Genomic Alterations in Cervical Carcinoma: Losses of Chromosome Heterozygosity and Human Papilloma Virus Tumor Status

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ABSTRACT

Specific human papilloma virus (HPV) types appear to be necessary etiological factors for most cervical cancers, yet additional genetic alterations seem to be required for their development and progression. The aim of this study is to determine the likely chromosomes location of genetic alterations that are required for their development and progression. The HPV status of the tumors was also determined. LOH was found to involve 19 chromosome arms in 20–43% of the tumors. Chromosome arms 6p, 3p, and 18q are most frequently involved in LOH in 43, 39, and 35% of the informative carcinomas, respectively. The respective regions involved in 6p21.1–23, 3p13–25.3, and 18q12.2–21.2. LOH is also limited to specific band segments within other regions. Similar high incidences of LOH of the same 3p segments have been reported in cervical carcinomas from different parts of the world. The same 3p and 6p segments are involved in many types of common cancers, whereas 18q changes are less frequent in other cancers. Chromosome arms 1q, 2q, 3q, 4q, 5p, 5q, 6q, 7q, 8q, 11q, 13q, 16p, 18p, and 19p are involved in LOH in 20–33% of the cervical tumors. Chromosome 11 alterations are among the most frequently found in many different types of neoplasias. In this study, 11p was involved in 16% of the tumors, and 11q was involved in 22%. Chromosome 17 alterations are found in more cancers than those of any other chromosome, frequently involving the p53 gene on 17p. LOH of 17p was found in 5 (15%) cervical tumors; 2 of these were HPV negative and expressed mutant p53. In such HPV-negative tumors, direct mutation of the wild-type p53 appears to replace the inactivation of the p53 product by oncogenic HPV types. Tumors with LOH at many loci were, on the average, at more advanced stages, as were tumors with mutant p53. The higher overall incidence of LOH in cervical carcinomas as compared to other cancers, and the diversity of LOH patterns found, suggest that different cervical carcinomas probably arise and progress in part, because of the loss of function of different yet finite sets of tumorigenicity suppressor-like genes and genes that are involved in tumor progression and metastasis. The findings also indicate that certain chromosome segments that are often altered in cervical carcinomas are also frequently altered in several different types of cancers. It remains to be determined whether the same or different genes located within these segments are involved in the different cancer types.

INTRODUCTION

Cervical carcinomas lead to approximately 500,000 deaths annually in the world. Although specific HPV3 types appear to be necessary etiological factors for most cervical cancers, additional genetic alterations seem to be required for their development and progression. Knowledge of these genetic changes, in conjunction with the viral status of these cancers, might lead to improved methods of prognosis and the development of more effective therapeutic strategies. Consequently, a combined molecular, viral, and cytogenetic examination of these tumors was undertaken. This first report of the series contains the results of assays for the LOH and HPV status of the tumors. Subsequent reports will compare these results with the cytogenetic findings in some of the same tumors; compare the LOH, cytogenetic, and HPV findings with the degree of aggressiveness of the tumors; and report on the findings of a much more detailed analysis of the genetic alterations found in chromosomes 3 and 6 than those described below.

LOH of specific chromosomes or chromosome segments occurs in many neoplasias and generally indicates the sites of tumorigenicity suppressor-like genes. Loss of the wild-type function of these genes appears to be a necessary step in the origin or progression of the malignant phenotype (1). Primarily in neoplasias of the hematopoietic system, and in some solid tumors, the presence of specific chromosome changes can serve as a prognostic indicator and as a guide for therapy (2). Our earlier studies revealed nonrandom structural and numerical chromosome aberrations in cervical carcinomas (3–7). With the exception of one report (8), there have been no LOH studies for all of the chromosomes of a sizable sample of cervical carcinomas. In addition, there have been very few studies correlating LOH and cytogenetic findings of the same set of any type of solid tumors. Most reports of LOH findings in cervical carcinomas, to be reviewed in the discussion, are restricted to only one or a few chromosomes; thus, the relative frequency of involvement of different chromosomes or chromosome segments in LOH has not been well established.

MATERIALS AND METHODS

Although specific HPV3 types appear to be necessary etiological factors for most cervical cancers, additional genetic alterations seem to be required for their development and progression. Knowledge of these genetic changes, in conjunction with the viral status of these cancers, might lead to improved methods of prognosis and the development of more effective therapeutic strategies. Consequently, a combined molecular, viral, and cytogenetic examination of these tumors was undertaken. This first report of the series contains the results of assays for the LOH and HPV status of the tumors. Subsequent reports will compare these results with the cytogenetic findings in some of the same tumors; compare the LOH, cytogenetic, and HPV findings with the degree of aggressiveness of the tumors; and report on the findings of a much more detailed analysis of the genetic alterations found in chromosomes 3 and 6 than those described below.

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MATERIALS AND METHODS

Tumor samples from 38 cervical carcinomas were collected between 1988 and 1993. Seventeen were collected in Middlesex, United Kingdom, by one of the authors (N. B. A.), and 21 were collected by the Cooperative Tissue Network sponsored by the National Cancer Institute from various locations within the United States. The 38 cases consisted of 28 squamous cell carcinomas, 7 adenocarcinomas, and 3 adenosquamous carcinomas. The histopathological type and stage of each of the tumors, as well as the ages of the patients, are given in Fig. 1, a and c.

When the study was started, Southern blotting with RFLP markers was used to estimate LOH frequencies. Because this required large amounts of DNA, the fresh tumor tissue was dissected as free of excessive nontumor tissue as possible, with the aid of a dissecting microscope before DNA extraction. Later, the DNA of the tumors was reexamined with microsatellite markers because they are much more informative. Only the data obtained with the latter method are presented here, with the exception of those obtained with the RFLP marker D17S34 (Fig. 1c).

To evaluate the extent to which contamination from nontumor cells masked LOH in the tumor cells, an aliquot of each primary tumor was also injected s.c. in nude mice to eliminate the contaminating constitutive cells that do not grow in the nude mouse. Tumors developed from 11 of 38 primary samples. The findings from some of these are illustrated in Fig. 3.
**GENOMIC ALTERATIONS IN CERVICAL CARCINOMA**

### Table

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**Fig. 1.** See opposite page for legend.
**LOH Methods with PCR**

DNA was extracted from the cervical carcinomas, tumors that grew in nude mice, and the normal constitutive cells of the same patients with the proteinase K-SDS-phenol method described by Sambrook et al. (9). Primers for 75 highly polymorphic microsatellite loci of Weissenbach et al. (10), listed in Fig. 1, a—c, were selected so that at least one marker was located on each autosome arm. Additional markers were used for the arms of the larger chromosomes and where necessary to minimize the number of uninformative cases. The sex chromosomes were not included so that effort could be concentrated on all of the other autosome arms.

The PCR method followed that recommended in the Génététique microsatellite map catalogue (11) with some modifications. The reaction mixture consists of 1 µl (20 ng) DNA, 2 µl of 10× buffer (Boehringer Mannheim), 50 µM of each dNTP, 5 pmol each of the appropriate R and F synthetic oligonucleotide primers, 0.5 units of Taq DNA polymerase (Boehringer Mannheim), 1 µCi of any [α-32P] dNTP added as a tracer, and water to make a final volume of 20 µl. An overlay of 25 µl mineral oil was then applied.

**PCR Conditions.** A DNA thermal cycler (Perkin Elmer Cetus 480) was programmed to perform the following steps. The first denaturation was at 95°C for 5 min, followed by 35 cycles of denaturation at 94°C for 40 s and annealing at 52°C for 45 s. One cycle of an elongation step at 72°C for 5 min ends the procedure. The PCR products are then diluted with an equal volume of formamide to which 0.25% bromphenol blue and 0.25% xylene cyanol are added. The products were then denatured at 95°C for 5 min and run on 6% polyacrylamide gels containing 7 M urea. Electrophoresis was conducted in 0.5× Tris-boric acid-EDTA buffer. The gels were then dried and exposed to X-ray film for 10–24 h, depending on the radioactivity of the bands.

LOH was considered to have occurred either when an allelic band present in the constitutive cell lane was absent in the tumor lane, or when it was one-third or less the density of the corresponding band in the tumor lane (Fig. 3, a—d).

**Detection and Typing of HPV**

Approximately 50–100 ng of extracted DNA from cancer tissue were used for the detection of HPV DNA by PCR. All samples were initially tested by the MY09/MY11 L1 consensus primer system, which amplifies a 450-bp fragment in the L1 open reading frame. Presence of a specific HPV-amplified product was demonstrated by hybridization with a mixture of PCR probes generated from within the L1 fragment by specific nested primers for HPV types 11, 16, 18, and 51 using viral genomes cloned in plasmids as targets. A β-globin fragment was coamplified in each reaction as a control. HPV type was determined by dot blot hybridization with type-specific oligonucleotide probes as described (12). More than 25 different HPV types were evaluated. In addition, the first 21 samples were also tested for HPV DNA by Southern blot hybridization using 5 µg of extracted DNA digested by PstI, as described previously (13).

**RESULTS**

The findings for each of the 75 loci on the 22 autosomes of each of the 38 tumors are given in Fig. 1, a—c, and summarized in the histogram in Fig. 2. In Fig. 1, a—c, the tumors are listed in the order of the number of allelic losses they contain. The map locations of the marker loci are based on published reports (4), and are given for each chromosome starting with the end of the short arm. Although the chromosome arm location of all the probes used has been verified, the

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<th>Tumor No.</th>
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**Fig. 1.** Chromosome arms, loci probed (D segments), and their map locations are shown. For sources of these data, see the text. Additional markers were used for the arms of the larger chromosomes and where necessary to minimize the number of uninformative cases. The sex chromosomes were not included so that effort could be concentrated on all of the other autosome arms.

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*Human Gene Mapping, International Workshops on Human Gene Mapping and the Individual Chromosome International Workshops on Human Chromosome Mapping Reports. Most of these have been published in Cytogenet. Cell Genet. See volume indexes for specific ones. A list of citations of all of these reports is in press in Cytogenet. Cell Genet., Vol. 71, 1996.*
exact band locations may not be completely accurate at a high level of resolution. The HPV findings are also given for all the tumors in Fig. 1, a–c. Patient age and histopathological tumor type and grade are given in Fig. 1, a and c. Those tumors that expressed mutant p53 (data from Ref. 14) are also indicated in Fig. 1, a and c.

Thirty-nine chromosome arms were tested in each of the 38 tumors, in the passaged tumor cells when available, and in the matched constitutive cell sample for each of the 75 marker loci (except D17S34; see Fig. 1c). LOH involved all the markers of a particular chromosome in a given tumor or, more often, only the markers on an individual arm or a segment of the arm. The number of tumors informative for each locus ranged from 15 (40%) to 37 (97%), with a mean of 27 (71%). In the first line of the summarized data at the bottom of Fig. 1, a–c, are the number of tumors with LOH of one or more markers of each of the chromosome arms. In the second line, these values are given as the percentage of informative cases with LOH for one or more of the markers on that arm, and these values are also shown in the histogram in Fig. 2. When scored in this manner, the range is from 29 (76%) to 38 (100%) of the tumors that are informative for at least one of the loci tested on a particular arm.

In the last two lines of Fig. 1, a–c, the frequencies are given for LOH of one or more markers on the entire chromosome. The latter values are of limited significance because the allelic losses are frequently restricted to one or more specific segments of the chromosome. These values are presented primarily to facilitate comparison with the results of others in other types of tumors, in which the findings were presented in this manner. Although this same limitation applies to some extent to the presentation of the values in terms of chromosome arms, it would be tedious to summarize the data in terms of individual loci, and also difficult to compare the results with those of others. However, this information is given for each locus of each tumor in Fig. 1, a–c.

In cases in which tumors developed in the nude mice, this DNA was run together with DNA extracted directly from the tumor and with DNA from the constitutive cells (Fig. 3, a, b, and d). (The results shown in Fig. 1, a–c, for tumors 3, 6, 19, and 23 are from the tumors passaged in the nude mouse.) These comparisons revealed no apparent masking in some tumors to masking of several losses in other tumors, but, as a whole, the masking effect was not large. Because the number of cases examined in this manner was small, the results cannot be quantified. The essential point is that LOH frequencies given are underestimations to some degree, particularly in tumors in which there is considerable infiltration of the tumor tissue by constitutive cells. However, this error is not likely to significantly alter the often large ratio of LOH incidences found between different chromosome segments. The good agreement between our LOH frequencies and those of other authors who examined some of the same chromosomes (see “Discussion”) also support this assumption. Chromosome gains and microsatellite instability (amplification) were also found in some cases. These findings will be given in a future report in conjunction with the cytogenetic findings.

Essentials of the LOH Findings. The most common sites of LOH involved 19 chromosome arms in 20–43% of the informative tumors. Chromosome arms 6p, 3p, and 18q are most frequently involved in LOH in 43, 39, and 35% of the informative carcinomas, respectively. For chromosome 6 the region involved is 6p21.1–23, for chromosome 3 the region is 3p13–25.3, and for chromosome 18 the region is 18q12.2–21.2. Chromosome regions 3q25.3–29 and 6q21–27 were also involved in 22% of the cases. The specific band segments involved within these regions are shown in Fig. 1, a and c, and some are discussed further below. In addition to 3q and 6q, chromosome arms 1q, 2q, 4p, 4q, 5p, 5q, 7q, 8q, 11q, 13q, 16p, 18p, and 19p were involved in LOH in 20–33% of the tumors. All other autosome arms had LOH in 3–19% of the cases (Figs. 1 and 2). On the basis of
GENOMIC ALTERATIONS IN CERVICAL CARCINOMA

Fig. 3. Representative autoradiograms of PCR gels demonstrating the allelic loss at some of the chromosome loci with high incidences of LOH [i. e., chromosome arms 3p (a), 3p (b), 6p (c), and 13q (d)]. The source of the DNA is indicated: T, original primary tumor; N, normal constitutive cells of the same subject; P, cells of the same tumor after passage in the nude mouse. Loci designations and tumor numbers correspond to those given in Fig. 1, a–c. Much lighter allelic bands in the tumor DNA lanes represent the loss of that allele; light bands represent the DNA of contaminating constitutive cells. This is proved by the absence of the band in the cells after passage of the tumor in the nude mouse in which the nontransforming constitutive cells do not grow. Lane pairs not labeled in d are examples of informative cases (heterozygous) with no LOH at the D13S159 locus.

Separate entirely chromosomes, 14 were involved in LOH in 24–45% of the 38 tumors (chromosomes 1–9, 11, 13, 16, 18, and 19). All other chromosomes had LOH in 21% or fewer of the tumors (Fig. 1). The number of losses in different tumors varied considerably, ranging from 36 (tumor 6) to three tumors in which no losses were detected (tumors 15, 33, and 38). In the latter cases, DNA from constitutive cells may have masked some losses in the tumor cells. LOH of 17p was found in 5 cases (15%) with probes that map to p13.1 and p13.3. The p53 locus is at p13.1. Nine of 38 cases were HPV negative; 3 of these had LOH of the 17p markers, 2 of which expressed mutant p53. A third case that also expressed mutant p53 contained HPV-33 sequences. Two of the HPV-positive tumors also expressed mutant p53 (Fig. 1c). We reported on some of these findings previously (14) and will expand on these results below.

DISCUSSION

One aim of this study is to determine the relative incidence of LOH in cervical carcinomas because former studies, except for that of Mitra et al. (8), were restricted to examinations of one or a few chromosomes. Other aims, to be reported separately, are to correlate the LOH data with the cytogenetic, HPV, and p53 findings and with the degree of aggressiveness of the tumors.

The finding of 35–43% LOH for segments of chromosomes 3p, 6p, and 18q strongly implicate these as the sites of one or more genes likely to play an important role in the origin or progression of cervical carcinomas. The 15 chromosome arms with LOH of 20–33% are also likely to contain such genes. More such sites appear to be involved in cervical carcinomas than in many other solid tumors that have been similarly examined, although many of the same chromosome regions are involved. This suggests that cervical carcinomas can arise or progress because of different genetic alterations, with or without the involvement of HPVs. This is not surprising considering the histopathological and clinical diversity of these tumors.

Mitra et al. (8) examined 53 primary cervical carcinomas collected in New Delhi, India, using 57 probes for RFLP sites. In comparing their results with ours, it must be taken into account that the markers used are at different map locations, that the RFLP probes resulted in a smaller number of informative loci, that there are differences in the number of informative cases for each chromosome arm, and that the geographic origin of the samples differ. They tested each locus on 35 to all 53 cases of their sample. Four (8%) to 52 (98%) of the tumors, compared to our range of 16 (62%) to 37 (97%), proved to be informative for each locus. Our results agree in respect to LOH of ≥20% of informative cases for chromosome arms 1q, 3p, 3q, 4q, 5p, 5q, 6p, 6q, and 18q. On the other hand, they found <20% LOH in some chromosome arms, where we found LOH frequencies of ≥20%. This involves 2q, 4p, 7q, 8p, 11q, 13q, 16q, 19p, and 19q, although in some of the cases, like the 11q, the differences are small. Conversely, they found LOH frequencies exceeding 20% for 11p and 20p, where we did not. There is agreement that LOH in chromosome arms 1p, 2p, 7p, 12p, 12q, 14q, 15q, 16q, 17q, 19q, 21q, and 22q is below 20%, often considerably so.

Implications of LOH on Chromosomes 3p, 6p, 6q, 7q, 11q, 13q, 17p, 18p, and 18q in Cervical and Other Cancers. The possible significance of the chromosome band segments for which we found the highest LOH frequencies in cervical carcinomas, or where the findings are of particular interest, will be discussed next, primarily in respect to the findings of others in cervical carcinomas and the involvement of these same segments in other cancers. In addition, we note some of the genes that have been mapped within these segments that appear to play a role in malignancy. The specifics of the findings in different tumors are given in reviews by others (8, 15–17).

Chromosome 3. As noted, the second highest LOH frequency in our sample is for loci on 3p found in 15 of 38 (39%) tumors (Fig. 1a). LOH involved either one, two, or all three of the 3p segments probed [p13 (7 cases), p22.1–24.1 (8 cases), and p25.1–25.3 (7 cases)]. In 8 cases, there was loss of the 3q marker at 3q25.3–29. Seven of these were the same cases that had 3p losses. In 4 of these 7 cases, there was LOH of all informative loci, suggesting the loss of an entire homologue. There was only one case in which there was a loss in 3q without a loss in 3p.

Mitra et al. (8), whose cases came from India, found LOH of the D3S2 locus in 40% and of the D3S32 locus in 32% of the tumors. Because these are located, respectively, at 3p21 and 3p21.3–22.1, a putative suppressor would be expected to be located within p22.1 because this is the only band of overlap with the D3S1298 probe used that maps to p22.1. This, and our finding of losses in other bands of 3p, suggesting that several loci may be involved, is supported further by the results of others. Chung et al. (18) in Hong Kong found LOH at the RAF-1 locus on 3p25) in 10 of 12 and at the D3S3 locus (3p14) in 10 of 11 informative cases. Jones and Nakamura (19), using several probes for 3p loci, found LOH for segment 3p13–14.3 in 6 of 8 cervical cancers and also in endometrial cancers, and of 3p21.1–22 in cervical and ovarian cancers, all in Japan. Kohno et al. (20), also in Japan, found LOH at the 3p13–21.1 region in 21 of 47 (45%) of the cervical cancers. Karlsen et al. (21) in Norway found LOH at 3p21 in 19 of 27 (70%) and at 3p21–22 in 13 of 23 (56%) of informative cervical carcinomas. Aburatani et al. (22) reported 11 deletions clus-
Correlating LOH on 3p to the stage of cancer, Kohno et al. (20) found allelic losses of 3p13–21.1 in 3 of 12 (25%) histopathological stage I cervical carcinomas, as compared to 18 of 35 (51%) in stage II–IV tumors. This is consistent with the trend we observed (Fig. 1a). Although the number of cases is too small to allow statistical analysis, the combined evidence suggests that the alterations of one or more genes on 3p may be related to invasion and metastasis.

That 3p is likely to contain several suppressor sequences is also indicated by other related findings. Killary et al. (23) demonstrated suppression of tumorigenicity in nude mice of interspecific microcell hybrids into which a 2-Mb fragment from the human chromosome 3p21–22 segment was introduced. They then showed that many of 89 small cell and 32 non-small cell lung carcinomas and cell lines had allelic losses at 3p21.2–21.3 (24). They have since isolated a portion of the 2-Mb fragment that retains the ability to suppress tumorigenicity.5 Boldog et al. (25) cloned the hereditary renal carcinoma-associated gene (HRCA1) from the t(3;8)(p14.2;q24.1) translocation breakpoint in hereditary renal carcinoma, but they have not yet functionally demonstrated that it is a suppressor. A suppressor in this region is also considered to be critical in the development of nonfamilial renal carcinomas (at least two other suppressors on 3p also appear to be involved in these cancers).

The 3p13–21.1 region is often involved in LOH or deleted in cancers of the breast, lung, kidney, and some other cancers (16, 17). In addition, the 3p22.1–24.1 segment we found to be involved in cervical carcinomas is adjacent to the site of the hMLH1 gene at 3p21.3 and is implicated in hereditary nonpolyposis colorectal cancer (26). Because the region of LOH often extends beyond the map position of the probe, it is possible that the hMLH1 gene is within the region of loss. The same or another gene on 3p is probably involved in testicular cancer and several other cancers. The gene for the familial adenomatous polyposis binding protein, β-catenin, has been mapped to 3p22 (27–29). β-Catenin probably represents a downstream modulator of adenomatous polyposis activity. It is interesting that it is located at 3p22, which is so often involved in many different cancers in addition to those of the cervix. Loci on 3p have also been shown to be associated with breast cancer and in several neoplasias of the hematopoietic system (2). As a whole, chromosome 3 is the third most frequent chromosome involved in LOH in all types of cancers (16).

The fragile site FRA3B at 3p14.2 is one of the most frequent and most sensitive sites induced by the carcinogen aphidicolin. We are currently better localizing the LOH segments on chromosome 3 in cervical carcinoma, and where possible, determining whether this involves the same genes as those affected in other tumors.

Chromosome 6. As noted, LOH of 6p loci was the most frequent abnormality detected in this study. In 43% of the tumors, there was LOH of at least one, but more often of two or all three loci at p21.1–21.2, p21.3–22.3, and p23. These losses occurred together with one or both of the 6q markers at q21–23.3 and/or q25.2–27 (Fig. 1a). Although LOH of only 6p markers was found in 9 of 15 tumors with LOH of any chromosome 3 loci, only one case was found in which there was loss of a 6q without loss of a 6p locus. In four cases, there was LOH at all informative loci on the 6 marker, suggesting complete loss of one of the homologues (Fig. 1a). The incidence of 43% of LOH for 6p markers exceeds those of 20 and 26% that Mitra et al. (8) obtained with the two 6p markers they used. In a cytogenetic analysis of two cell lines established from two cervical carcinomas, Mitra et al. (30) found that both lines contained an (5)(p10) and del(6)(q15q26). This is consistent with the above reviewed results.

The same segments of the 6p and 6q we found involved in LOH in cervical carcinomas have been involved in ovarian carcinomas (31–33), small cell lung cancer (34), and colorectal carcinomas (35). The same 6q segments are also involved in breast carcinomas (36) and several other neoplasias (2, 17). As is the case for chromosome 3, we are now better localizing the LOH segments on chromosome 6 in cervical carcinomas.

Chromosome 7. We found LOH within 7q31–35 in 12 of 36 (33%) informative tumors. In this case, the substantial discrepancy between our findings and those of Mitra et al. (8), who found LOH in the 7q32–qter in 2 of 18 (11%) informative cases, may be because of small sample sizes. No other investigators have evaluated LOH of chromosome 7 in these tumors. Chromosome 7q has been implicated as containing one or more suppressor loci in several different neoplasias. We reported earlier cytogenetically detected deletions with breakpoints varying from 7q11 to q34 in carcinomas of the prostate, colorectum, and testes (37). Monosomy and trisomy of chromosome 7 is frequent in prostatic cancers, and the frequency of tumors with such aneuploidy increases as the aggressiveness of the tumor increases (38–41). Trisomy of chromosome 7 also occurs in colorectal carcinomas (42, 43). Trisomy or tetrasyony of chromosome 7 has been reported in renal carcinomas, apparently in conjunction with the unusual finding of trisomy in the nonmalignant parenchyma. However, the consistency and significance of this finding is controversial (44). We will report on the incidence of excess copies of chromosome 7 in cervical carcinomas in a future report. Thus, chromosome 7 is likely to contain one or more genes that are involved in cervical carcinomas and other neoplasias, and a dosage effect may also be involved.

Chromosome 11. We and others have shown that in cell hybrids, chromosome 11 of a normal cell carries genes that suppress the tumorigenicity of cervical carcinoma-derived D98AH2 (HeLa) cells (45–48). We have also shown that there is a deficiency in all or part of chromosome 11 in several types of cancers, including cervical cancer (4, 5, 7). Jesudasan et al. (49) reported molecular rearrangements in the 11q13 band in several cervical carcinoma-derived cell lines, including D98AH2 cells.

Compared to our finding of LOH of one or more markers on chromosome 11 in 32% of cervical carcinomas, Hampton et al. (50), using 16 polymorphic markers and 32 cervical carcinomas, found 14 (44%) with LOH; 7 (22%) of these were in the 11q22–24 segment. We also had 22% LOH (8 of 36 informative tumors) on 11q, with LOH at 11q13–23 in 3 cases and at 11q23.3–24 in 5 other cases (Fig. 1b). Hampton et al. (50) had 28% of what they claimed were non-clonal and 16% clonal LOH on 11p as compared to our 16% LOH for 11p markers. However, they found that all clonal cases with LOH at 11p (5 of 20) were accompanied by LOH on 11q, suggesting loss of an entire chromosome. In contrast, we had only two such cases, and in one of these there was no LOH of the interstitial locus at 11p14. In the other case, the interstitial locus was not informative. Thus, in four of our cases, there were 11p losses without 11q losses, and in six cases, there were 11q losses without 11p losses. The cytogenetic findings in some of these cases were consistent with the LOH findings that will be shown in a future report. Mitra et al. (8) found LOH of one or more markers of 11p in approximately 42% of their cases and of 11q in 19% of the cases, the latter agreeing closely with the aforementioned findings; the former, however, was higher. Whether this is

5 S. Naylor, personal communication.
an effect of small numbers or a result of the difference in the geographical source of the tumors remains to be resolved.

The combined evidence suggests that there are likely to be several different genes with suppressor-like functions on chromosome 11 that play a role in cervical cancer. This would not be surprising considering that chromosome 11 is the second most frequent chromosome that is structurally or numerically aberrant or involved in LOH in all neoplasias (16). Specific translocations and rearrangements of chromosome 11 in several different cancers have lead to the cloning of breakpoints and genes (2). The three tumors with LOH at 11q13—23 (Fig. 1b) are of special interest because of other evidence for the presence a suppressor gene in this region between the cyclin D1 and INT2 genes (49).

Of particular relevance to the interaction of cellular genes with those of HPV's in cervical carcinoma is the evidence of Smits et al. (51) that the 11p11—15 segment is likely to contain a gene or genes involved in the regulation of HPV-16 early enhancer promoter and in the suppression of the transforming activity of the viral DNA. If this is correct, then in tumors that have not lost this segment of chromosome 11, the activity of the viral early promoter should be downregulated, thereby suppressing HPV-induced transformation characteristics. In cervical carcinoma, where this short arm segment is lost fairly often, this suppression should not occur, and such tumors would be expected to be more aggressive.

Koi et al. (52) were able to achieve growth arrest of rhabdomyosarcoma cells by introducing into them a human 11p15 subchromosomal transferable fragment. We reported that a deficiency in the number of chromosome 11s and/or loss of chromosome 11p occurred in approximately one-third of all cervical carcinomas (53, 54), which agrees well with the present findings of LOH of one or more markers of chromosome 11 in 32% of the tumors. It will be interesting to determine whether tumors that are positive for HPV-16 (and perhaps for other HPV types with similar transforming sequences) and have LOH or deletions of the p11—15 region fall into a category of more aggressive tumors.

Chromosome 13. In our material, this carrier of the retinoblastoma suppressor gene (RB1) is involved in the LOH in 26% of cervical carcinomas, placing it fifth in rank of the chromosome arms with LOH in our series. Mitra et al. (8) had a similar incidence of LOH. Chromosome 13 is fourth in the rank of chromosomes with LOH in all types of cancers, including several very common cancers such as those of the breast, lung, stomach, kidney, liver, and bone (17). One proposed mechanism of action of the high-risk oncogenic HPV types in cervical carcinomas is the inactivation of the RB1 gene product by direct mutation of RB1 as described in many other cancers (18, 19). It is interesting that 3 of 10 tumors with LOH on 13q in this series are HPV negative, suggesting the possibility that in these cases an oncogenic event was the direct mutational loss of function of the RB1 gene. We plan to test this possibility.

Chromosome 17. This carrier of the p53 gene at 17p13.1 was involved in LOH in 5 (15%) tumors; 3 of these expressed mutant p53, of which 2 were HPV negative, and the third contained HPV-33 sequences (see below). These three cases were among the top four cases in respect to the number of loci involved in LOH, and all three were of pathological stage IIIB (Fig. 1c). We reported the findings on p53 in the first 27 cases included previously, and the p53 data in Fig. 1, a and c, are from that report (14). A total of 8 (12.3%) of 65 HPV-negative tumors reported in the literature, including this report, were found to have p53 mutations (8, 14, 59—66). Thus, analogous to what has been found in many other cancers, loss of wild-type p53 suppressor gene function appears to be one of the important events in the transformation or progression of cervical carcinomas. Most often this occurs when the E6 oncogene product of the high-risk HPV types inactivates the p53 product or, less frequently and in the absence of HPV sequences, because of a direct mutation of p53 as described previously. The tumor that had LOH of both 17p markers, expressed mutant p53, and contained HPV-33 sequences is interesting in respect to the association of mutant p53 with HPV-33 reported by Lo et al. (66), who suggest that the E6 protein of HPV-33 may have a reduced binding affinity for p53 protein and, hence, may be less effective in inactivating the wild-type p53.

Wrede et al. (57), Crook et al. (59), and Tsuda et al. (60) reviewed the evidence indicating that cervical tumors with mutant p53 are more aggressive than those without this mutation and occur in older women. In our sample, the mean age of the 30 women with HPV-positive tumors is 42.1 years. In comparison to this, the mean for the five cases with LOH on 17p is 55 years and for the three cases with mutant p53 is 62 years. All three of the latter tumors are at the highest pathological stage (stage IIIb) and are among the four tumors with the greatest number of loci involved in LOH (Fig. 1c). It will clearly require a larger series of cases to determine the validity of this trend.

Chromosome 17 is first in rank among those chromosomes with LOH in all types of cancer; most of them are common cancers such as those of the colon and breast (16, 17). The related alteration of the p53 gene is found in more cancers than any other gene. In addition to the p53 gene, several other cancer-related genes are on chromosome 17, including the BRCA1 gene, which, when mutated and inherited, predisposes to breast and ovarian cancers (67). One or more of these genes is related to familial breast and ovarian cancer.6

Chromosome 18. The LOH incidence of 26% for 18p and of 35% for 18q, which we found, strongly suggests that these arms, particularly 18q, contain genes that play an important role in cervical carcinoma. This is the first time this high incidence of LOH on 18q has been reported in cervical cancer. Mitra et al. (8) found only 2 of 21 (10%) informative cases with LOH on 18q. Also, in contrast to our findings, they report a higher incidence of 6 of 16 (38%) informative cases with LOH on 18p. The discrepancy may be due to the smaller number of informative cases in their study and/or the difference in the map location of the probes. LOH of this chromosome, particularly 18q21—qter, appears to be an important event in colon, breast, ovarian, gastric, kidney, prostate, and non-small cell cancers of the lung (17) and usually involves the DCC colon cancer tumor suppressor gene at 18q21.1. This band was definitely involved in 6 of 12 tumors with LOH of 18q in this study and could not be excluded as being involved in the other 6 cases because the appropriate loci were uninformative (Fig. 1c). The LOH of chromosome 18 has been associated with poor prognosis and with progression from the intramucosal to the invasive stage in colon cancers (1). Germ-line mutations of a gene on chromosome 18 may play a role in the familial forms of breast and ovarian cancers (68). It is interesting that non-tumorigenic HPV-18-immortalized keratinocyte cell lines that were chemically transformed to tumorigenicity showed 18q deletions and loss of DCC expression (69). It will be interesting to determine whether DCC alterations are involved in cervical carcinomas and, if so, whether any are familial.

Conclusions. The higher overall incidence of LOH in cervical carcinomas as compared to many other cancers, and the diversity of LOH patterns found, suggest that different groups of cervical carcinomas probably arise and/or progress, in part, because of the loss of function or alterations of different yet finite sets of tumorigenicity suppressor genes and genes that are involved in tumor progression and metastasis. This is not surprising considering the fact that both squamous cell and adenocarcinomas, varying considerably in their degree

6 See the 17 papers devoted to this in Vol. 52, No. 4 of Am. J. Hum. Genet., 1993.
of differentiation and other histopathological characteristics, were examined. Also, cases infected with different types of HPV, as well as HPV-negative cases, were examined. The relatively small number of cases in each of the different histopathological and HPV groups precludes attempts to establish correlations at this stage.

The findings also indicate that certain restricted chromosome segments that are frequently genetically altered in cervical carcinomas are also frequently altered in several other types of cancers. That p53 and Rb1 genes are involved in some cases is certain. It remains to be determined whether the other segments with a high incidence of LOH involve the same genes or different genes located within these segments in other types of cancers. The high degree of coincidence of these segments makes it seem likely that the same genes are involved in at least some of the cases. We intend to clarify this question because genes that are common to several cancer types would be particularly interesting candidates for cloning attempts.

Recently, Mitra et al. (70) reported that allelic loss and microsatellite instability in the region of the DSS406 locus at 5p15 may play a role in the early development of cervical cancers and might serve as a potential marker for progression of precancerous cervical lesions. This was based, in part, on a incidence of 55.6% (25 of 45 cases) LOH at this locus at 5p15.1–15.2 in invasive carcinomas. Our incidence at the adjacent 5p13.1–14 segment is 20% for all cases and is consistent with the findings of Mitra et al. (70) of 27.6% at 5p14 in the invasive cases. If these findings are correct, then the critical alteration appears to be within and restricted to the 5p15 segment, for which we did not use a probe. As these authors indicate, a larger study will be necessary to validate this indication for a potentially valuable prognostic marker. However, our findings and those of others make it seem likely that one such marker will not be adequate. Additional investigations are likely to lead to the assembly of a battery of such markers that might provide reliable prognostic indications in most if not all cases.

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205

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