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# Localization of HeLa Cell Tumor-Suppressor Gene to the Long Arm of Chromosome 11

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## Summary

**Cytogenetic and molecular genetic analyses of human intraspecific HeLa × fibroblast hybrids have provided evidence for the presence of a tumor-suppressor gene(s) on chromosome 11 of normal cells. In the present study, we have carried out extensive RFLP analysis of various nontumorigenic and tumorigenic hybrids with at least 50 different chromosome 11–specific probes to determine the precise location of this tumor-suppressor gene(s). Two different hybrid systems, (1) microcell hybrids derived by the transfer of a normal chromosome 11 into a tumorigenic HeLa-derived hybrid cell and (2) somatic cell hybrids derived by the fusion of the HeLa ($D98^{OR}$) cells to a retinoblastoma (Y79) cell line, were particularly informative. The analysis showed that all but one of the nontumorigenic hybrid cell lines contained a complete copy of the normal chromosome 11. This variant hybrid contained a segment of the long arm but had lost the entire short arm of the chromosome. The tumorigenic microcell and somatic cell hybrids had retained the short arm of the chromosome but had lost at least the q13-23 region of the chromosome. Thus, these results showed a perfect correlation between the presence of the long arm of chromosome 11 and the suppression of the tumorigenic phenotype. We conclude therefore that the gene(s) involved in the suppression of the HeLa cell tumors is localized to the long arm (q arm) of chromosome 11.**

## Introduction

Somatic cell hybrids generated by the fusion of two or more different cells of same or different species have been extremely useful in the genetic analysis of malignancy. Early studies of Harris et al. (1969) and Harris (1971) that involved the fusion of tumorigenic and nontumorigenic mouse cells led to the conclusion that tumorigenicity behaves as a recessive trait. From other studies that utilized the fusion of intraspecific rodent cells and interspecific rodent × human cells, definite conclusions were not drawn, owing to the chromosomal instability of these hybrid cells (Klinger et al. 1978; Kucherlapati and Shin 1979). Chromosomally stable intraspecific human cell hybrids derived by the fusion of tumorigenic HeLa cells to normal human cells provided the evidence that tumorigenicity indeed behaves as a recessive trait (Stanbridge 1976). These hybrids were completely nontumorigenic. After a prolonged passage in culture, rare tumorigenic segregants were isolated, possibly owing to the loss of specific chromosomes that contained the tumor suppressor sequences. Cytogenetic analysis of these hybrids revealed a statistical correlation between the loss of a single copy each of chromosome 11 and chromosome 14 and the development of the tumorigenic phenotype (Stanbridge et al. 1981). Independent studies by Klinger (1980, 1982), using HeLa × fibroblast hybrids, confirmed the association between the loss of chromosome 11 and few other chromosomes and the development of the tumorigenic cells. It was impossible, by the karyotypic analysis, to determine the parental origin of the chromosome 11 lost in these tumorigenic hybrid cells.

The evidence for the loss of a normal chromosome 11 in the tumorigenic hybrids was provided by molecular genetic studies involving the use of chromosome-specific RFLP probes. Srivatsan et al. (1986) and Kaelbing and Klinger (1986) showed that the loss of a single normal chromosome 11 was sufficient for the reexpres-

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sion of the tumorigenic phenotype. The conclusive evidence that a normal chromosome 11 is indeed involved in tumor suppression came from the studies of Saxon et al. (1986). They were able to show the suppression of the tumorigenic phenotype by the introduction of a normal chromosome 11 into a tumorigenic HeLa × fibroblast hybrid cell by the microcell transfer technique (Saxon et al. 1985). A selection process involving the loss of the introduced chromosome led to the reappearance of the tumorigenic cells. The reintroduction of the chromosome 11 once again resulted in the suppression of the tumorigenic phenotype. Thus, these studies indicate the presence of "tumor-suppressor" sequences on normal chromosome 11 and that the loss of them leads to the development of tumorigenic phenotype.

In the present study, we have attempted to identify the precise location of this tumor-suppressor gene(s) by using the chromosome 11–specific RFLP probes. We have analyzed various nontumorigenic and tumorigenic hybrids for an association between the loss of specific sequences on chromosome 11 and the reversion to the tumorigenic phenotype. This analysis localized the tumor-suppressor gene(s) to the long arm (q arm) of the chromosome, possibly to the q13-q23 region.

## Material and Methods

### *Cell Lines*

The parental cell lines and the nontumorigenic and tumorigenic hybrids derived from the introduction of a normal chromosome 11 into HeLa cells are presented in table 1. All the hybrid cell lines were tested for tumorigenicity by injection into *nude* mice. The cell lines were all grown in minimum essential medium containing essential amino acids and 10% FCS.

### *Plasmid and Phage DNAs*

Chromosome 11– and chromosome 13–specific RFLP probes used in the present analysis were obtained from a number of laboratories and from the American Type Culture Collection. Plasmids were grown in *Escherichia coli* strain HB101 and purified by ethidium bromide–CsCl density gradient centrifugation (Clewell and Helinski 1969). Phage particles and phage DNAs were prepared by the method of Lawn et al. (1978). The insert RFLP probes were cut out from the plasmid and phage DNAs by digestion with appropriate restriction enzymes and isolated by separation on 0.8%–1.0% low-melting-point agarose gels. Oligonucleotide labeling of the probe DNAs was performed according to the method of Feinberg and Vogelstein (1983). Genomic DNAs were prepared according to the method of Jolly et al. (1982).

### *Blot Hybridization Analysis*

Genomic DNAs were digested with restriction enzymes appropriate for the different RFLP probes. The information about the restriction enzymes used, the size of the polymorphic alleles, and the chromosomal location of the informative probes is presented in table 2. DNA samples were digested in a buffer containing 33 mm Tris-HCl (pH 7.8), 60 mm potassium acetate, 10 mM $MgCl_2$, and 1 mM DTT at 37°C overnight, for all the enzymes except *Taq*I. The *Taq*I digestion was performed in the same manner at 65°C overnight.

**Table 1**

**Description of Parental and Hybrid Cell Lines**

| Parental Cell Lines (fibroblast = HeLa) | Hybrid Cell Lines | |
|---|---|---|
| | Nontumorigenic | Tumorigenic |
| GM77 × D98/AH-2 . . . . . | ESH5-119SA | ESH5-130SA |
| | | ESH-136SA |
| IMR-90 × D98/AH-2 . . . . . | ESH39E-C5 | ESH39L-16SA |
| | ESH39E-C14 | ESH39L-13SA |
| 110.1[a] × ESH15[b] . . . . . | 110.1/ESH15.1 | 110.1/ESH15.3 |
| | 110.1/ESH15.5 | 110.1/ESH15.4 |
| | 110.1/ESH15.6 | |
| Y79[c] × D98[ORd] . . . . . | HHY17p2c | HHY17p2c Tuo |

[a] Mouse (A9) cell line containing a translocated X:11 (11pter>11q23::X26>Xqter) chromosome as the only human chromosome.

[b] Tumorigenic revertant cell line derived from the fusion of diploid fibroblast 75-55C and HeLa cells.

[c] Retinoblastoma cell line.

[d] Ouabain-resistant clone of parental HeLa cells, D98/AH-2.

Digested DNAs were subjected to the Southern hybridization analysis as described elsewhere (Srivatsan et al. 1986).

## Results

### *RFLP Analysis of the HeLa X Fibroblast Hybrids*

Genomic DNAs isolated from parental cell lines and from nontumorigenic and tumorigenic hybrids (table 1) were subjected to RFLP analysis using various chromosome 11–specific probes (table 2). The short arm, 11p15-specific, c-Ha-ras, and insulin probes (Bell et al. 1982; Der et al. 1982) were informative for these hybrid cell lines. The 6.6-kb c-Ha-ras probe (Der et al. 1982) hybridized to fragments of 4.0 kb and 2.5 kb with fibroblast GM77 DNA and to a homozygous fragment of 3.0 kb with HeLa cell DNA (fig. 1*a*). Both cellular DNAs contained a common fragment of 2.3 kb. The nontumorigenic hybrid ESH5-119SA contained allelic fragments of 4.0 kb, 3.0 kb, and 2.5 kb, which represented both the parental DNAs. Hybridization of the ras probe to DNA from the two tumorigenic hybrid cell lines was quite different. While the tumorigenic hybrid cell line ESH5-136SA had lost a copy of the fibroblast chromosome 11, the 2.5-kb allelic fragment, the tumorigenic hybrid cell line ESH5-130SA did not lose any of the fibroblast-specific alleles. Thus there was no correlation between the loss of this region of chromosome 11 and the reversion to the tumorigenic phenotype.

Similar results were obtained by hybridization analysis of IMR90 × HeLa cell hybrids by the insulin probe. The insulin probe, phins 310 (Bell et al. 1982), hybridized to heterozygous fragments of 2.4 kb and 2.3 kb with the IMR90 DNA and to a homozygous fragment of 0.75 kb with the HeLa cell DNA (fig. 1*b*). The DNA isolated from the nontumorigenic hybrid cell lines ESH 39E-C5 and ESH39E-C14 contained the 2.4-kb fragment of the fibroblast parent and the 0.75-kb fragment of the HeLa parent. These two cell lines had lost the 2.3-kb allelic fragment of the fibroblast. On the other hand, tumorigenic hybrid cell lines ESH 39L-16SA and ESH 39L-13SA, in addition to the HeLa-specific 0.75-kb fragment, had retained the 2.3-kb allelic fragment but had lost the 2.4-kb allelic fragment of the fibroblast parent. Thus, the results obtained with the insulin probe reiterated the possible noninvolvement of the

**Table 2**

**Chromosome 11– and Chromosome 13–specific RFLPs in Parental Cell Lines**

| Polymorphic Probe, Chromosomal Location | Restriction Enzyme | Polymorphic Fragment (kb) | | | | Reference |
|---|---|---|---|---|---|---|
| | | ESH15 | 110.1 | D98$^{OR}$ | Y79 | |
| Insulin, 11p15 | *Pvu*II | .75, 2.2 | .75 | .75 | .80 | 2 |
| c-Ha-ras, 11p15 | *Taq*I | 3.0, 3.5 | 2.5 | 3.0 | 2.5 | 7 |
| | *Pvu*II | 2.8, 3.2, 4.3 | 2.5 | 2.8 | 2.5 | |
| γ-Globin, 11p15 | *Hpa*I | 7.6 | 7.6 | 7.6, 13.0 | 7.6 | 22 |
| Catalase, 11p12 | *Bgl*II | NT | NT | 7.5 | 7.5 | 27 |
| D11S149, 11p$^{11}$-11q$^{13}$ | *Pvu*II | 3.2 | 5.2 | 3.2 | 3.2 | 36 |
| D11S146, 11q | *Msp*I | 3.8, 4.3 | 4.3 | 4.3 | 3.8, 4.3 | 36 |
| Sea-oncogene, 11q | *Hin*dIII | 18.0, 19.0 | NT | 19.0 | 18.0, 19.0 | 8 |
| SS6, 11q13 | *Bam*HI | 5.6/2.8, 8.4 | 8.4 | 5.6/2.8, 8.4 | 8.4 | 3 |
| | *Taq*I | 2.2, 3.0 | NT | 3.0 | 2.2, 3.0 | |
| E45-RL4, 11q13-23 | *Kpn*I | 8.6, 10.3 | NT | 10.3 | 8.6, 10.3 | 17 |
| p2-7-1D6, 11q22 | *Taq*I | 4.5, 6.7 | NT | 4.5, 6.7 | 4.5 | 34 |
| π2-14, 11q22 | *Pvu*II | NT | NT | 11.0, 12.5 | 11.0, 12.5 | 34 |
| | *Rsa*I | 2.3, 3.0 | NT | 3.0 | 2.3, 3.0 | |
| APO-A-I genomic (5′) 11q23 | *Apa*I | 2.5, 4.0 | None | 2.5 | 2.5, 4.0 | 13 |
| APO-A-IV, 11q23 | *Xba*I | 10.0, 20.0 | None | 20.0 | 10.0, 20.0 | 13 |
| π6-3, 11q22-23 | *Msp*I | NT | None | 3.85, 9.50 | 3.85, 9.50 | 34 |
| D11S147, 11q | *Pst*I | 4.0, 5.0 | NT | 5.0 | 5.0 | 36 |
| Hu10, 13q13 | *Xmn*I | NT | NT | 8.5, 10.0 | 10.0 | 9 |
| pTH162, 13q14 | *Bgl*II | NT | NT | 5.0, 7.7 | 6.1, 8.2 | 35 |
| p9D11, 13q22 | *Taq*I | NT | NT | 7.6 | 7.6, 11.0 | 4 |

NOTE.—NT = not tested; none = absence of the probe-specific sequences in the t(x; 11) chromosome.

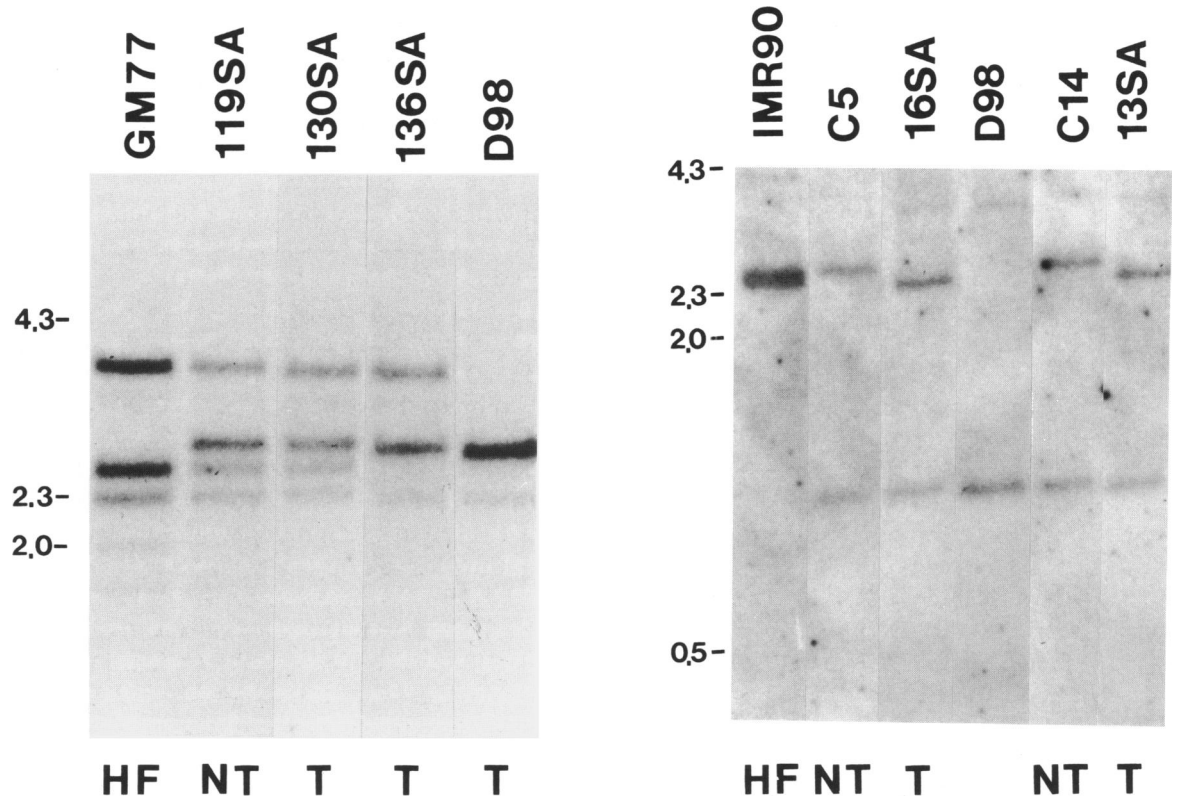


**Figure 1** Hybridization analysis of fibroblast × HeLa hybrids with short arm–specific chromosome 11 sequences. *a*, DNAs derived from GM77 × HeLa hybrids and digested with *Taq*I and hybridized to the c-Ha-ras probe (11p15). Only one of the two tumorigenic hybrids, ESH5-136SA, has lost the fibroblast-specific 2.5-kb allelic fragment. The other tumorigenic hybrid, ESH5-130SA, has retained both copies of fibroblast chromosome 11. Thus the p15 region does not correlate with the suppression of the tumorigenic phenotype. *b*, DNAs derived from the IMR90 × HeLa hybrids and digested with *Pvu*II and hybridized to the phins 310, insulin probe (11p15). Both nontumorigenic and tumorigenic hybrid cell lines contain one of the two fibroblast chromosomes, indicating the possible noninvolvement of this region in tumor suppression. Numbers on the left side represent the molecular-weight markers from phage lambda digested with *Hind*III. NT = nontumorigenic; T = tumorigenic cell lines; HF = human fibroblast cell lines.

p15 region of chromosome 11 in suppression of the tumorigenic phenotype. Supportive evidence for the involvement of long arm in suppression of the tumorigenic phenotype was provided by extensive analysis of other HeLa × normal chromosome 11 hybrids (see below).

### *RFLP Analysis of the HeLa × Retinoblastoma Hybrids*

Development of retinoblastoma has been correlated with a loss of genetic information on chromosome 13 (Cavenee et al. 1983; Friend et al. 1986; Fung et al. 1987; Lee et al. 1987). We and others have shown involvement of gene(s) on chromosome 11 in suppression of HeLa cell tumorigenicity (Kaelbling and Klinger 1986; Saxon et al. 1986; Srivatsan et al. 1986). Hybrid clones isolated from the fusion of retinoblastoma cells to the HeLa cells were found to be nontumorigenic on injection into *nude* mice (Pasquale et al. 1988). A tumorigenic segregant clone was isolated from one of the nontumorigenic hybrids. The nontumorigenic mass culture (HHy17P2C) and the tumorigenic revertant (HHy17P2C TuO) were subjected to the RFLP analysis with chromosome 13– and chromosome 11–specific probes, to identify the genetic locus involved in the derivation of tumorigenic revertant cells.

Various chromosome 13–specific RFLP probes, including those derived from the RB-1 gene locus, were used in the analysis (Cavenee et al. 1983; Dryja et al. 1984; Nakamura et al. 1987; T'Ang et al. 1989). Three probes—Hu10, pTHI62, and 9D11—were informative (table 2). Analysis of nontumorigenic (HHy17p2c) and tumorigenic (HHy17p2c Tuo) hybrid cell lines with these probes indicated the presence of a copy of HeLa chromosome 13 in both the hybrid cell lines (data not

shown). Thus, these results indicated that chromosome 13 was not involved in the reappearance of the tumorigenic phenotype.

Results of analysis of the different DNA samples with the short arm–specific c-Ha-ras (11p15), D11S16 (11p13), and catalase (11p12) probes (Der et al. 1982; Korneluk et al. 1984; Feder et al. 1985) are shown in figure 2. The parental cell lines Y79 and HeLa were polymorphic with the ras probe, containing 2.5-kb and 3.0-kb fragments, respectively (fig. 2*a*). The common fragment of 2.3 kb was also observed in these two cell lines. Both nontumorigenic and tumorigenic hybrid cell lines contained the Y79-specific 2.5-kb allele in addition to the 3.0-kb HeLa allele. Hence, the p15 region of chromosome 11 did not correlate with the development of the tumorigenic phenotype. Similar conclusions were drawn by hybridization analysis with Wilms (WAGR) locus probes D11S16 and catalase (Korneluk et al. 1984; Feder et al. 1985). Probe D11S16 (11p13) hybridized to heterozygous allelic fragments of 11.0 kb and 7.6/6.5/4.0 kb with the Y79 DNA and to a homozygous allele containing the 6.5/4.0/3.0-kb fragments with the HeLa cell DNA (fig. 2*b*). The common fragment of 5.4 kb was also observed in the two DNAs. Both the nontumorigenic and the tumorigenic hybrid DNAs contained the Y79-specific 11.0-kb fragment in addition to the HeLa-specific 6.0/4.0-kb allelic fragments. These results thus indicated the presence of the p13 region of normal chromosome 11 (that of Y79) in the tumorigenic hybrid cell line. The catalase probe hybridized to a homozygous fragment of 7.5 kb with the two parental DNAs (fig. 2*c*). However, a gene dosage analysis performed using c-Ha-ras as a comparative probe indicated the presence of equal copies of the catalase (11p12) region in both the nontumorigenic and tumorigenic hybrid cell lines. The results implicated the possible retention of the catalase region of Y79 chromosome 11 in the tumorigenic hybrid cell line. Thus the presence of p15-p12 region of Y79 chromosome 11 in the tumorigenic revertant cell line (fig. 2) was an indication of noninvolvement of this region of short arm in tumor suppression.

Correlation between the presence of the long arm and tumor suppression was observed by hybridization of DNA samples to the q23-specific APOAI probe (Karathanasis et al. 1983; Frossard et al. 1986). The retinoblastoma cell line (Y79) was polymorphic, containing 4.0-kb and 2.5-kb allelic fragments (fig. 3*a*), whereas the HeLa cell line ($D98^{OR}$) was homozygous, containing only the 2.5-kb allele. Both parental cell lines contained a 1.5-kb common fragment. The Y79-specific 4.0-kb allelic fragment was present in the nontumorigenic hybrid cell line but not in the tumorigenic revertant cell line. Both the hybrid cell lines contained the 2.5-kb polymorphic allele, derived from the HeLa parent. Therefore, the loss of the long arm of chromosome 11 derived from Y79 correlated with the reappearance of the tumorigenic phenotype. Additional analysis with the APOAI probe by using other restriction enzymes revealed the presence of only one copy each of the Y79 and HeLa chromosome 11's in the nontumorigenic hybrid cell line (data not shown). The tumorigenic hybrid cell line had retained the single copy of the HeLa chromosome but had lost at least a segment of the long arm of Y79 chromosome 11.

Evidence that the long-arm segment of HeLa chromosome 11 was indeed retained in the tumorigenic revertant cell line was provided by hybridization analysis with the q13-specific SS6 probe, the human homologue of the MMTV int-2 locus (Casey et al. 1986). This probe hybridized to heterozygous fragments of 8.4 kb and 5.6/2.8 kb with HeLa cell DNA (fig. 3*b*). The Y79 parent contained the homozygous allelic fragment of 8.4 kb. The nontumorigenic hybrid cell line, 17p2c, contained both the 8.4-kb and 5.6/2.8-kb alleles. Since the analysis with the APOAI probe has already indicated the presence of a single copy of Y79 chromosome 11 in this cell line (fig. 3*a*), the 8.4-kb fragment observed with the SS6 probe represented the single copy of the Y79 chromosome 11. The 5.6/2.8-kb fragment was of course derived from the HeLa parent. The tumorigenic hybrid cell line, 17p2cTuo, had retained the 5.6/2.8-kb allelic fragment of the HeLa parent but had lost the 8.4-kb allelic fragment, the long-arm segment of Y79 chromosome 11. Thus, hybridization analysis with APOAI and SS6 probes (figs. 3*a*, 3*b*) suggested involvement of the long arm of Y79 chromosome 11 in suppression of the tumorigenic phenotype.

Deletion of the long-arm segment was found to be due to loss of sequences (hemizygous deletion) rather than to mitotic recombination. While hemizygous deletion would result in retention of a single copy of the region (hemizygosity), mitotic recombination would lead to chromosome homozygosity resulting in two copies of the region. In the present analysis, copy-number determination made by rehybridization of the SS6 blot with the c-Ha-ras probe, and a chromosome 13–specific probe (probes that have already been shown to be present in equal copies in both the nontumorigenic and tumorigenic hybrid cell lines) indicated the presence of a single copy of the SS6 (q13) region in the tumorigenic cells (fig. 3*b*). Hence, reversion to the tumorigenic

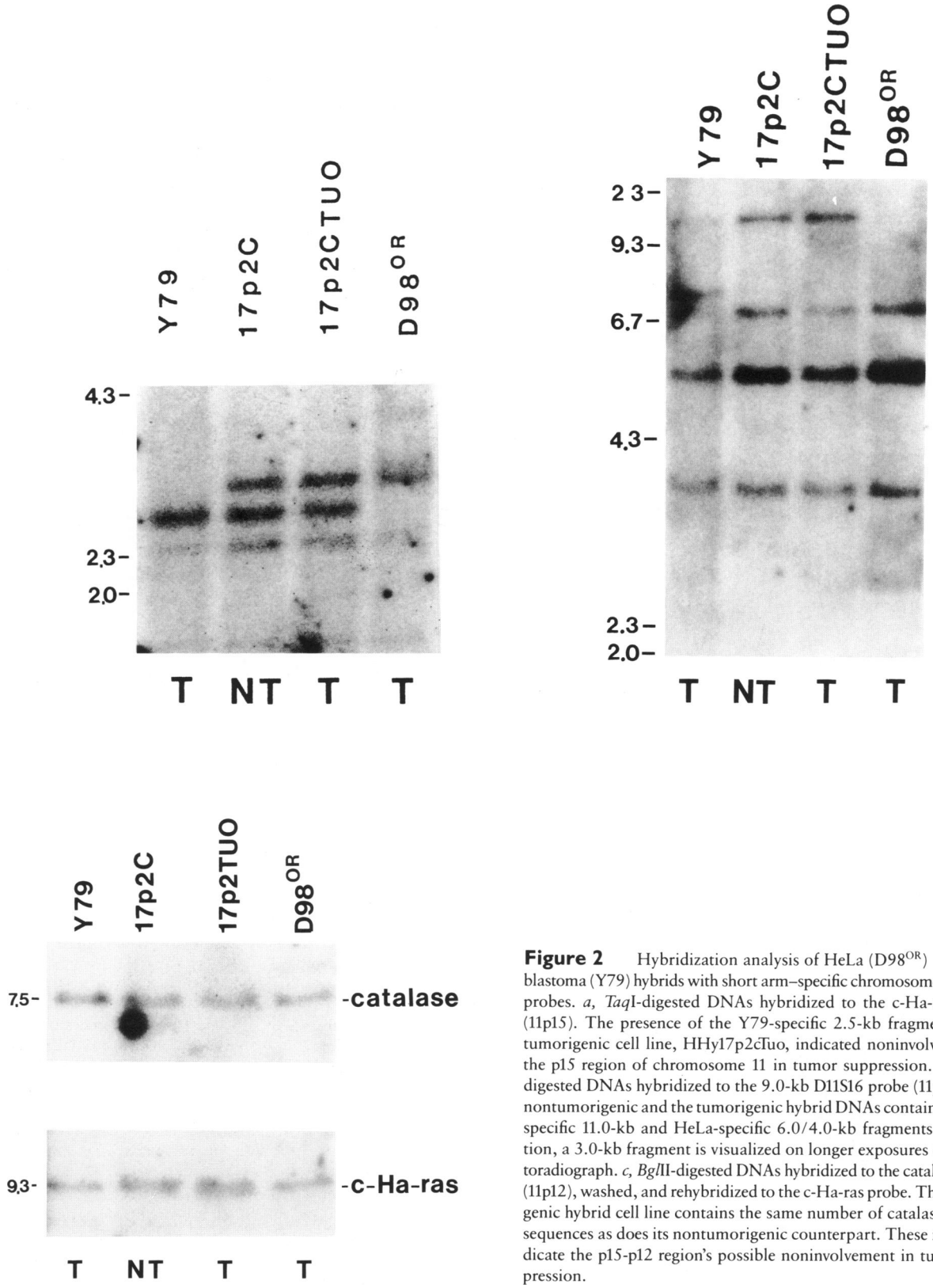


**Figure 2** Hybridization analysis of HeLa ($D98^{OR}$) × retinoblastoma (Y79) hybrids with short arm–specific chromosome 11 RFLP probes. *a, Taq*I-digested DNAs hybridized to the c-Ha-ras probe (11p15). The presence of the Y79-specific 2.5-kb fragment in the tumorigenic cell line, HHy17p2cTuo, indicated noninvolvement of the p15 region of chromosome 11 in tumor suppression. *b, Msp*I-digested DNAs hybridized to the 9.0-kb D11S16 probe (11p13). The nontumorigenic and the tumorigenic hybrid DNAs contain the Y79-specific 11.0-kb and HeLa-specific 6.0/4.0-kb fragments. In addition, a 3.0-kb fragment is visualized on longer exposures of the autoradiograph. *c, Bgl*II-digested DNAs hybridized to the catalase probe (11p12), washed, and rehybridized to the c-Ha-ras probe. The tumorigenic hybrid cell line contains the same number of catalase-specific sequences as does its nontumorigenic counterpart. These results indicate the p15-p12 region's possible noninvolvement in tumor suppression.

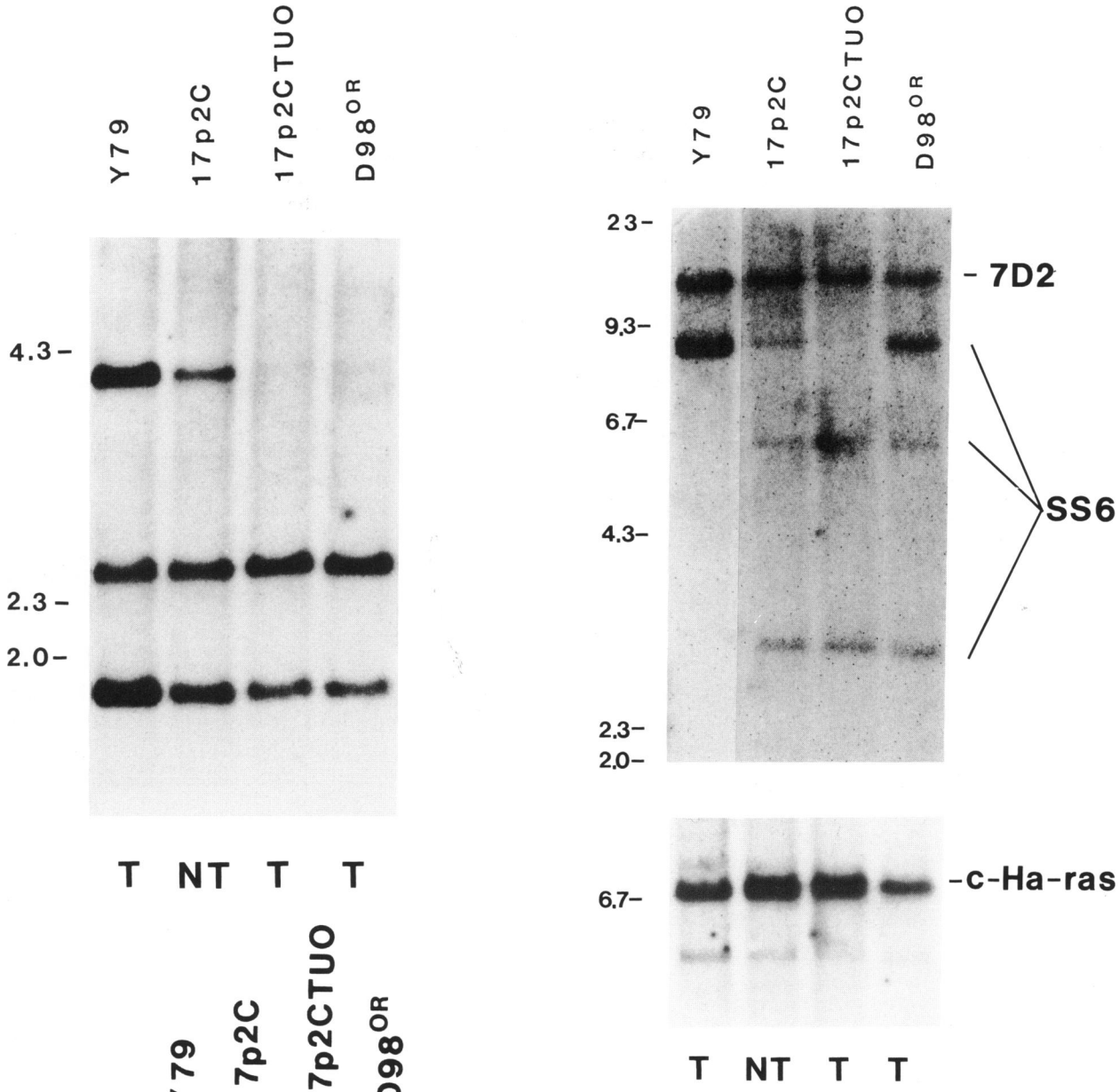


**Figure 3** RFLP analysis of HeLa × retinoblastoma hybrids with the long arm–specific chromosome 11 probes. *a, Apa*I-digested DNAs hybridized to the q23-specific chromosome 11 probe, the 1.4-kb genomic fragment of the APOAI gene. The nontumorigenic cell line contains the Y79-specific 4.0-kb fragment and the 2.5-kb fragment, the fragment common to both the Y79 and HeLa cell lines. The tumorigenic cell line has lost the Y79-specific 4.0-kb allelic fragment but has retained the 2.5-kb fragment. This demonstrates a correlation between loss of the q arm of normal chromosome 11 and reversion to the tumorigenic phenotype. *b, Bam*HI-digested genomic DNAs first hybridized to the q13-specific SS6 probe. The probe was then washed off, and the blot rehybridized to 7D2, a probe for chromosome 13 (13q14), and to c-Ha-ras, a probe for chromosome 11 (11p15), both of which have been shown to be present in equal copies in both the non-tumorigenic (17p2c) and the tumorigenic (17p2c Tuo) cell lines. The results show the presence of only a single copy of the chromosome 11 q13 region, the 5.6/2.8-kb allelic fragment of the HeLa genome, in the tumorigenic hybrid cell line. This cell line has lost the single copy of the Y79 chromosome 11, the 8.4-kb allelic fragment. *c, Msp*I-digested DNAs were hybridized to the distal probe, φ6-3 (11q22-23). Both the nontumorigenic and the tumorigenic cell lines contain a copy each of the polymorphic 9.50-kb and 3.85-kb fragments, a copy of each of the q22-23 region of the Y79 and HeLa chromosome 11s. The results thus indicate the retention of the q22-23 region of Y79 chromosome 11 in the tumorigenic cell line that has lost the q13-23 (SS6 and APOA I probes) region.

phenotype was observed to be due to hemizygous loss of sequences from the long arm of Y79 chromosome 11.

Presence of the deletion was confirmed by the identification of the distal breakpoint region by using other long arm–specific probes. Three different probes—P2-7-1D6, $\phi$2-14, and $\phi$6.3—localized to the q22-23 region of the chromosome (Maslen et al. 1988) were informative. The results indicated the loss of Y79 chromosome–specific P2-7-1D6 and $\phi$2-14 sequences in the tumorigenic cell line (data not shown). However, sequences homologous to the $\phi$6-3 probe were not deleted (table 2, fig. 3*c*). Probe $\phi$6-3 hybridized to heterozygous fragments of 9.5 kb and 3.85 kb with the DNA from both the Y79 and HeLa cell lines. A homozygous fragment of 3.0 kb and a common fragment of 2.5 kb were also observed in the two DNAs. Analysis of the hybrid cell lines revealed the presence of the polymorphic allelic fragments of 9.5 kb and 3.85 kb in both the nontumorigenic and tumorigenic hybrid cell lines. Since it has already been shown that the tumorigenic cell line had retained the single copy of the HeLa chromosome 11 (fig. 3*b*), the result with the $\phi$6-3 probe represented the presence of an additional copy of chromosome 11 (polymorphic chromosome), that of the Y79 parent in this hybrid cell line. Also, these results indicated that the distal breakpoint region of the deletion would be near the q22-23 region. The proximal breakpoint region of the deletion was not precisely localized with the available probes. However, cytogenetic studies carried out at the 450-band level revealed the presence of two normal appearing chromosome 11's in both the nontumorigenic and tumorigenic hybrid cell lines (A. Banerjee, personal communication). Hence, the reversion to the tumorigenic phenotype seems to be due to the selective loss of sequences in the q13-23 region of the Y79 chromosome 11.

### *RFLP Analysis of the ESH15 × t(X;11) Microcell Hybrids*

Microcell hybrids were derived by transferring a t(X;11) chromosome (from the cell line 110.1) into a 6TG(6-thioguanine)—resistant, tumorigenic hybrid cell, ESH15(TI), which in turn was derived by a fusion of a normal fibroblast (75-55C) to HeLa cells (Der and Stanbridge 1978). The translocation breakpoints of the t(X;11) chromosome are 11pter>11q23::Xq26>Xqter. Five HPRT$^+$ clones were isolated from the ESH15 × t(X;11) fusions and tested for tumorigenicity in *nude* mice. Since HPRT$^+$ clones were derived from a number of microcell fusion experiments, the hybrid cell lines represented individual clonal isolates. While three of the clones (clones 110.1/ESH, 15.1,110.1/ESH 15.5, and 110.1/ESH 15.6) were found to be nontumorigenic, the other two clones (110.1/ESH 15.3 and 110.1/ESH 15.4) were tumorigenic like the parental ESH15 cells (Saxon et al. 1986; P. J. Saxon, personal communication). The DNA from all of these cell lines was tested for the presence of the t(X;11) chromosome in order to determine the correlation between tumorigenicity and the presence or absence of this chromosome.

Hybridization analysis of genomic DNAs with short arm–specific chromosome 11 probes is shown in figure 4. The c-Ha-ras probe (11p15) was polymorphic for *Taq*I-digested parental DNAs (fig. 4*a*). DNA isolated from the cell line 110.1 [containing the t(X;11) chromosome] contained the polymorphic 2.5-kb allelic fragment, and DNA isolated from the tumorigenic recipient cell line ESH15 contained the normal fibroblast (75-55c)-specific 3.5-kb and HeLa(D98/AH-2)-specific 3.0-kb allelic fragments. The 2.3-kb fragment represented the common fragment derived from both the parental DNAs. All the hybrids except 110.1/ESH15.4 contained the 3.5-kb, 3.0-kb, and 2.3-kb allelic fragments of the ESH15 parent. Cell line 110.1/ESH15.4 contained only the 3.0-kb and 2.3-kb alleles. This cell line did not contain the 3.5-kb allelic fragment. Two of the nontumorigenic hybrids (.1 and .5) and the tumorigenic hybrids (.3 and .4) contained the 2.5-kb fragment of the t(X;11) chromosome. However, the third nontumorigenic hybrid clone, 110.1/ESH15.6, did not contain the t(X;11)-specific 2.5-kb fragment. Therefore, the p15 region of chromosome 11 did not correlate with the suppression of tumorigenicity.

Further evidence for noninvolvement of the short arm in tumor suppression was provided by hybridization analysis with a probe localized to the pericentromeric region, D11S149 (Nakamura et al. 1989). This probe hybridized to a 5.2-kb fragment with the t(X;11) chromosomal DNA and to a fragment of 3.2 kb with the ESH15 cellular DNA (fig. 4*b*). Hybridization to a mouse DNA–specific fragment of 2.2 kb was also observed with the 110.1 cell line. The t(X;11) chromosome– specific fragment of 5.2 kb was present in the nontumorigenic cell line 110.1/ESH 15.5 and in the tumorigenic cell line 110.1/ESH15.3. This fragment was absent in the nontumorigenic cell line 110.1/ESH 15.6. Also, there was no change in the number of copies (either loss or duplication) of the ESH15-specific alleles in the three hybrid cell lines (when the number of copies was calculated by rehybridization to the p15-specific ras probe). Thus, the analysis of the ESH15× t(X;11) microcell

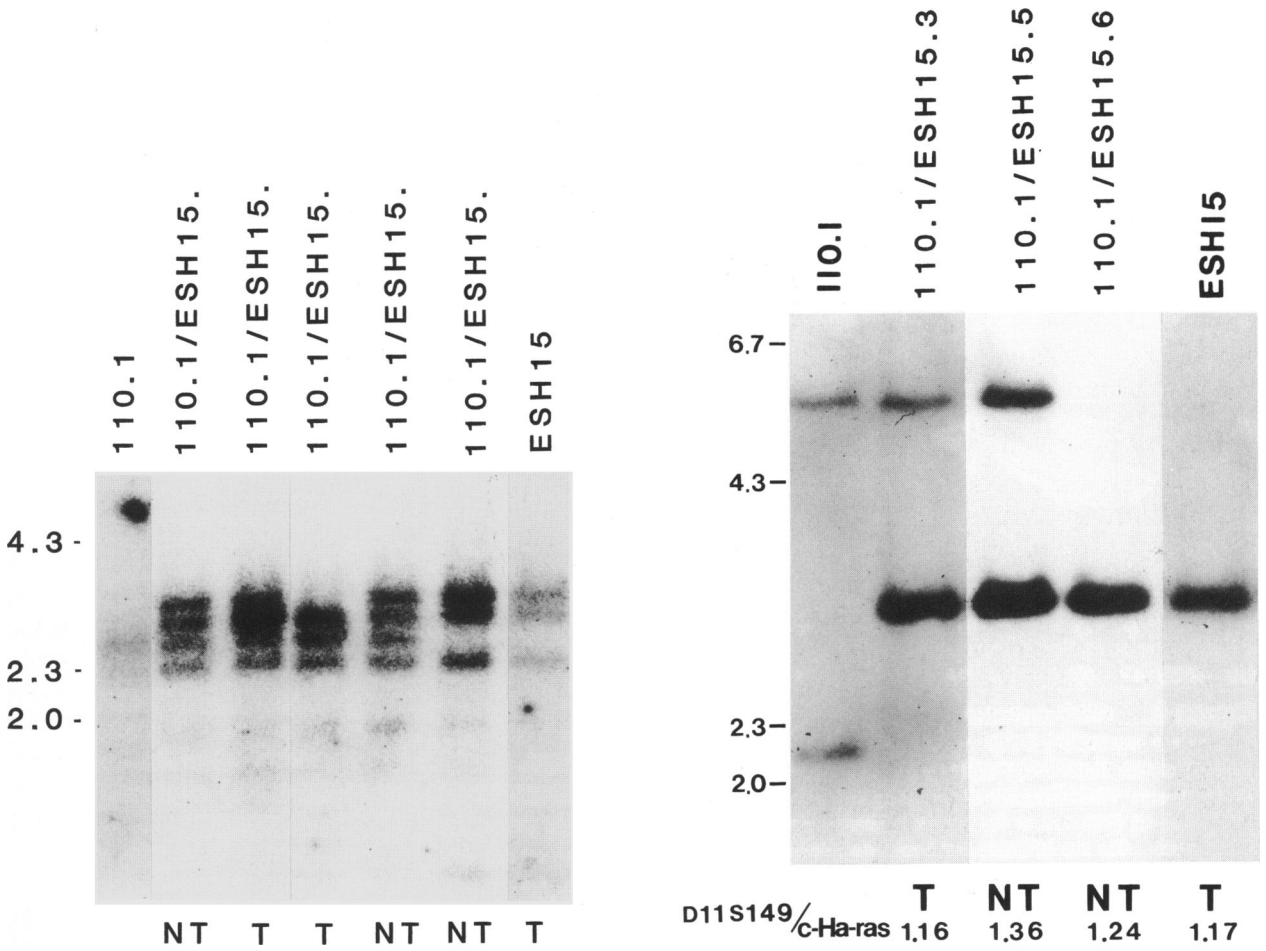


**Figure 4** Hybridization analysis of the ESH15 × 110.1 microcell hybrids with short arm–specific chromosome 11 probes. *a*, *Taq*I-digested DNAs hybridized to the c-Ha-ras probe (11p15). The t(x;11) chromosome (contained in cell line 110.1)–specific 2.5-kb allelic fragment is observed in two nontumorigenic cell lines (clones .1 and .5) and in the two tumorigenic cell lines (clones .3 and .4). This fragment is absent in the nontumorigenic cell line 110.1/ESH15.6. *b*, *Pvu*II-digested DNAs hybridized to the pericentrometric probe, D11S149 (11p11-13). The t(x;11)-specific 5.2-kb allelic fragment is present in the nontumorigenic hybrid cell line 110.1/ESH 15.5 and in the tumorigenic hybrid cell line 110.1/ESH15.3 but is absent in the nontumorigenic hybrid cell line 110.1/ESH15.6. These results suggest noninvolvement of entire short arm of chromosome 11 in suppression of the tumorigenic phenotype. Numbers at the bottom represent the ratio of the copies of the ESH15-specific D11S149 sequences vis-à-vis the ESH15-specific c-Ha-ras sequences. The analysis shows the presence of equal copies of the ESH15-specific chromosome 11 short arm in all of the hybrid cell lines.

hybrids with the ras and D11S149 probes (figs. 4*a*, 4*b*) indicated noninvolvement of the entire short arm of chromosome 11 in tumor suppression.

RFLP analysis with long arm–specific probes showed a correlation between the presence of the long-arm segment and tumor suppression. Figure 5 shows the analysis of the DNAs with the long arm(11q13)–specific SS6 probe (Casey et al. 1986). HeLa cells (D98/AH-2), from which ESH 15 was derived, were polymorphic for this probe, giving rise to 8.4-kb and 5.6/2.8-kb fragments. Hybridization of the 5.6-kb fragment was weaker in comparison to the 2.8-kb fragment, as reported by Casey et al. (1986). The donor cell line (110.1) contained a single copy of the 8.4-kb fragment, and the recipient cell line (ESH 15) contained two copies of the 8.4-kb fragment and one copy of the 5.6/2.8-kb fragments (according to calculations on the basis of the densitometric tracings).

The allelic composition of the long arm of chromosome 11 in the nontumorigenic hybrid, 110.1/ESH15.6 (not containing the short arm), was compared with those of the nontumorigenic hybrid cell lines 110.1/ESH15.1

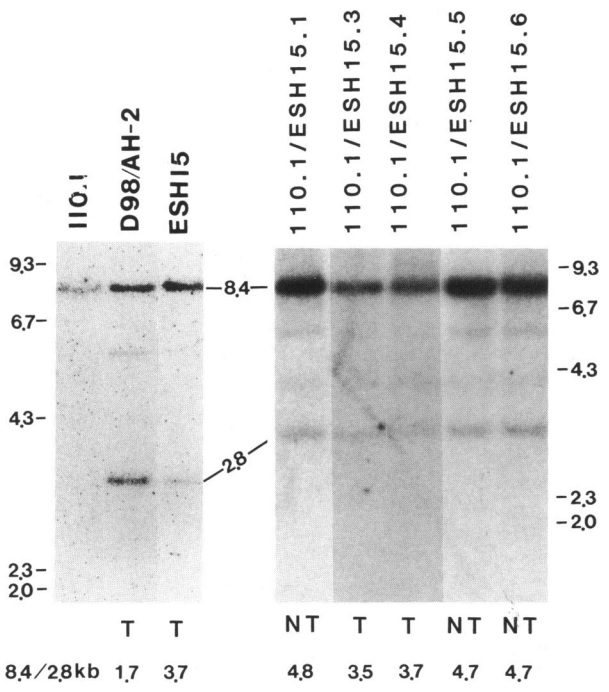


**Figure 5** RFLP analysis of genomic DNAs isolated from ESH 15 × 110.1 microcell hybrids with the long arm–specific chromosome 11 probe. *Bam*HI-digested DNAs were hybridized to the SS6 probe (11q13). Hybridization of the probe to the 5.6-kb fragment is less intense than it is to the 2.8-kb fragment reported by Casey et al. (1986). For the different cell lines, the intensity ratio of the 8.4-kb fragment vis-à-vis the 2.8-kb fragment is presented at the bottom of the figure. A ratio of 1.7 represents one copy of the 8.4-kb allele. The nontumorigenic cell line 110.1/ESH15.6, like the other nontumorigenic cell lines, contains an additional copy of the long-arm segment of chromosome 11 [possibly derived from the t(x;11) chromosome]. On the other hand, the tumorigenic cell lines 110.1/ESH15.3 and 110.1/ESH/15.4 contain the same number of copies of the 8.4-kb fragment as does the parental cell line, ESH15. These two cell lines seem to have lost the q13 region of the t(x;11) chromosome. Thus, there is a correlation between the presence of the long arm of normal chromosome 11 and the suppression of the tumorigenic phenotype.

and 110.1/ESH15.5 and with that of the tumorigenic hybrid cell line 110.1/ESH15.3 (all three cell lines contain the short arm). All the cell lines contained the 8.4-kb and 5.6/2.8-kb alleles (fig. 5). However, a comparison of the intensity of the 8.4-kb fragment indicated the presence of three copies of the 8.4-kb allele in the three nontumorigenic hybrid cell lines (clones .1, .5, and .6). The tumorigenic hybrid cell line 110.1/ESH15.3 did not contain any 8.4-kb allele other than those (two copies) already present in the ESH15 cells. The additional copy of the 8.4-kb allelic chromosome observed in the nontumorigenic cell lines (clones .1, .5, and .6) was most likely derived from the t(x;11) chromosome, because all of these hybrid clones were selected in HAT medium. Besides, it has already been shown that the nontumorigenic hybrid cell line 110.1/ESH15.6 did not contain the short arm and that there was no duplication of the ESH15-specific sequences (figs. 4*a*, 4*b*). Hence, three copies of the 8.4-kb allelic chromosome in this cell line would represent the addition of a copy of the t(X;11) chromosome to the two copies of the ESH15 parental cell line.

The tumorigenic hybrid cell line 110.1/ESH 15.3 contained only two copies of the 8.4-kb allelic fragment, as did the ESH15 cells (fig. 5). This cell line had lost the additional copy of the 8.4-kb allele that is observed in all of the nontumorigenic hybrid cell lines. The parental origin of the lost chromosomal sequences could be either the t(X;11) chromosome or the tumor-related HeLa chromosome. Hybridization analysis with the ras probe (table 2) indicated the presence of only one HeLa-specific chromosome 11 in the parental ESH15 hybrid cell line (data not shown). This single copy of the HeLa chromosome 11 was retained in this hybrid cell line when the latter was analyzed with the SS6 probe (fig. 5). Hence, it seems that the reversion to the tumorigenic phenotype was due to the loss of sequences from the long arm of a fibroblast chromosome 11, most likely that of the t(X;11) chromosome. Similarly, the loss of the q13 region of t(X;11) chromosome was also observed in the tumorigenic hybrid cell line 110.1/ESH15.4 (fig. 5). Thus, when use was made of the hybrid system (ESH 15 × 110.1 hybrids) in which chromosome 11 was clearly shown to be involved in the suppression of the tumorigenic phenotype, these results strongly suggested the presence of the tumor-suppressor gene(s) in the long arm (q arm) of chromosome 11.

Analysis of the hybrid cell lines with the q23-specific APOAI and D11S29 probes (Frossard et al. 1986; Warnich et al. 1986) indicated noninvolvement of this region in tumor suppression (data not shown). The sequences homologous to these probe regions were absent in the t(X;11) chromosome, the chromosome shown to be responsible for the suppression of the tumorigenic phenotype in the ESH15 × t(X;11) hybrids (Saxon et al. 1986). This observation in combination with the result obtained with the D11S149 probe (fig. 4*b*) localizes the tumor-suppressor gene(s) to the q13-23 region.

## Discussion

Previous cytogenetic and molecular genetic studies on nontumorigenic and tumorigenic hybrids derived

from fibroblast × HeLa cell fusions have indicated the presence of a tumor-suppressor gene(s) on the normal chromosome 11 (Stanbridge et al. 1981; Klinger 1982; Kaelbling and Klinger 1986; Srivatsan et al. 1986). The role of chromosome 11 in tumor suppression was confirmed by suppression of the tumorigenic phenotype on the introduction of the chromosome into the tumorigenic HeLa cells and HeLa cell–derived hybrid cell lines (Saxon et al. 1986). In the present study, HeLa × normal chromosome 11 hybrids were analyzed in detail by using the chromosome 11–specific RFLP probes to determine the location of this tumor-suppressor gene(s).

Analysis with short arm–specific probes indicated noninvolvement of the short-arm segment in tumor suppression. Four different probes—insulin, c-Ha-ras, D11S16, and D11S149—were informative. While tumorigenic hybrids of the HeLa × fibroblast fusions contained at least the p15 region (c-Ha-ras- and insulin-specific sequences), tumorigenic hybrid cell lines derived from the other fusions contained sequences of other short-arm regions as well. RFLP analysis using the C-Ha-ras, D11S16, and D11S149 probes indicated the presence of both the p15-p12 region of normal chromosome 11 in the tumorigenic hybrid cell line derived from the HeLa × retinoblastoma fusion (fig. 2) and the entire short-arm segment in the tumorigenic microcell hybrids (fig. 4). Also, one of the nontumorigenic microcell hybrids (110-1/ESH15.6) did not contain the short arm–specific sequences of normal chromosome 11. Thus, the short arm of normal chromosome 11 did not correlate with the suppression of tumorigenic phenotype in the different hybrid cell systems.

A number of long arm–specific sequences were useful in localizing the tumor-suppressor gene(s) to this segment of the chromosome. Heterozygous allelic chromosome 11s were observed for both Y79 and HeLa cell lines by different probes using various restriction enzymes (table 2). However, RFLP analysis indicated the presence of only one copy each of Y79 and HeLa chromosome 11's in the nontumorigenic hybrid cell line 17p2c. The tumorigenic cell line 17p2cTuo had retained the HeLa chromosome 11 but had lost the sequences of the q13-23 region of Y79 chromosome 11 (figs. 3*a*, 3*b*). Thus, the RFLP analysis implicated the long arm in the suppression of the tumorigenic phenotype. Additional evidence for the involvement of the long arm in tumor suppression was provided by the ESH15 × t(X;11) microcell hybrids. Analysis with the q13-specific SS6 probe indicated the presence of the t(X;11) chromosomal region (normal chromosome 11) in the nontumorigenic hybrid cell line 110.1/ESH15.6, which had lost the short arm of this chromosome (fig. 5). The tumorigenic hybrid cell lines, on the other hand, had lost the SS6 probe region of the normal chromosome 11 while retaining the short-arm segment. Thus, the different hybrid cell lines showed a perfect correlation between the presence of the long arm of normal chromosome 11 and the suppression of the tumorigenic phenotype.

Detailed molecular genetic and cytogenetic studies have implicated the short arm of chromosome 11 in the development of bladder carcinomas, breast carcinomas, rhabdomyosarcoma, Beckwith-Wiedemann syndrome, and Wilms-aniridia (Koufos et al. 1984, 1985; Orkin et al. 1984; Reeve et al. 1984; Fearon et al. 1985; Ali et al. 1987; Scrable et al. 1987). While the precise location of genes implicated in bladder carcinomas and breast carcinomas is not yet known, molecular genetic studies have localized the genes involved in the development of rhabdomyosarcoma and Beckwith-Wiedemann syndrome to p15 and has localized the gene involved in the development of Wilms-aniridia syndrome to the p13 regions of the chromosome. The localization of the Wilms tumor-suppressor gene(s) to chromosome 11 was further confirmed by the suppression of the tumorigenic phenotype on the introduction of a normal chromosome 11 into a Wilms tumor cell line (Weissman et al. 1987). Since the HeLa tumor-suppressor gene(s) seems to be localized to the long arm, this gene(s) is probably unrelated to the genes implicated in the various tumors mentioned above. In one study at least, it has been shown that the HeLa tumor-suppressor gene(s) is possibly different from the Wilms tumor-suppressor gene (Pasquale et al. 1988).

Abnormalities in the long arm of chromosome 11 have also been observed in other malignancies, including multiple endocrine neoplasia type 1, insulinomas (11q13 locus), and tumors associated with Ataxia-telangiectasia (11q22-23), Ewing sarcoma (11q23), and acute nonlymphoblastic leukemias (Ture-Cavel et al. 1984; Gatti et al. 1988; Larrson et al. 1988; Stark et al. 1989). It remains to be seen whether the HeLa tumor-suppressor gene(s) is related to any of these genes. It is also interesting to note that the two regions q13 and q23, which are implicated in various tumors, are locations of constitutional fragile sites (Yunis 1986).

It will be important to determine the frequency of 11q sequences lost in cervical carcinoma specimens from patients, since the HeLa cell line was derived from a cervical carcinoma. In addition, a tumor-suppression and molecular genetic study involving the transfer of chromosome 11 into several other cervical carcinoma

cell lines will be necessary to determine the generality of our findings. Studies performed so far indicate a significant role for chromosome 11 in the development of cervical carcinomas.

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