, R. & O'Connell, P. (1990) *Cell* **62**, 187–192.
11. Wallace, M. R., Marchuk, D. A., Andersen, L. B., Letcher, R., Odeh, H. M., Saulino, A. M., Fountain, J. W., Brereton, A., Nicholson, J., Mitchell, A. L., Brownstein, B. H. & Collins, F. S. (1990) *Science* **249**, 181–186.
12. Kinzler, K., Nilbert, M., Vogelstein, B., Bryan, T., Levy, D., Smith, K., Preisinger, A., Hamilton, S., Hedge, P., Markham, A., Carlson, M., Joslyn, G., Groden, J., White, R., Miki, Y., Miyoshi, Y., Nishisho, I. & Nakamura, Y. (1991) *Science* **251**, 1366–1370.
13. Sager, R. (1989) *Science* **246**, 1406–1412.
14. Hansen, M. F. & Cavenee, W. K. (1987) *Cancer Res.* **47**, 5518–5527.
15. Fournier, R. E. K. & Ruddle, F. H. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 319–323.
16. Ege, T., Ringertz, N. R., Hamberg, H. & Sidebottom, E. (1977) *Methods Cell Biol.* **15**, 339–357.
17. Weissman, B. E., Saxon, P. J., Pasquale, S. R., Jones, G. R., Geiser, A. G. & Stanbridge, E. J. (1987) *Science* **236**, 171–180.
18. Riccardi, V. M., Sujansky, E., Smith, A. C. & Francke, U. (1978) *Pediatrics* **61**, 604–610.
19. Koufos, A., Hansen, M. F., Lampkin, B. C., Workman, M. L., Copeland, N. G., Jenkins, N. A. & Cavenee, W. K. (1984) *Nature (London)* **309**, 170–172.
20. Trent, J. M., Stanbridge, E. J., Macbride, H. L., Meese, E. U., Casey, G., Araujo, D. E., Witkowski, C. M. & Nagle, R. B. (1990) *Science* **247**, 568–571.
21. Shimizu, M., Yokota, J., Mori, N., Shuin, T., Shinoda, M., Masaaki, T. & Oshimura, M. (1990) *Oncogene* **5**, 185–194.
22. Scrable, H. J., Witte, D. P., Lampkin, B. C. & Cavenee, W. K. (1987) *Nature (London)* **329**, 645–647.
23. Scrable, H., Witte, D., Shimada, H., Seemayer, T., Wang-Wuu, S., Soukup, S., Koufos, A., Houghton, P., Lampkin, B. & Cavenee, W. K. (1989) *Genes Chromosomes Cancer* **1**, 23–35.
24. McAllister, R. M., Melnyk, J., Finkelstein, J. Z., Adams, E. C., Jr., & Gardner, M. B. (1969) *Cancer* **24**, 520–526.
25. Lugo, T. G., Handelin, B., Killary, A. M., Housman, D. E. & Fournier, R. E. K. (1987) *Mol. Cell. Biol.* **7**, 2814–2820.
26. Koi, M., Morita, H., Yamada, H., Satoh, H., Barrett, J. C. & Oshimura, M. (1989) *Mol. Carcinog.* **2**, 12–21.
27. Koi, M., Shimizu, M., Morita, M., Yamada, H. & Oshimura, M. (1989) *Jpn. J. Cancer Res.* **80**, 413–418.
28. Stanbridge, E. J. (1981) *Isr. J. Med. Sci.* **17**, 563–568.
29. Benedict, W. F., Weissman, B. E., Mark, C. & Stanbridge, E. J. (1984) *Cancer Res.* **44**, 3471–3479.
30. Kugoh, H. M., Hashiba, H., Shimizu, M. & Oshimura, M. (1990) *Oncogene* **5**, 1637–1644.
31. Glaser, T., Gerhard, D., Payne, C., Jones, C. & Housman, D. (1985) *Cytogenet. Cell Genet.* **40**, 643.
32. Antonarakis, S. E., Boehm, C. D., Giardina, P. J. V. & Kazazian, H. H., Jr. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 137–141.
33. Shih, C. & Weinberg, R. (1982) *Cell* **29**, 161–169.
34. Sotelo-Avila, C. & Gooch, M. (1976) *Perspect. Pediatr. Pathol.* **3**, 255–272.
35. Koufos, A., Hansen, M. F., Copeland, N. G., Jenkins, N. A., Lampkin, B. C. & Cavenee, W. K. (1985) *Nature (London)* **316**, 330–334.
36. Koufos, A., Grundy, P., Morgan, K., Aleck, K. A., Hadro, T., Lampkin, B. C., Kalbakji, A. & Cavenee, W. K. (1989) *Am. J. Hum. Genet.* **44**, 711–719.
37. Reeve, A. E., Sih, S. A., Raizis, A. M. & Feinberg, A. P. (1989) *Mol. Cell. Biol.* **9**, 1799–1803.
38. Bonetta, L., Kuehn, S. E., Huang, A., Law, D. J., Kalikin, L. M., Koi, M., Reeve, A. E., Brownstein, B. H., Yeger, H., Williams, B. R. G. & Feinberg, A. P. (1990) *Science* **250**, 994–997.
39. Misra, B. C. & Srivatsan, E. S. (1989) *Am. J. Hum. Genet.* **45**, 565–577.
40. Pereira-Smith, O. M. & Smith, J. R. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 6042–6046.
41. Diller, L., Kassel, J., Nelson, C. E., Gryka, M. A., Litwak, G., Gebhardt, M., Bressac, B., Ozturk, M., Baker, S. J., Vogelstein, B. & Friend, S. H. (1990) *Mol. Cell. Biol.* **10**, 5772–5781.
42. Baker, S. J., Markowitz, S., Fearon, E. R., Willson, J. K. V. & Vogelstein, B. (1990) *Science* **249**, 912–915.
43. Chen, P.-L., Chen, Y., Bookstein, R. & Lee, W.-H. (1990) *Science* **250**, 1576–1580.
44. Mikkelsen, T. & Cavenee, W. K. (1990) *Cell Growth Differ.* **1**, 201–207.
45. Malkin, D., Li, F. P., Strong, L. C., Fraumeni, J. F., Jr., Nelson, C. E., Kim, D. H., Kassel, J., Gryka, M. A., Bischoff, F. Z., Tainsky, M. A. & Friend, S. H. (1990) *Science* **250**, 1233–1238.
46. Srivatava, S., Zou, Z., Pirollo, K., Blattner, W. & Chang, E. H. (1990) *Nature (London)* **348**, 747–749.