Journal of Cellular Biochemistry 42:135–142 (1990)

Transfer of a Normal Human Chromosome 11 Suppresses Tumorigenicity of Some but Not All Tumor Cell Lines

Mitsuo Oshimura, Hiroyuki Kugoh, Minoru Koi, Motoyuki Shimizu, Hideto Yamada, Hitoshi Satoh, and J. Carl Barrett

Laboratory of Cytogenetics, Kanagawa Cancer Center Research Institute, Asahi-ku 54-2, Yokohama 241, Japan (M.O., H.K., M.K., M.S., H.Y.); Laboratory of Molecular Carcinogenesis, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709 (H.S., J.C.B.)

The complete suppression of tumorigenicity of a human cervical cancer cell (HeLa) and a Wilms' tumor cell line (G401) following the introduction via microcell fusion of a single chromosome t(X;11) has been demonstrated by Stanbridge and coworkers. To determine whether other tumor cell lines are suppressed by chromosome 11, we performed chromosome transfer experiments via microcell fusion into various human tumor cell lines, including a uterine cervical carcinoma (SiHa), a rhabdomyosarcoma (A204), a uterine endometrial carcinoma (HHUA), a renal cell carcinoma (YCR-1), and a rat ENU-induced nephroblastoma (ENU-T1). We first isolated a mouse A9 cell containing a single human chromosome 11 with integrated pSV2-neo plasmid DNA. Following microcell fusion of the neo-marked chromosome 11 with the various tumors mentioned above, we isolated clones that were resistant to G418 and performed karyotypic analyses and chromosomal in situ hybridization to ensure the transfer of the marked chromosome. Whereas the parental cells of each cell line were highly tumorigenic, SiHa and A204 microcell hybrid clones at early passages were nontumorigenic in nude mice and HHUA was moderately tumorigenic. On the other hand, YCR-1 and ENU-T1 microcell hybrid clones were still highly tumorigenic following the introduction of chromosome 11. Thus, the introduction of a normal chromosome 11 suppresses the tumorigenicity of some but not all tumors, suggesting that the function of the putative suppressor gene(s) on chromosome 11 is effective only in specific tumors.

Key words: tumor-suppressor gene, microcell-fusion, pSV2-neo, nude mouse, mono-chromosome

The suppression of tumorigenicity of tumor cells by hybridization with normal cells has led to the hypothesis that normal cells contain gene(s) that suppress the neoplastic potential of tumor cells [1]. Several studies have mapped putative tumor-suppressor

Abbreviations used: DMEM, Dulbecco's modified Eagle minimum essential medium; FCS, fetal calf serum, ENU, *N*-ethylnitrosourea; HPRT, hypoxanthine/guanine phosphoribosyl transferase.

Received March 23, 1989; accepted September 22, 1989.

genes to specific chromosomes derived from normal cells by analysis of the loss of chromosomes in the hybrid cells that reexpress tumorigenicity [2–6].

A more direct approach to the identification of a chromosome(s) carrying tumor-suppressor gene(s) is the introduction of specific chromosomes into the tumor cells of interest. Saxon et al. first demonstrated the complete suppression of tumorigenicity of HeLa cells by introduction of a single human chromosome 11 via microcell fusion [7]. The same approach was also used to demonstrate the presence of tumor-suppressor gene(s) for Wilms' tumor on human chromosome 11 [8]. In these studies, a normal human chromosome with a translocation between chromosome 11 and X chromosome was used. The translocated portion of the X chromosome encodes the hypoxanthine/guanine phosphoribosyl transferase (HPRT) enzyme. HPRT-deficient HeLa tumor cells were used as recipient cells for microcell transfer, and cells with a transferred chromosome (X;11) containing HPRT gene activity were selected in medium containing hypoxanthine, aminopterin, and thymidine (HAT media). In contrast, chromosomes tagged by DNA transfection with a dominant selectable gene, e.g., pSV2-neo gene [9,10] or pSV2-gpt gene [11], can be transferred into any mammalian cells without the requirement for isolating mutant cells that lack the enzymes. By this technique, we derived a pSV2-neo tagged chromosome 11 from normal human fibroblasts.

In the present study, we examined whether the suppression of tumorigenicity by human chromosome 11 reported for HeLa cells was observed with other tumorigenic cell lines.

MATERIALS AND METHODS

Cells

Mouse A9 cells containing a pSV2-neo-tagged chromosome 11, A9(neo11)-1 [10], were used as chromosome donors in microcell transfer to the following tumor cell lines: SiHa human cervical carcinoma [12], A204 human rhabdomyosarcoma [13], HHUA human uterine endometrial carcinoma [14], YCR-1 human renal cell carcinoma [15], and ENU-T1 rat nephroblastoma [16]. All the cell lines were maintained in Dulbecco's modified Eagle's media supplemented with 10% fetal calf serum. The cells were screened for mycoplasma contamination and found to be negative.

Microcell Mediated Chromosome Transfer

The methods used were similar to those described by Fournier and Ruddle [17] with some modifications [18]. Microcells containing only one or a few chromosomes from A9(neo11)-1 cells were prepared and fused to the recipient cells of interest. Resulting microcell hybrids containing the human 11neo chromosome were isolated in growth medium containing 800–2,000 μg/ml of G418 antibiotic. Clones were grown for several passages to obtain sufficient cells and then assayed for tumorigenicity and analyzed karyotypically.

Chromosome Analysis

Metaphase chromosomes were prepared by methods previously described [19]. Chromosomes were analyzed by quinacrine plus Hoechst 33258 staining method, which can identify each human chromosome and chromosomal segment and can also distinguish human centromeric regions from those of mouse chromosomes [20]. Twenty or more well-banded metaphases were fully analyzed for each karyotype.

Chromosomal In Situ Hybridization

The in situ hybridization method of Harper et al. [21] was used to ensure the presence of the transferred human chromosome 11 containing integrated pSV2-neo plasmid DNA. The pSV2-neo plasmid DNA was labeled by nick-translation using [^{3}H]dATP (53.6 μCi/mmol), [^{3}H]dGTP (34.5 μCi/mmol), and [^{3}H]dTTP (103 μCi/mmol). Specific activities of 4–5 $\times 10^7$ cpm/μg of DNA were obtained. After hybridization and washing, the slides were coated with NTB2 nuclear track emulsion (Kodak) and exposed for 2 weeks at 4°C. Exposed slides were developed in Kodak D-19 developer for 3 min, fixed, stained with quinacrine mustard stain for 30 min, and analyzed.

Tumorigenicity Assay

For assays of tumorigenicity, cells at passages 4–6 were trypsinized, harvested, and suspended in serum-free medium. Cells (5×10^6 or 1×10^7) in 0.2 ml media were inoculated subcutaneously into 4 to 6 week old athymic ICR nu/nu mice. Animals were examined for tumor formation at regular intervals for up to 120 days. Tumorigenicity was measured by the ability of the cells to form progressively growing tumors.

RESULTS AND DISCUSSION

In order to transfer a normal human chromosome 11 into different tumor cell lines via microcell fusion, we first isolated mouse A9 cells containing a single human chromosome 11 with integrated pSV2-neo plasmid DNA [18]. The chromosomal location of the pSV2-neo gene in the A9(neo11)-1 cells was determined by chromosomal in situ hybridization. A high percentage of the autoradiographic grains following hybridization with a [^{3}H]pSV2neo probe were located on chromosome 11 in metaphase A9(neo11)-1 cells. In total, 187 grains were scored on 77 metaphase spreads and 45 (24%) of them were on chromosome 11. The location of the pSV2-neo integration sites is estimated to be 11p11 since 58% of all grains on chromosome 11 were localized to this region [18].

Chromosome analyses showed a modal chromosome number of 63–66 for SiHa cervical carcinoma cells with 14–19 marker chromosomes. The cells contained three copies of an apparently normal chromosome 11 (Table I). All the metaphases of the A204 rhabdomyosarcoma and 90% of the uterine endometrial carcinoma HHUA cells showed an apparently normal karyotype (46,XX), which is extremely unusual for tumor cell lines. Therefore, these cells contained two copies of chromosome 11. Renal carcinoma YCR-1 cells had a modal chromosome number of 58–61 with 23–28 marker chromosomes, most of which were of unknown origin. No normal chromosome 11 was observed in the metaphases analyzed. The modal chromosome number of the rat ENU-induced nephroblastoma (ENU-T1) cells was 44 with one to three marker chromosomes.

Microcell-mediated transfers of the neo-tagged human chromosome 11 were performed using the A9(neo11)-1 described above as the chromosome donor. In three to five successive experiments with the five tumor cell lines, eight to 15 independent G418-resistant microcell hybrids from each tumor were isolated. Chromosome analyses with Q-banding plus Hoechst 33258 staining of these microcell hybrids showed that five clones of SiHa cells, five clones of A204 cells, six clones of HHUA, three clones of YCR-1 cells, and four clones of ENU-T1 cells contained one or two extra copies of an

TABLE I. Tumorigenicity in Nude Mice of Microcell Hybrids From Various Tumor Cell Lines Following Transfer of Normal Human Fibroblast-derived Chromosome 11

Tumor cell lines and their microcell hybrids	Percentage of metaphase spreads with indicated number of intact chromosome 11[a]						Tumorigenicity[b]	
	0	1	2	3	4	5	No. tumors/ injected site	(Latency period in days)
Human uterine cervical carcinoma								
Parental SiHa				100			13/14	(< 30)
Microcell hybrids								
#11-1[c]					20	80	0/6	(> 120)
#11-7[c]					10	90	0/3	(> 120)
#11-9[c]					100		0/3	(> 120)
#11-11[c]					100		1/3	(90/> 120)
#11-11 tumor[c]				90	10		3/3	(< 30)
#11-17[c]					50	50	0/3	(> 120)
Human rhabdomyosarcoma								
Parental A204			100				9/9	(< 7)
Microcell hybrids								
#11-15				100			0/6	(> 120)
#11-20[c]				100			0/3	(> 120)
#11-22				100			0/3	(> 120)
#11-23				100			0/3	(> 120)
#11-25				100			0/3	(> 120)
Human uterine endometrial carcinoma								
Parental HHUA			100				5/5	(< 30)
Microcell hybrids								
#11-1[c]				100			0/5	(> 120)
#11-5				100			1/6	(35/> 120)
#11-6[c]				100			4/9	(< 30)
#11-7[c]				100			4/9	(< 35)
#11-8[c]				100			5/11	(< 25)
#11-9				100			6/11	(< 25)
Human renal cell carcinoma								
Parental YCR-1	100						17/17	(< 14)
Microcell hybrids								
#11-1[c]			100				8/8	(< 14)
#11-1 tumor		10	90					
#11-2			100				5/5	(< 14)
#11-3[c]		20	80				5/5	(< 14)
#11-3 tumor		10	90					
Rat ENU-induced nephroblastoma								
Parental ENU-T1	100						6/6	(< 14)
Microcell hybrids								
#11-1		100					7/7	(< 15)
#11-5		100					8/6	(< 17)
#11-5 tumor	10	90						
#11-6		100					5/6	(< 16)
#11-9	10	90					6/6	(< 14)

[a]Twenty or more metaphases were analyzed.
[b]Observed for up to 120 days after inoculation of 10^7 cells at passages 4–8 into 4–6 week old ICR nu/nu athymic mice, except for ENU cells of 5×10^6.
[c]The introduction of chromosome 11 was confirmed by chromosomal in situ hybridization.

"intact" chromosome 11 (Table I). No extra mouse chromosomes detected by bright fluorescent centromeric regions with Hoechst stain were observed; this, however, does not exclude the possible presence of mouse sequences in the microcell hybrids, which will be discussed later. Chromosome in situ hybridization further confirmed the presence of the neo-tagged normal human chromosome 11 in the microcell hybrids containing extra copies of chromosome 11 examined (Table I). For example, 98 total grains were scored on the metaphase chromosomes of HHUA microcell hybrid #11-7 cells; among them, 40 grains were on 11p and 80% of all the grains on chromosome 11 were again located at 11p11-12 (Fig. 1a,b). Essentially the same results were obtained from the analyses of the other clones (data not shown).

All the microcell hybrids from the five tumor lines were further analyzed for tumorigenicity in nude mice, and the results are summarized in Table I. Parental SiHa cells formed tumors at 13 of 14 inoculated sites with a latency period of 30 days [18]. In contrast, four of five SiHa microcell hybrids with the introduced chromosome 11 were nontumorigenic for >120 days after injection [18]. One clone (#11-11) formed a tumor at one of three inoculated sites at 90 days after injection (Table I). This tumor was removed and karyotyped after growth in culture. Chromosomal analysis revealed the loss of one copy of chromosome 11 in this tumor, and chromosomal in situ hybridization failed to detect the neo-tagged chromosome 11. Furthermore, reinjection of tumor-derived cells of clone #11-11 into nude mice resulted in the formation of tumors at all sites with a 30 day latency (Table I). Taken together, these results indicated that

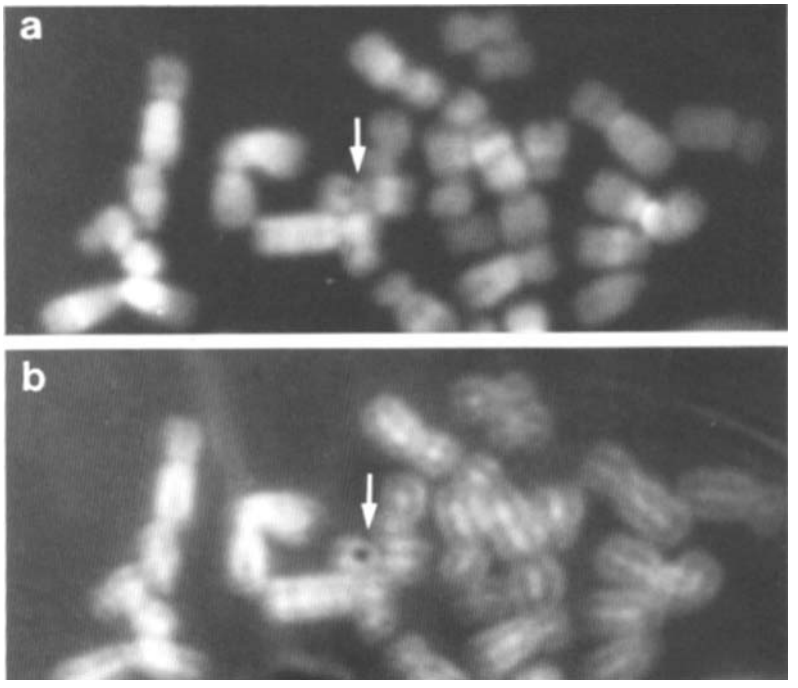

Fig. 1. Chromosomal in situ hybridization showing a normal human chromosome tagged with pSV2neo-gene in HHUA microcell hybrid clone #11-7. **a:** Q-banded, partial metaphase of HHUA #11-7; **b:** the same metaphase of HHUA #11-7 focused on the autoradiographic grain. Arrows indicate the chromosome 11 tagged with pSV2neo-gene; the integration site is 11p11-12.

introduction of single copy of normal human chromosome 11 into SiHa cells suppresses the tumorigenicity of this tumor. A clone that lost this chromosome reexpressed tumorigenicity. These results have been reported previously [18].

Parental A204 cells formed tumors at 100% of the inoculated sites with a latency period of <7 days, whereas all five A204 microcell hybrids were nontumorigenic for >120 days after injection. In contrast, five of six HHUA microcell hybrids were tumorigenic with variable take incidences. The tumor-take incidence of the tumorigenic microcell hybrids ranged from 17 to 55%. One clone (#11-1) did not form tumors over the observation period of 120 days. In contrast, the parental HHUA cells formed progressive tumors in 100% of the inoculated sites within 30 days after inoculation (Table I).

Both parental YCR-1 and ENU-T1 cells as well as all their microcell hybrids containing neo-tagged chromosome 11 formed tumors at 100% of the inoculated sites within 14 days and 17 days, respectively. Direct chromosome analyses of two tumors from YCR-1 microcell hybrids (#11-1 and -3) and one tumor from ENU-T1 microcell hybrid (#11-5) showed more than 80% of metaphases to have retained an extra copy of chromosome 11. Therefore, it is unlikely that the cells that formed tumors in these microcell hybrids had lost the introduced chromosome 11. This is also supported by the evidence that the parental cells and their microcell hybrids had similar tumor latency periods.

Thus, the results have clearly shown that the introduction of one or two extra copies of human chromosome 11, derived from normal human fibroblasts, into SiHa and A204 cells leads to complete suppression of tumorigenicity of these cells, moderate suppression of tumorigenicity in HHUA cells, and no suppression of YCR-1 or ENU-T1 cells. These observations suggest that the function of the putative tumor suppressor gene(s) on chromosome 11 is effective only in specific tumors.

A possible concern is whether mouse sequences play a role in the suppression of tumorigenicity. This is unlikely for several reasons. First, multiple independent clones following transfer of human chromosome 11 were isolated and suppression was correlated with the presence of the human chromosome 11 in SiHa and A204 tumors. If mouse sequences were involved in the tumorigenic potential of the cells, this clear correlation would not be expected. Second, we have isolated clones following transfer of a human chromosome 12, and no suppression of tumorigenicity was observed [18] even though the chromosome was from a mouse/human hybrid.

The mechanism of the suppression of the tumorigenic phenotype of these cells has not been determined. Stanbridge [22] suggested that the putative tumor suppressor gene on chromosome 11 is a dominantly acting gene that prevents cells from expressing the neoplastic state and is analogous to the tumor suppressor genes involved in retinoblastoma and Wilms' tumor [23,24]. These genes are inactive or lost in the tumor cell lines, and reintroduction of an active gene by chromosome transfer results in suppression of tumorigenicity. The suppression of tumorigenicity after transfer of a human chromosome 11 may also result from a change in gene balance or gene dosage [25]. The expression of tumorigenicity may be regulated by a balance between positive, tumor growth-stimulating genes (e.g., oncogenes), and negative, tumor growth-regulating genes (e.g., tumor-suppressor genes). Introduction of one or two extra copies of a normal chromosome 11 carrying the latter gene(s) could affect the dosage of this gene. Since the dosage of the positive factors may vary between subclones of the original parental cells,

this may result in different abilities of a single normal chromosome to suppress tumorigenicity. This may explain the results obtained with the human uterine endometrial carcinoma HHUA cells (Table I).

Stanbridge et al. have reported that the introduction of a single X-autosome translocation chromosome containing a large portion of human chromosome 11 is sufficient for the suppression of tumorigenicity of another cervical carcinoma cell line HeLa [7] and the G401 Wilms' tumor cell line [8]. Our results also suggest the presence of putative tumor-suppressor gene(s) for SiHa cervical cancer cells, and A-204 rhabdomyosarcoma cells on human chromosome 11, although it is not known whether the same gene(s) is responsible for the suppression of tumorigenicity of these cells. Mapping of the tumor-suppressor gene on human chromosome 11 might clarify this question. In our experiments, we analyzed only microcell hybrids that contained an intact transferred human chromosome. Among the G418-resistant clones obtained after transfer of neo-tagged human chromosome 11, a considerable number of clones contained no intact human chromosome 11. Some of these clones may contain additional chromosomal fragments derived from the transferred chromosome 11. Precise chromosome analysis using high-resolution banding techniques and/or RFLP analysis of chromosome 11 in conjunction with tumorigenicity studies of these clones may facilitate the chromosomal sublocation of the putative tumor-suppressor gene(s). These studies are currently in progress.

Furthermore, it will be interesting to determine whether microcell hybrids, whose tumorigenicity was suppressed, terminally differentiate in vivo as reported for cell-cell hybrids [26,27]. An examination of the in vitro properties of suppressed microcell hybrids may provide information on the underlining mechanisms for the suppression of tumorigenicity and can be useful for developing new in vitro assays that can distinguish tumorigenic and nontumorigenic cells.

ACKNOWLEDGMENTS

The authors thank Prof. N. Wake, Kyusyu University, for his continuous encouragement and helpful suggestions throughout these studies. This study was supported in part by a Grants-in-Aid for Cancer Research from the Ministry of Education, Science and Culture, Japan, a Grants-in-Aid for Comprehensive Ten-year Strategy for Cancer Control from the Ministry of Health and Welfare of Japan, and a grant from the Science and Technology Agency of Japan.

REFERENCES

1. Klein G, Bregula U, Wiener F, Harris H: J Cell Sci 8:659, 1971.
2. Stanbridge EJ, Flandermeyer RR, Daniels DW, Nelson-Rees WA: Somatic Cell Genet 17:699, 1981.
3. Evans EP, Brutenshaw MD, Brown B, Hennion R, Harris H: J Cell Sci 56:113, 1982.
4. Klinger HP, Shows TB: JNCI 71:559, 1983.
5. Benedict WF, Weissman BE, Mark C, Stanbridge EJ: Cancer Res 44:3471, 1984.
6. Oshimura M, Koi M, Ozawa N, Sugawara O, Lamb PW, Barrett JC: Cancer Res 48:1823, 1988.
7. Saxon PJ, Srivatsan ES, Stanbridge EJ: EMBO J 15:3461, 1986.
8. Weissman BE, Saxon PJ, Pasquale SR, Jones GR, Geiser AG, Stanbridge EJ: Science 236:175, 1987.
9. Lugo TG, Handelin B, Killary AM, Housman DE, Fournier REK: Mol Cell Biol 17:2814, 1987.
10. Koi M, Shimizu M, Morita M, Yamada H, Oshimura M: Jpn J Cancer Res 80:413, 1989.
11. Saxon PJ, Stivatsan ES, Leipzig GV, Sameshima JH, Stanbridge EJ: Mol Cell Biol 5:140, 1985.

12. Friedl F, Kimura I, Osato T, Ito V: Proc Soc Exp Biol Med 135:543, 1970.
13. Giard DJ, Aaronson SA, Todaro GJ, Arnstein P, Kersey JH, Dosik H, Parks WP: JNCI 51:147, 1973.
14. Ishiwata I, Ishiwata C, Soma M, Aral J, Ishikawa H: Gynecol Oncol 17:281, 1984.
15. Kubota V, Shiun T, Yao M, Inoue H, Yoshioka T: FEBS Lett 212:159, 1987.
16. Nagashima V, Ohaki Y, Umeda M, Oshimura M, Misugi K: Arch Virch B (in press).
17. Fournier REK, Ruddle FH: Proc Natl Acad Sci USA 74:919, 1977.
18. Koi M, Morita H, Yamada, Satoh H, Barrett JC, Oshimura, M: Mol Carcinogen 2:12, 1989.
19. Oshimura M, Hesterberg TW, Barrett JC: Cancer Res 44:5017, 1984.
20. Yoshida MC, Ikeuchi T, Sasaki M: Proc Jpn Acad Sci 51:181, 1975.
21. Harper MEA, Ulrich A, Saunders GF: Proc Natl Acad Sci USA 78:4458, 1981.
22. Stanbridge EJ: Bioassay 3:252, 1985.
23. Knudson AG: Cancer Res 45:1437, 1985.
24. Cavenee WK, Dryja TP, Phillips RA, Benedict WF, Godbout R, Callie BL, Morphe AL, Strong LC, White RL: Nature 79:305, 1983.
25. Oshimura M, Barrett JC: Environ Mutagen 8:129, 1986.
26. Stanbridge EJ, Araujo D, Ross P, Bulter G, McCullough J, Wilkinson J: Cancer Res 46:4759, 1986.
27. Stanbridge EJ, Wilkinson J: Proc Natl Acad Sci USA 75:1466, 1978.