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Deletion and Translocation of Chromosome 11q13 Sequences in Cervical Carcinoma Cell Lines

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Summary

Molecular genetic studies on HeLa cell–derived nontumorigenic and tumorigenic hybrids have previously localized the HeLa cell tumor-suppressor gene to the long arm of chromosome 11. Extensive molecular and cytogenetic studies on HeLa cells have shown chromosome band 11q13 to be rearranged in this cell line. To determine whether q13 rearrangement is a nonrandom event in cervical carcinomas, six different human papilloma virus (HPV)–positive (HeLa, SiHa, Caski, C4-I, Me180, and Ms751) and two different HPV-negative (C33A and HT3) cell lines were studied. Long-range restriction mapping using a number of q13-specific probes showed molecular rearrangements within 75 kb of INT2 probe in three HPV-positive cell lines (HeLa, SiHa, and Caski) and in an HPV-negative cell line (HT3). FISH using an INT2 YAC identified a breakpoint within the sequences spanned by this YAC in two of the cell lines, HeLa and Caski. INT2 cosmid derived from this YAC showed deletion of cosmid sequences in two other cell lines, SiHa and C33A. These two cell lines, however, retained cosmid sequences of Cyclin D1, a probe localized 100 kb proximal to INT2. Deletions being the hallmark of a tumor-suppressor gene, we conclude that the 100-kb interval between the two cosmids might contain sequences of the cervical carcinoma tumor-suppressor gene.

Introduction

Cervical cancer is one of the major form of human cancers in developing countries. Epidemiological studies have shown a strong correlation between the development of these tumors and the presence of human papilloma viruses (HPVs) (Pfister 1987). However, it is well documented that viral infection is not sufficient to induce cancer (Pater et al. 1986; Wilczynski et al. 1988). Other events, such as activation of oncogenes and inactivation of tumor-suppressor genes, may be required. Oncogene activation has not been implicated as an important factor in cervical cancer development, with the exception of few reports that have indicated amplification of cMyc and HRAS1 in some advanced-stage tumors (Ocadiz et al. 1987; Riou et al. 1988). Thus, tumor-suppressor genes may play a role in the development of these tumors.

Loss-of-heterozygosity (LOH) studies have shown deletion of chromosomes 3p and 11q in a significant fraction of cervical carcinomas (Yokota et al. 1989; Srivatsan et al. 1991). LOH studies using microsatellite repeat polymorphic probes have also identified interstitial deletion of 11q13 in some of these tumors (Hampton et al. 1994). Functional studies involving the transfer of chromosome 11 into two different cervical carcinoma cell lines, HeLa and SiHa, confirmed the existence of a tumor-suppressor gene on chromosome 11 (Saxon et al. 1986; Koi et al. 1989). An extension of these studies on HeLa cell–derived nontumorigenic and tumorigenic hybrids localized this gene to the long arm of chromosome 11 (Misra and Srivatsan 1989). Detailed RFLP and molecular cytogenetic analyses of HeLa cells indicated 11q13 to be rearranged in these cells (Jesudasan et al. 1994). It is likely that this rearrangement could have resulted in the inactivation of a tumor-suppressor gene localized to chromosome 11q13. Therefore, to examine whether 11q13 rearrangement is a nonrandom event in cervical cancer, we analyzed seven additional cervical cell lines (five HPV positive and two HPV negative), using 11q13-specific probes.

Material and Methods

Cell Lines

Blood lymphocytes were obtained from different individuals. Fibroblast cell lines GM0077, GM1604, and IMR-90 were grown in Dulbecco's modified Eagle's medium

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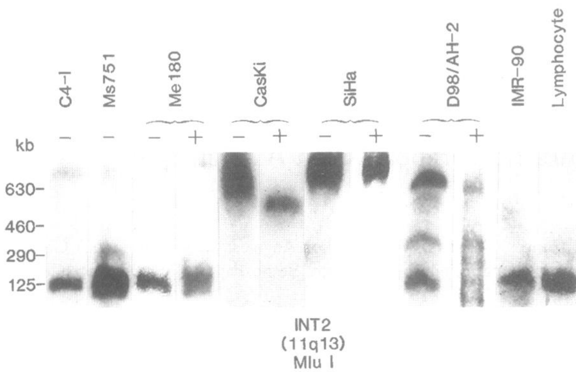

Figure 1 PFGE analysis of HPV-positive cervical cell line DNAs. *Mlu*I-digested DNA samples embedded in agarose plugs were separated on 0.9% agarose gels and were transferred onto Nytran membranes. Hybridization to 11q13-specific INT2 locus probe SS6 shows Caski and SiHa cells to contain a single 600-kb fragment. The two cell lines do not contain the 125-kb fragment observed in normal lymphocytes and in fibroblast IMR-90. While HeLa cells contain fragments of 550 kb, 350 kb, and 125 kb, the other three cell lines—C4-I, Me180, and Ms751—contain only the 125-kb fragment. On treatment with 5-aza-CR, a mobility shift is observed in Caski cell–specific and HeLa cell–specific fragments. However, the drug treatment does not result in conversion of larger-size fragments to normal size fragments in these two cell lines. The 600-kb fragment in SiHa cells remains unaltered after the drug treatment. The 350-kb and 125-kb fragments in drug-treated HeLa cells possibly represent a fraction of cells that could escape demethylation. A minus sign (−) denotes analysis without 5-aza-CR treatment, and a plus sign (+) denotes analysis with 5-aza-CR treatment. *Saccharomyces cerevisiae* chromosomes were used as molecular-weight markers.

(DMEM) containing 10% FCS. Cervical cell lines HeLa, C4-I, and Me180 contain HPV18 sequences, and cell lines Caski, SiHa, and Ms751 contain HPV16 sequences. HT3 and C33A are HPV-negative cell lines. HeLa is an adenocarcinoma, and the others are squamous-cell carcinomas. All cervical carcinoma cell lines were grown in minimal essential medium containing 10% FCS.

YAC and Cosmid Probes

A YAC containing INT2 sequences was isolated by screening the CEPH genomic YAC library constructed in the pYAC-4 vector system. Screening was carried out with a modified pool strategy using PCR (Green and Olson 1990; Nelson et al. 1991). INT2-specific primers 5′ GCTTGTTGTCTGTCGGAT 3′ and 5′ CCAGGCTCCAGGTCACTCG 3′ were used for screening the YAC library. A positive signal was observed in the primary pool of the CEPH library. By PCR assay performed on subpools and sub-subpools, a single INT2 YAC clone was isolated. This YAC also contained Cyclin D1 sequences, a probe localized 100 kb proximal to INT2. The size of the YAC was determined to be 275 kb by pulsed-field gel electrophoresis (PFGE) analysis. This YAC was confirmed to be nonchimeric by hybridization to metaphase chromosomes from normal cells.

Ten different cosmids were isolated by screening a Los Alamos chromosome 11 cosmid library, with the INT2 YAC used as a probe. Cosmid 129F8 localized to the proximal end of the YAC contained Cyclin D1 genomic sequences. Cosmid 31A9 contained INT2 genomic sequences. Long-range restriction mapping showed Cyclin D1 and INT2 cosmids to be localized within 100 kb.

Treatment with 5-aza-cytidine (5-aza-CR)

Cells were treated with a 5-μM concentration of 5-aza-CR for 24 h, as proposed elsewhere (Jones and Taylor 1980; Rosl et al. 1988), and were allowed to grow for 48 h in DMEM containing 10% FCS. Cells were then harvested for PFGE analysis.

Polymorphic Probes and Blot Hybridization Analysis

Various chromosome 11 probes used have all been described elsewhere (Misra and Srivatsan 1989). Digestion of genomic DNAs with restriction enzymes, separation of fragments on agarose gels, and Southern hybridization of fragments immobilized on Nytran membranes with ^{32}P-labeled probes were performed as described elsewhere (Saxon et al. 1986).

PFGE

The PFGE analysis was performed as described elsewhere (Higgins et al. 1989). In brief, DNA in agarose plugs was prepared from cells suspended in PBS (2×10^7 cells/ml). Plugs were incubated in 0.5 M EDTA, 1% sarkosyl, pH 8.0, containing proteinase K (2 μg/ml) at 50°C for 48 h, were washed twice in Tris-EDTA buffer (10 mm Tris-HCl, pH 7.5, 1 mM EDTA), and were treated twice with freshly prepared 40 μg phenylmethylsulfonylfluoride/ml at 50°C for 2 h. Plugs then were either stored in 0.5 M EDTA or subjected to restriction-enzyme analysis. Digestion with rare-cutting restriction enzymes was performed in buffer conditions specified by the manufacturer, at 37°C for *Mlu*I, *Not*I, and *Sac*II and at 50°C for *Bss*HII, overnight. High-

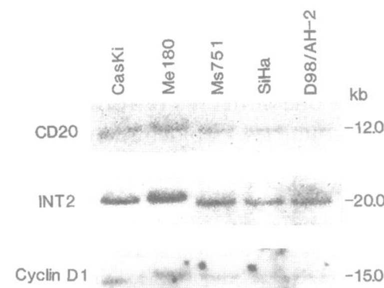

Figure 2 Copy-number analysis of cervical cell lines. *Hin*dIII-digested DNA samples (5 μg) separated on 0.8% agarose gels were hybridized sequentially to INT2, CD20, and Cyclin D1. Results show similar copies of the three probe regions in all five cell lines, indicating absence of INT2 amplification in these cell lines.

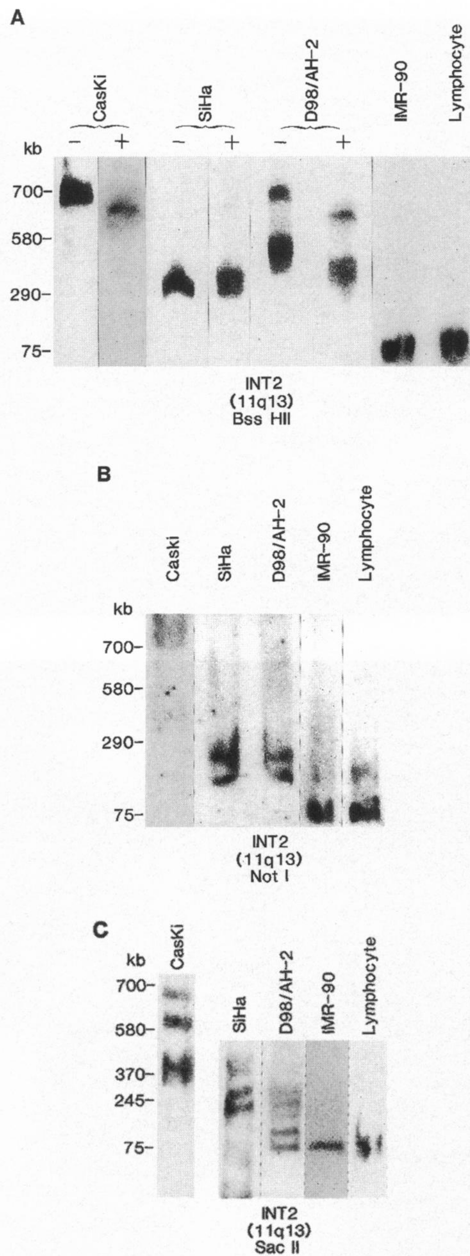

Figure 3 Hybridization of *Bss*HII-, *Not*I-, and *Sac*II-digested samples to INT2 probe. PFGE analysis after digestion with *Bss*HII (*A*), *Not*I (*B*), and *Sac*II (*C*) shows altered INT2 fragments in all three cervical cell lines. A normal appearing *Sac*II band was also observed in HeLa cells. However, normal size bands were not seen in Caski or SiHa cells. Mobility shift persisted after treatment with 5-aza-CR-2.5 μM. Thus, these results and the results obtained with *Mlu*I digestion (fig. 1) strongly suggest molecular alteration of INT2, the 11q13 region in HeLa, SiHa, and Caski cell lines.

molecular-weight DNA fragments embedded in agarose plugs were separated on 0.9% agarose gels by using the Bio-Rad CHEF DR II PFGE system. Electrophoresis was performed in 0.5 × TBE (45 mM Tris-borate, pH 8.25, 1 mM EDTA), pH 8.25, buffer with a constant voltage of 12 V/cm. Pulses of 60 s and 90 s each for 12 h were used for the separation of 50-kb–1.0-Mb fragments. Gels were stained in 1 μg ethidium bromide/ml for 30 min and were destained in water for 20 min before photography. DNA fragments were nicked using a UV cross-linker (Bio-Rad GS gene linker) and were denatured in 0.5 M NaOH, 1.5 NaCl buffer twice for 30 min. DNA fragments were transferred onto Nytran membranes by using the alkaline transfer buffer and were immobilized at 80°C under vacuum for 2 h. Southern blot hybridizations were performed at 55°C, as described elsewhere (Saxon et al. 1986), and the blots were exposed to x-ray films.

Cytogenetics

Cytogenetic analyses of the cervical carcinoma cell lines were performed by established methods. Twenty metaphases per cell line were analyzed. The number of metaphase chromosomes obtainable from Me180 and HT3 cells was insufficient for cytogenetic studies. FISH analyses using the chromosome 11–specific centromeric (D11Z1; Oncor) and the chromosome 11–painting (P5202-BIO; Oncor) probes were performed per manufacturer's instructions. Dual color hybridization using the centromeric (digoxigenin) and painting (biotin) probes was performed as described elsewhere (Jesudasan et al. 1994). In brief, the painting probe (200 ng/slide) was denatured at 70°C for 10 min and preincubated with human Cot1 DNA (2 μg/slide) at 37°C for 2 h. Centromeric (alpha-satellite) probe was denatured in hybrisol VII (Oncor) at 70°C for 5 min and was added to the preincubated painting probe just before being added onto the slide. Hybridizations were performed at

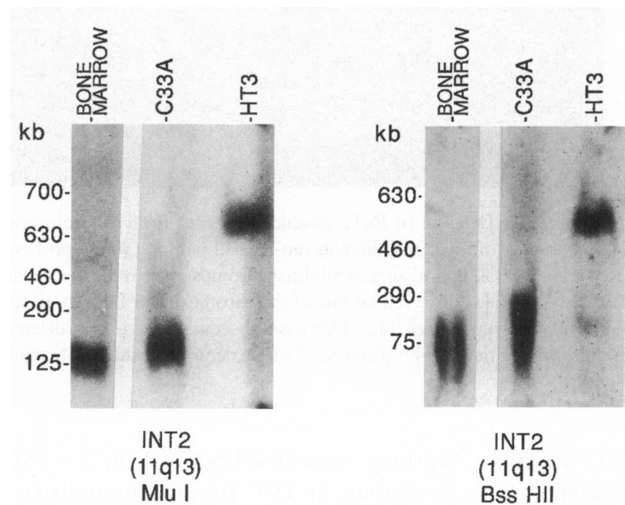

Figure 4 PFGE analysis of HPV-negative cell-line DNAs. Hybridization of the digested DNAs to the INT2 probe shows mobility shift for the restriction fragments from HT3 cell DNA. C33A cells contain normal appearing fragments as seen in normal bone marrow.

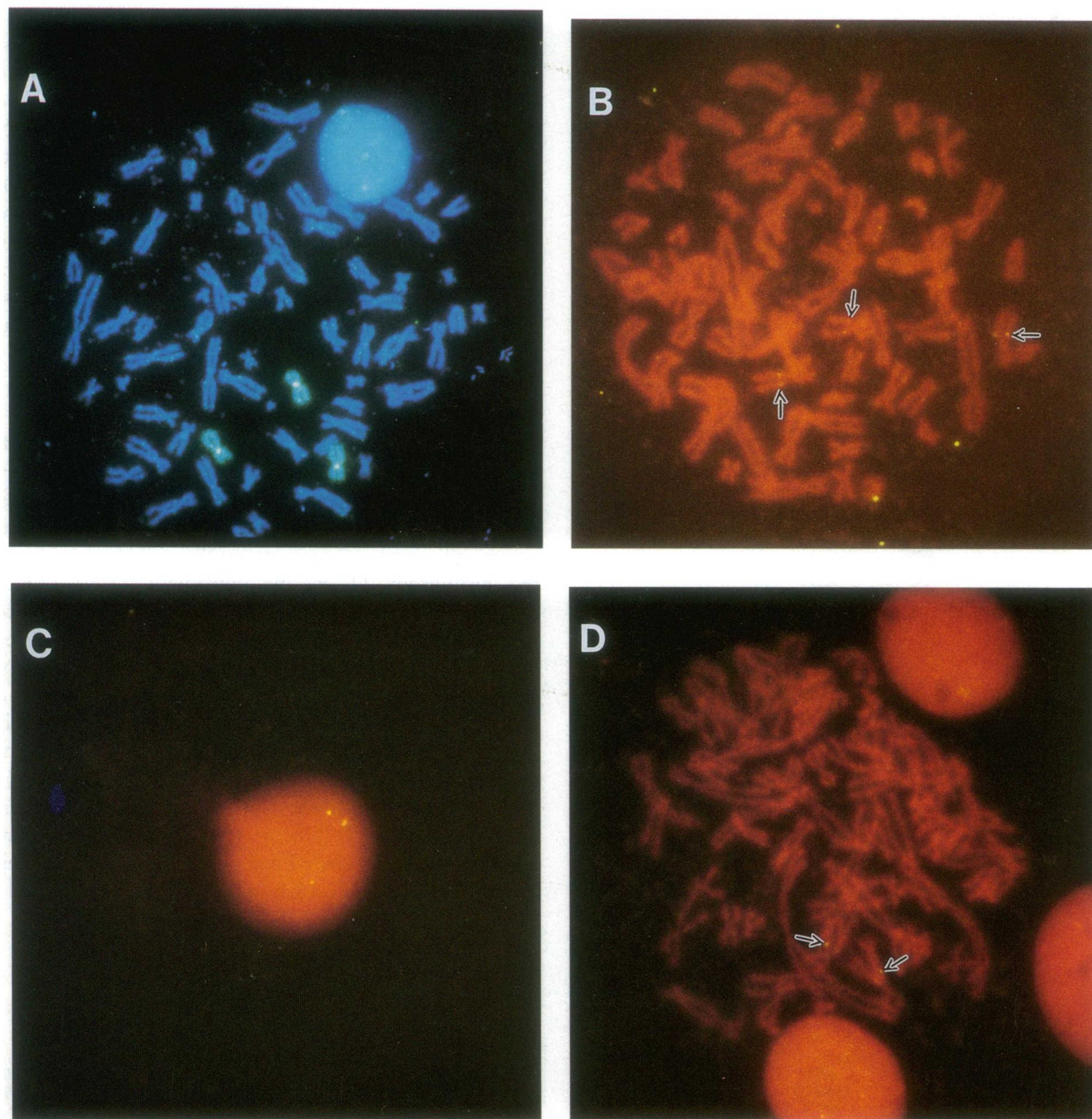

Figure 5 Deletion of INT2 cosmid sequences from a chromosome 11 of SiHa cells. *A,* Hybridization of metaphase spreads to biotin-labeled 11 centromeric (*orange*) and digoxigenin-labeled painting (*blue*) probes, showing the presence of three normal appearing chromosomes 11. *B,* Cyclin D1 cosmid, 129F8, hybridizing to all three chromosomes in the metaphase spreads. *C,* Interphase nucleus showing cosmid signal on three chromosomes 11. A weak signal is observed on one of the chromosomes 11, in both the metaphase and the interphase. *D,* INT2 cosmid 31A9 showing hybridization to only two chromosomes 11. 31A9 cosmid sequences are thus deleted from the third chromosome 11, indicating a breakpoint between these two cosmids, within the 100-kb distance. Arrows denote hybridization signals on the chromosomes.

37°C overnight. Washing was carried out once in 2 × SSC containing 50% formamide at 45°C for 20 min and twice in 2 × SSC at 37°C for 5 min. Hybridization signals were detected by using the detection system supplied by the manufacturer. Photographs were taken by using a Nikon fluorescent microscope and Ektachrome 100 film.

FISH Using YAC and Cosmid Probes

Labeling of the YAC and cosmid probes and hybridization of the probes to metaphase spreads were performed as described elsewhere (Jesudasan et al. 1994). In brief, probe was denatured at 7O°C for 10 min, annealed with human cot1 DNA (final concentration 2 μg/200 ng of the

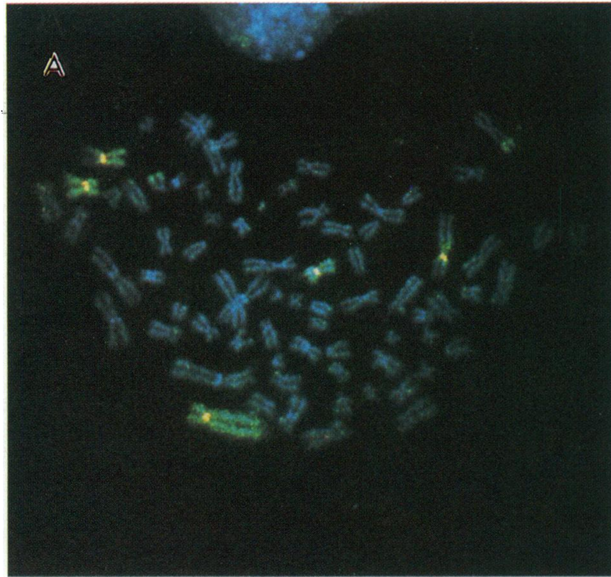

Figure 6 Marker chromosomes 11 in Caski cells. *A,* FISH on metaphase spreads to chromosome 11 centromeric and chromosome 11–painting probes, showing the presence of three chromosomes 11, two der(11)s, and translocation of chromosome 11 sequences to a marker chromosome. *B,* GTG-banding, suggesting presence of q13 material at qter of a der(11) and 11q13-q12 at pter of the marker der(14). Chromosome 11 insertions are indicated by arrows.

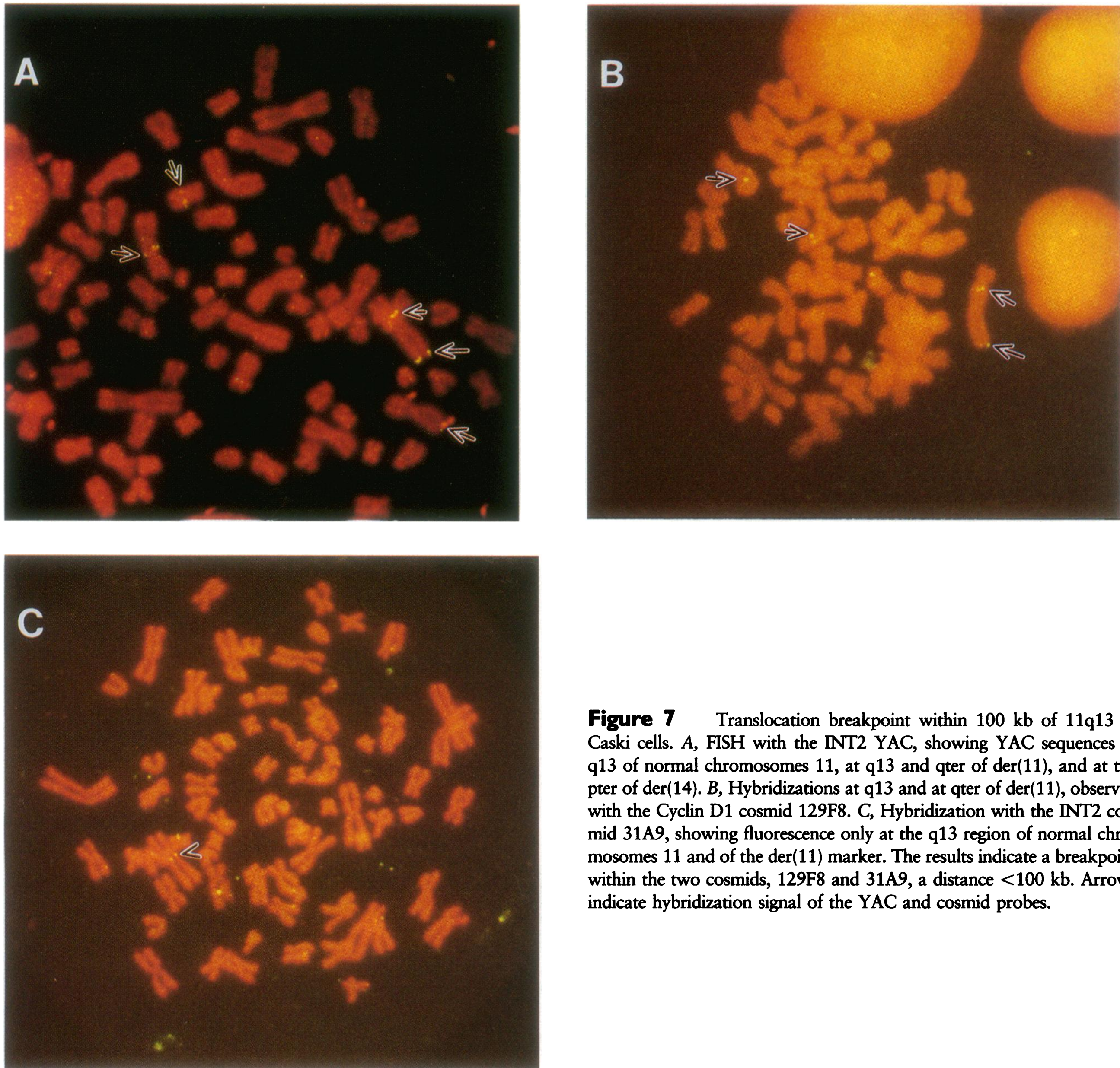

Figure 7 Translocation breakpoint within 100 kb of 11q13 in Caski cells. *A*, FISH with the INT2 YAC, showing YAC sequences at q13 of normal chromosomes 11, at q13 and qter of der(11), and at the pter of der(14). *B*, Hybridizations at q13 and at qter of der(11), observed with the Cyclin D1 cosmid 129F8. *C*, Hybridization with the INT2 cosmid 31A9, showing fluorescence only at the q13 region of normal chromosomes 11 and of the der(11) marker. The results indicate a breakpoint within the two cosmids, 129F8 and 31A9, a distance <100 kb. Arrows indicate hybridization signal of the YAC and cosmid probes.

probe) at 37°C for 2 h, and hybridized to the metaphase spreads (200 ng of the probe/slide) at 37°C overnight. Washing was performed once in 2 × SSC containing 50% formamide at 45°C for 20 min and twice in 2 × SSC at 50°C for 10 min. For cosmid probes, additional washing was carried out in 1 × SSC at 60°C for 10 min. Hybridization signals were detected as described above.

Results and Discussion

Molecular Rearrangement of 11q13 Sequences in HPV-Positive Cell Lines HeLa, Caski, and SiHa

RFLP studies on primary cervical tumors have shown deletion of chromosome 11q sequences in a significant fraction of tumors (Srivatsan et al. 1991; Hampton et al. 1994). However, conventional Southern blotting did not reveal molecular alterations for any of the chromosome 11 probes in primary tumors or cervical cell lines (data not shown). Detailed molecular cytogenetic (i.e., FISH) studies on HeLa cells have shown structural alterations involving chromosome band 11q13 in these cells (Jesudasan et al. 1994). Therefore, to examine whether molecular alteration of chromosomal band 11q13 occurs in other HPV-positive cervical tumors, six HPV-positive cell lines—HeLa, C4-I, Caski, SiHa, Ms751, and Me180—were subjected to long-range restriction-mapping analysis.

PFGE studies using seven different 11q13 probes showed

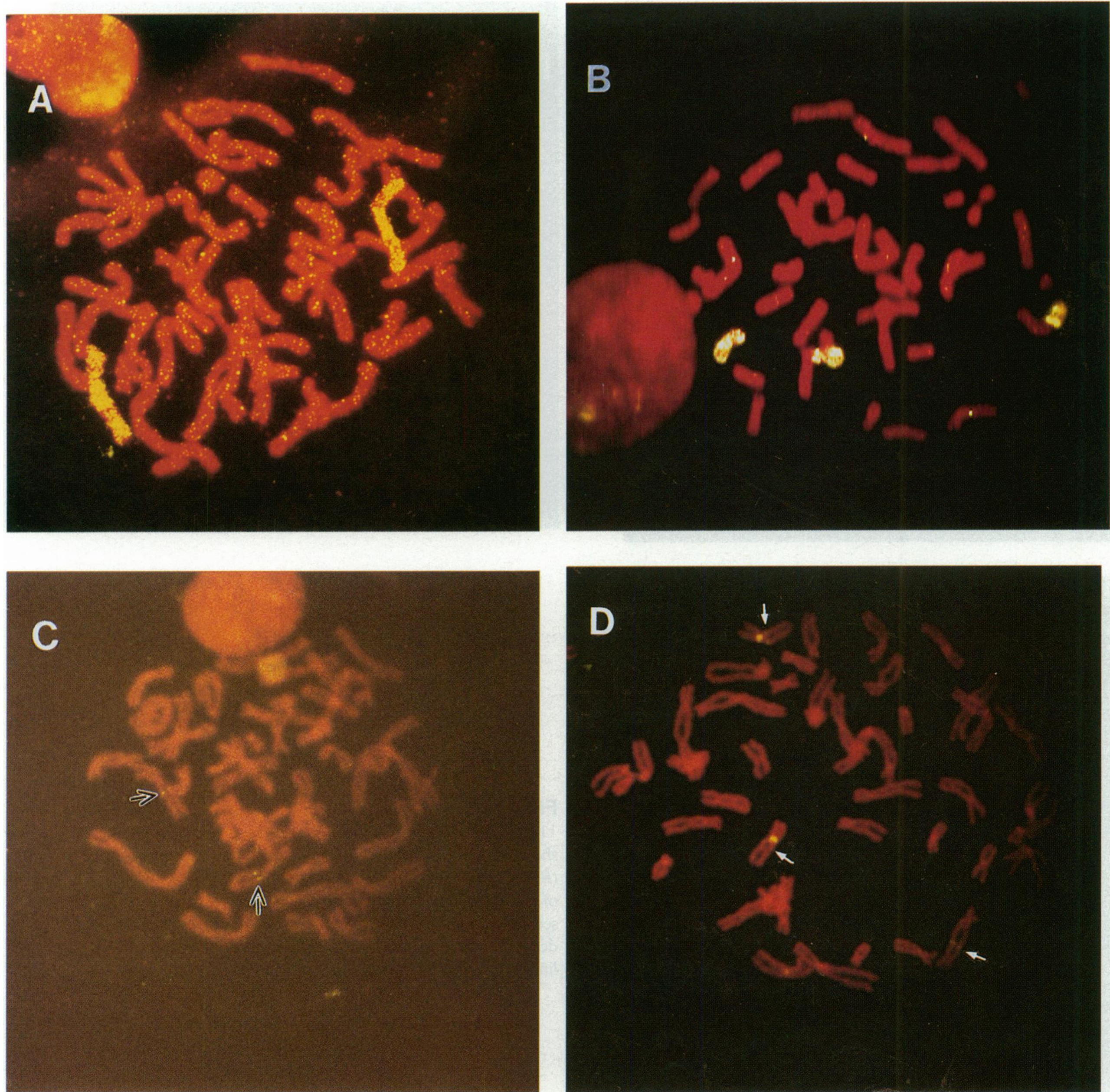

Figure 8 Translocation of INT2 YAC sequences in C33A cells. A majority of cells contain two chromosomes 11, as seen by painting with the 11 probe (*A*). Translocation of a part of chromosome 11 is also observed in some (i.e., 20%) of the metaphases (*B*). YAC hybridization shows signal (*C*) on two chromosomes 11 in most of the cells; and in the presence of the chromosome 11 centromeric probe (*D*), YAC signal is observed at the translocation site of the marker chromosome, as well as in that of the two chromosomes 11, in a minority of cells.

molecular alterations for the INT2 probe. This probe detected a single *Mlu*I fragment of 600 kb in Caski and SiHa cells (fig. 1). These two cell lines did not contain the 125-kb fragment observed in normal cells. The HeLa (D98/AH-2) cells contained fragments of 550 kb and 350 kb, in addition to the normal size, 125-kb fragment. The other three HPV-positive cell lines—C4-I, Me 180, and Ms 751—contained only the normal size, 125-kb fragment.

That the mobility shift in Caski, SiHa, and HeLa cell DNAs was not due to partial digestion was indicated by the observation of normal size fragments on rehybridization of the blots to other 11q13 probes, e.g., CD20 (a 500-kb fragment) (data not shown). To rule out the possibility of abnormal PFGE fragments due to methylation, samples isolated after either 5-aza-CR (2.5 μM) treatment or 5-aza-deoxycytidine (5-aza-CdR; 0.3 μM) treatment were ana-

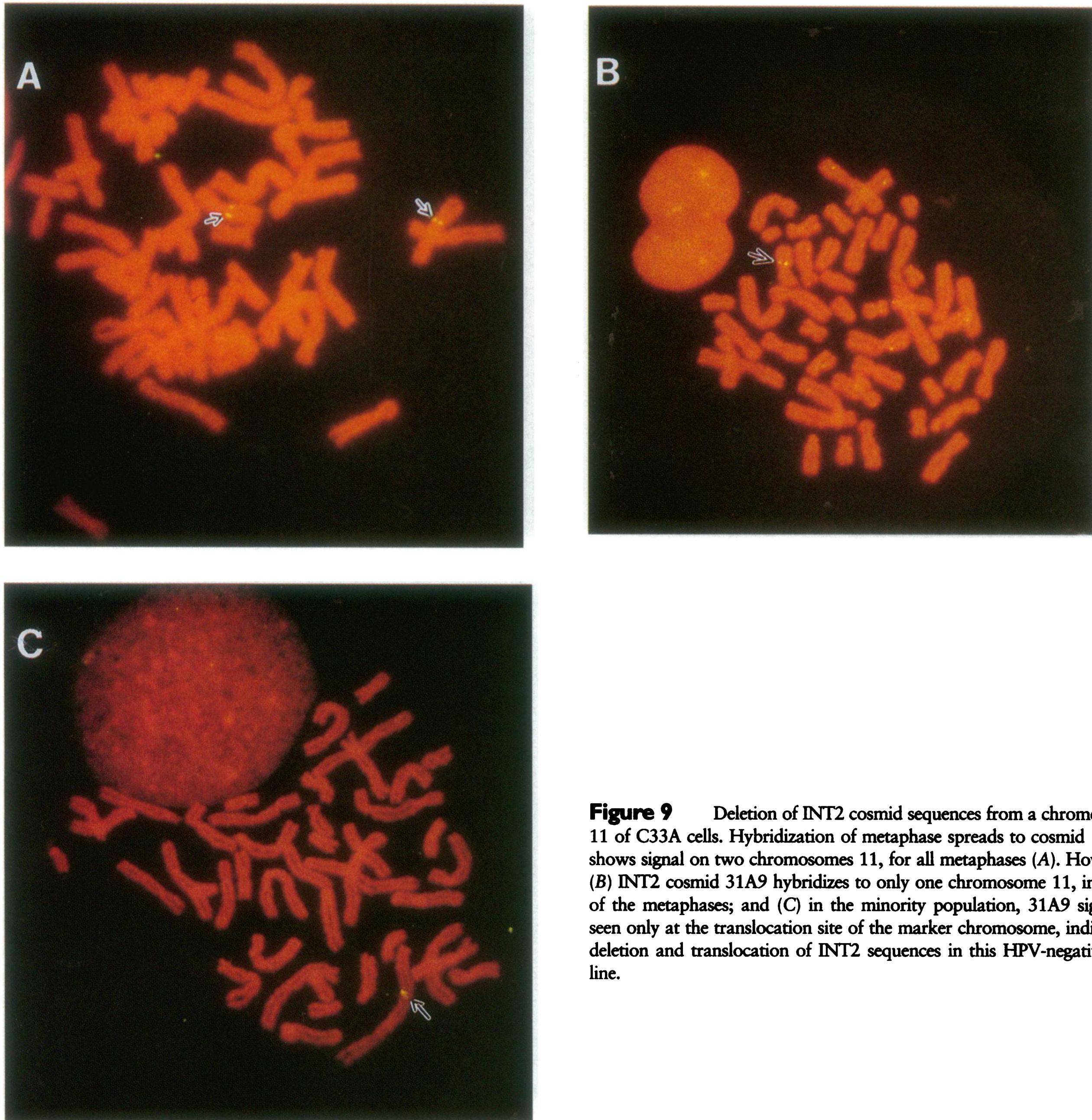

Figure 9 Deletion of INT2 cosmid sequences from a chromosome 11 of C33A cells. Hybridization of metaphase spreads to cosmid 129F8 shows signal on two chromosomes 11, for all metaphases (*A*). However, (*B*) INT2 cosmid 31A9 hybridizes to only one chromosome 11, in most of the metaphases; and (*C*) in the minority population, 31A9 signal is seen only at the translocation site of the marker chromosome, indicating deletion and translocation of INT2 sequences in this HPV-negative cell line.

lyzed. 5-aza-CR treatment did not alter the 600-kb *Mlu*I fragment in the SiHa cells (fig. 1). Though a mobility shift was observed in Caski and HeLa cell fragments after the drug treatment, the results still indicated molecular alteration of the INT2 region. Identical results were obtained for the cells treated with different concentrations of 5-aza-CR (2.5, 5.0, and 10 μm), as well as for cells treated with 5-aza-CdR. Methylation seems to have occurred as an event secondary to molecular rearrangement in Caski and HeLa cells. Further, mobility shift was not due to INT2 amplification, because INT2 was present in a number of copies that was similar to that of CD20 or Cyclin D1 in the different cervical cell lines (fig. 2).

Additional evidence for the molecular rearrangement of 11q13 sequences was provided by analysis using three other restriction enzymes: *Bss*HII, *Not*I, and *Sac*II. *Bss*HII digestion showed Caski and SiHa cells to contain single fragments, of 700 kb and 300 kb, respectively (fig. 3*A*). HeLa cells contained fragments of 650 kb and 400 kb. These three cell lines did not contain the 75-kb fragment observed in normal cells. While 5-aza-CR treatment did not alter the INT2 fragment in SiHa cells, the drug treatment

resulted in faster-migrating but still molecularly altered fragments in Caski and Hela cells (fig. 3*A*). The normal size, 75-kb fragment was absent in all three cell lines.

*Not*I digestion revealed fragments of 900 kb and 800 kb in Caski cells (fig. 3*B*). SiHa and HeLa cells contained 250-kb and 150-kb fragments. The normal size, 75-kb fragment was not observed in these cell lines. *Sac*II digestion yielded fragments of 650 kb, 600 kb, and 370 kb in Caski cells (fig. 3*C*). SiHa cells contained a 250-kb and a 200-kb fragment. Hela cells (D98/AH-2) contained a 200-kb, a 150-kb, and a normal size, 75-kb fragment. Thus, analysis with different restriction enzymes indicated molecular alteration of 11q13 sequences as the likely event in the three cell lines.

To demonstrate that the mobility shift was not due to RFLP, 11 different normal DNA samples (4 lymphocyte, 3 fibroblast, and 4 bone marrow; a total of 22 chromosomes) were analyzed using the INT2 probe. Only a single normal size fragment was observed for each of the restriction enzymes (*Mlu*I, *Bss*HII, *Not*I, and *Sac*II) used. Similarly, previous studies have not reported presence of polymorphism for the INT2 probe in PFGE gels (Janson et al. 1991; Brookes et al. 1992). Thus, these results indicated that the PFGE alterations in HeLa, SiHa, and Caski cells most likely represented molecular alterations and not RFLP.

Rearrangement of 11q13 Sequences in the HPV-Negative Cell Line HT3

To determine whether 11q13 rearrangement is a cervical tumor–related event, two HPV-negative cell lines, HT3 and C33A, were also analyzed. Mobility shift was observed for the INT2 probe in the HT3 cells, with two different restriction enzymes: *Mlu*I and *Bss*HII (fig. 4). C33A cells contained normal appearing bands for both the enzymes.

Confirmation of Molecular Alterations by FISH Analysis

In HeLa cells, the PFGE alterations were confirmed to be molecular rearrangement of 11q13 sequences by FISH using chromosome 11–painting and INT2-containing YAC probes (Jesudasan et al. 1994). In order to confirm the molecular alterations in the other cell lines, metaphase chromosomes were hybridized to chromosome 11–painting and YAC and cosmid probes. Since metaphase chromosomes were not obtainable from the HPV-negative HT3 cell line, C33A cells were analyzed using these probes.

Deletion of 11q13 Sequences in SiHa Cells

Standard cytogenetics has shown SiHa cells to contain three normal appearing chromosomes 11, which was confirmed by FISH using chromosome 11 centromeric and chromosome 11–painting probes (fig. 5*A*). FISH using INT2 YAC showed YAC sequences on all three chromosomes 11 (data not shown). PFGE studies have, however, indicated molecular rearrangements within 75 kb of the 11q13-specific INT2 probe (figs. 1 and 3). Thus, the combined cytogenetic and molecular genetic studies suggested a submicroscopic deletion of sequences near the INT2 locus from a chromosome 11 in SiHa cells. To identify this deletion, metaphase spreads were hybridized to the cosmid probes derived from the INT2 YAC. Cyclin D1 cosmid 129F8 had a hybridization pattern similar to that of the YAC, i.e., hybridization to all three chromosomes 11 (figs. 5*B* and *C*). However, hybridization of the chromosomes to INT2 cosmid 31A9 showed cosmid signal on only two chromosomes 11 (fig. 5*D*). Sequences of 31A9 were deleted from the third chromosome 11, thereby indicating interstitial deletion of 11q13 sequences, including that of INT2 in the SiHa cells.

Translocation of 11q13 Sequences in Caski Cells

FISH using chromosome 11 centromeric and chromosome 11–painting probes showed three copies of normal appearing chromosomes 11, two derivative chromosomes 11, and chromosome 11 material in a marker chromosome (fig. 6*A*). GTG-banding suggested that one of the der(11)s contained q13 material at 11qter (fig. 6*B*). The marker chromosome was identified as a der(14) chromosome containing 11q13-q12 material at the p-terminus.

FISH using biotin-labeled INT2 YAC showed YAC sequences at the q13 region of chromosomes 11 and at qter of the der(11) chromosome (fig. 7*A*). This indicated a translocation breakpoint within the sequences spanned by the INT2 YAC. Hybridization of the chromosomes to Cyclin D1 cosmid 129F8 again identified signals at q13 and at qter of the der(11) marker (fig. 7*B*). However, INT2 cosmid 31A9 hybridized only to the q13 region of this der(11) chromosome (fig. 7*C*). These results suggest that a breakpoint has occurred within the 100-kb distance between these two cosmids, 129F8 and 31A9.

Deletion and Translocation of 11q13 Sequences in C33A Cells

FISH by chromosome painting showed presence of two normal appearing chromosomes 11 (fig. 8*A*). In 20% of metaphases, we also observed chromosome 11 material in a marker chromosome (fig. 8*B*). Hybridization to the INT2 YAC confirmed the presence of two chromosomes 11 in the majority of cells (fig. 8*C*). The minority population contained the YAC sequences on two chromosomes 11 and at the translocation site of the marker chromosome (fig. 8*D*). Cosmid 129F8 hybridized to two chromosomes 11 in all metaphase spreads (fig. 9*A*). On the other hand, cosmid 31A9 hybridized to a single chromosome 11 in the majority of metaphases, indicating deletion of 31A9 sequences from the other chromosome 11 (fig. 9*B*). Twenty percent of metaphase spreads contained 31A9 sequences only at the translocation site of the chromosome 11 containing marker chromosome (fig. 9*C*). The two chromosomes 11 present in this minority population represent du-

plication of the 31A9-deleted chromosome 11. The results thus showed translocation to have occurred as an event secondary to deletion in these cells. The minority population was retained, possibly because of the low passage of this cell line in culture. Taken together, though the C33A cells contained a normal size PFGE fragment (fig. 4), the FISH studies revealed deletion and translocation of 11q13 sequences. Thus, presence of molecular alterations in four of eight cell lines suggests rearrangement of chromosome 11q13 sequences as a nonrandom event in cervical cancer.

Finally, we have observed INT2 YAC to be fairly unstable compared with other YACs in our possession. Similarly, cosmid 31A9 seems to be unstable, losing the insert sequences on growth in bacterial cells. Hybridization to a repeat probe shows 31A9 to contain repeat elements. It is likely that this instability leads to deletion of these sequences in tumors, which in turn may explain the fragility of the 11q13 region. Verification of this hypothesis must await sequencing of 31A9 and adjacent cosmids.

Amplification of the genes Cyclin D1, INT2, Hst-1, and Sea localized to chromosome 11q13 has been observed in squamous-cell carcinomas (Lammie and Peters 1991; Motokura et al. 1992; Motokura and Arnold 1993). Some of these tumors have also been shown to have amplified expression of Cyclin D1. In the present investigation, we did not observe amplification or amplified expression of any of these genes in the cervical cell lines (data not shown). Also, western blot analysis showed low-level expression of Cyclin D1 at different stages of the cell cycle in these cell lines (data not shown). The data therefore indicate that the 11q13 rearrangement may not result in the activation of these oncogenes.

Besides cervical cancer, chromosome 11q13 is also implicated in the development of a number of other human tumors, such as multiple endocrine neoplasia type 1, squamous-cell carcinomas of the head and neck, and neuroblastoma (Larsson et al. 1988; Jin et al. 1990; Lammie et al. 1991; Srivatsan et al. 1993; Ah-See et al. 1994). It remains to be determined whether a single gene is involved in the development of these different human tumors.

In conclusion, using PFGE, cytogenetic, and molecular cytogenetic approaches, we have observed molecular rearrangements including deletion of 11q13 sequences in both the HPV-positive and HPV-negative cervical cell lines. Since deletion of 11q13 sequences has also been observed in primary cervical tumors (Srivatsan et al. 1991; Hampton et al. 1994), these molecular alterations, specifically interstitial deletions in SiHa and C33A cells, most likely represent inactivation of a putative tumor-suppressor gene localized to chromosome band 11q13. Since it has been postulated that two mutational events are required for the inactivation of a tumor-suppressor gene (Knudson 1985), the second mutational event in the different cell lines could be a point mutation or a minor deletion. Detection of this second event would require the isolation of the gene in question. Cosmids 129F8 and 31A9 should prove useful in the isolation and cloning of this tumor-suppressor gene.

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References

Ah-See KW, Cooke TG, Pickford IR, Soutar D, Balmain A (1994) An allelotype of squamous carcinoma of the head and neck using microsatellite markers. Cancer Res 54:1617-1621

Brookes S, Lammie GA, Schuuring E, Dickson C, Peters G (1992) Linkage map of a region of human chromosome band 11q13 amplified in breast and squamous cell tumors. Genes Chromosom Cancer 4:290-301

Green ED, Olson MV (1990) Systematic screening of yeast artificial chromosome libraries by use of the polymerase chain reaction. Proc Natl Acad Sci USA 87:1213-1217

Hampton GM, Penny LA, Baergen RN, Larson A, Brewer C, Liao S, Busby-Earle RMC, et al (1994) Loss of heterozygosity in cervical carcinoma: subchromosomal localization of a putative tumor-suppressor gene to chromosome 11q22-24. Proc Natl Acad Sci USA 91:6953-6957

Higgins MJ, Hansen MF, Cavenee WK, LaLande M (1989) Molecular detection of chromosomal translocations that disrupt the putative retinoblastoma susceptibility locus. Mol Cell Biol 9:1-5

Janson M, Larsson C, Werelius B, Jones C, Glaser T, Nakamura Y, Jones CP, et al (1991) Detailed physical map of human chromosomal region 11q12-13 shows high meiotic recombination rate around the *MEN1* locus. Proc Natl Acad Sci USA 88:10609-10613

Jesudasan RA, Slovak ML, Sen S, Srivatsan ES (1994) Rearrangement of chromosome band 11q13 in HeLa cells. Anticancer Res 14:1727-1734

Jin Y, Higashi K, Mandahl N, Heim S, Wennerber J, Biorklund A, Dictor M, et al (1990) Frequent rearrangement of chromosomal bands 1p22 and 11q13 in squamous cell carcinomas of the head and neck. Genes Chromosom Cancer 2:198-204

Jones PA, Taylor SM (1980) Cellular differentiation, cytidine analogs and DNA methylation. Cell 20:85-93

Knudson AG (1985) Hereditary cancer, oncogenes and antioncogenes. Cancer Res 45:1437-1443

Koi M, Hiroyuki M, Hideto Y, Barrett JC, Oshimura M (1989) Normal human chromosome 11 suppresses tumorigenicity of human cervical tumor cell line SiHa. Mol Carcinog 2:12-21

Lammie GA, Fantl V, Smith R, Schuuring E, Brookes S, Michalides R, Dickson C, et al (1991) D11S287 a putative oncogene on chromosome 11q13 is amplified and expressed in squamous cell and mammary carcinomas and linked to BCL-1. Oncogene 6:439-444

Lammie GA, Peters G (1991) Chromosome 11q13 abnormalities in human cancer. Cancer Cells 3:413–420

Larsson C, Skogsied B, Oberg K, Nakamura Y, Nordenskjold M (1988) Multiple endocrine neoplasia type 1 gene maps to chromosome 11 and is lost in insulinoma. Nature 332:85–87

Misra BC, Srivatsan ES (1989) Localization of HeLa cell tumor-suppressor gene to the long arm of chromosome 11. Am J Hum Genet 45:565–577

Motokura T, Arnold A (1993) Cyclin D and oncogenesis: current opinion. Genet Dev 3:5–10

Motokura T, Kronenberg HM, Arnold A (1992) Cloning and characterization of human cyclin D3, a cDNA closely related in sequence to the PRAD1/Cyclin D1 proto oncogene. J Biol Chem 267:20412–20415

Nelson DL, Ballabio A, Victoria MF, Pieretti M, Bies RD, Gibbs RA, Maley JA, et al (1991) Alu-primed polymerase chain reaction for regional assignment of 110 yeast artificial chromosome clones from the human X chromosome: identification of clones associated with a disease locus. Proc Natl Acad Sci USA 88:6157–6161

Ocadiz R, Sauceda R, Cruz M, Graef AM, Gariglio P (1987) High correlation between molecular alterations of the c-myc oncogene and carcinoma of the uterine cervix. Cancer Res 47:4173–4177

Pater M, Dunne G, Ghatage P, Pater A (1986) Human papillomavirus types 16 and 18 sequences in early cervical neoplasia. Virology 155:13–19

Pfister H (1987) Human papillomaviruses and genital cancer. Adv Cancer Res 48:113–146

Riou G, Barrois M, Sheng Z, Duvillard P, Lhomme C (1988) Somatic deletions and mutations of C-Ha-ras gene in human cervical cancers. Oncogene 3:329–334

Rosl F, Durst M, zur Hausen H (1988) Selective suppression of human papillomarvirus transcription in non-tumorigenic cells by 5-azacytidine. EMBO J 7:1321–1328

Saxon PJ, Srivatsan ES, Stanbridge EJ (1986) Introduction of human chromosome 11 via microcell transfer controls tumorigenic expression of HeLa cells. EMBO J 5:3461–3466

Srivatsan ES, Misra BC, Venugopalan M, Wilczynski SP (1991) Loss of heterozygosity for alleles on chromosome 11 in cervical carcinoma. Am J Hum Genet 49:868–877

Srivatsan ES, Ying K-L, Seeger RC (1993) Deletion of chromosome 11 and 14q sequences in neuroblastoma. Genes Chromosom Cancer 7:32–37

Wilczynski SP, Bergen S, Walker J, Liao SY, Pearlman L (1988) Human papillomaviruses and cervical cancers. Hum Pathol 19:697–701

Yokota J, Tsukada Y, Nakajima Y, Gotoh M, Shimosato Y, Mori N, Tsunokawa Y, et al (1989) Loss of heterozygosity on the short arm of chromosome 3 in carcinoma of the uterine cervix. Cancer Res 49:3598–3601