Alterations of the p53 Gene in Human Primary Cervical Carcinoma with and without Human Papillomavirus Infection

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ABSTRACT

A previous report using cervical carcinoma cell lines suggests that the inactivation of two tumor suppressor gene products, p53 and pRB, either by complex formation with the E6 and E7 proteins of oncogenic human papillomaviruses (HPVs) or by mutation, may be an important step in cervical carcinogenesis (M. Scheffner et al., Proc. Natl. Acad. Sci. USA, 88: 5523-5527, 1991). The present study was designed to clarify the association between p53 inactivation and infection with oncogenic HPVs in primary carcinomas of human uterine cervix. We examined 36 primary cervical carcinomas for the presence of HPV DNAs by Southern blot analysis with probes specific for HPV-16, -18, -31, -33, -35, -39, -52, -56, and -58. HPV DNA sequences were detected in 19 of 36 tumors; 10 cases with HPV-16; 3 cases with -18; 3 cases with -58; 2 cases with -56; and one case with -52. The presence of HPV-16 and -18 in cervical carcinomas was further reexamined using polymerase chain reaction. HPV DNA sequences were detected in an additional 10 cases: 9 cases with -16 and one case with -18. The inactivation of the p53 gene by allelic loss or by point mutation was also examined. No allelic loss at the polymorphic site in codon 72 of the p53 gene was detected in any of 10 informative cases. Missense point mutations in the highly conserved regions of the p53 gene were demonstrable as single-stranded conformational polymorphisms of polymerase chain reaction-amplified DNA fragments and subsequently identified by direct DNA sequencing. Point mutations were detected in only two cases: one with an ATG→CTG transversion in codon 133 of exon 5, resulting in a Met→Leu substitution, and another with a CCC→TGG transition in codon 248 of exon 7, resulting in an Arg→Trp substitution. Both tumors with point mutations in p53 genes were among 10 tumors which contained a small copy number of HPV-16 DNA sequences (1 copy of HPV/10¹ to 10⁴ cells) detectable by polymerase chain reaction amplification but not by Southern blot analysis of genomic DNAs derived from the tumors. None of 19 tumors with a large copy number of HPV DNA sequences detectable by Southern blot analysis of more than 1 copy of HPV/2 to 10 cells) nor any of 7 tumors with undetectable HPV DNA sequences contained p53 gene mutations in the regions examined. These observations suggest that, in contrast to data derived from cultured cervical carcinoma cell lines, inactivation of the p53 gene by allelic loss or by point mutation is infrequent in clinical samples of cervical carcinomas of the human uterus, irrespective of the presence or absence of HPV infection.

INTRODUCTION

Carcinoma of the uterine cervix is one of the most common neoplasias among women. Epidemiological studies suggest the involvement of numerous risk factors in the etiology of cervical cancer. However, recent studies show strong associations between specific strains of HPVs and cervical cancer (1).

HPVs have been classified as either "high risk" or "low risk" based on their association with anogenital carcinomas or their precursor lesions (2). The "low-risk" HPVs such as types 6 and 11 are associated with condylomas, which rarely progress into malignancy. In contrast, HPV-16, -18, -31, -33, -35, -39, -52, -56, and -58 are variously frequently found in invasive carcinomas and therefore constitute high-risk types (3). Analysis of the viral genome has revealed that the two early genes, E6 and E7, of the high-risk HPVs are transforming genes, in contrast to these genes of the low-risk HPVs, the products of which are either nontransforming or very weakly transforming (2). In vitro studies indicate that the protein encoded by the E6 gene of HPV-16 and -18 can form a stable inactivating complex with p53 in the same manner as the simian virus 40 T-antigen and adenosine E1b proteins (4). Also, the protein from the E7 gene of high-risk HPVs has the same capacity to bind pRB as the simian virus 40 T-antigen and the adenosine E1a protein (5). On the other hand, E6 and E7 proteins of low-risk HPVs, HPV-6 and -11, do not associate with these suppressor gene products (4, 5). Loss of normal tumor suppressor function of these two genes can contribute directly to the progression of cellular transformation (2). It is not yet clear, however, whether both p53 and pRB are always inactivated in primary cervical carcinomas positive for HPV-16 and -18, or whether E6 and E7 proteins of other high-risk HPVs such as type-31, -33, -35, -39, -52, -56, and -58 also target p53 and pRB. The p53 gene encodes a nuclear phosphoprotein which is involved in the negative control of cell proliferation (6). Alteration of the p53 gene by base substitution, deletion, or insertion mutations or by allelic loss or rearrangements is observed in a wide variety of human cancers and is currently the most commonly found alteration associated with human cancers (7, 8). In colorectal tumors, 80% show alterations of the p53 gene in both alleles, one through deletion and the other through a base substitution mutation in highly conserved coding regions of the gene (7, 9).

A similar pattern of the inactivation of p53 is reported in lung, breast, and brain tumors (8, 10, 11). If one of the main targets of the E6 gene product of oncogenic HPVs is cellular p53 protein in cervical carcinoma in vivo, and if the inactivation of p53 is essential in the etiology of cervical carcinoma, one might predict alternative mechanisms for the inactivation of the p53 gene, by gene deletion or point mutation, in cervical carcinomas which lack active HPV infection. In support of this, it has been reported recently that two cervical carcinoma cell lines, C33A and HT-3, neither of which contains HPV DNA, have a point mutation in codons 273 and 245, respectively, whereas none of the HPV-positive cell lines contained p53 gene mutations (12, 13). It is still unclear, however, whether the inactivation of p53 gene plays a significant role in the etiology of cervical carcinoma, since the number of samples reported was small (6 HPV-positive and 2 HPV-negative altogether) and since the state of the p53 gene in primary cervical carcinoma is unknown. In addition, recent evidence has shown that even normal primary fibroblasts have a mutated p53 gene in the earliest of cell passages, making results obtained from long-term cell lines suspect (14).

These circumstances prompted us to investigate the association between p53 inactivation and high-risk HPV prevalence in primary tumors of the human uterine cervix. We examined 36
primary cervical carcinomas for the presence of the HPV genome by Southern blot analysis of genomic tumor DNA and PCR-amplified DNA. We have also studied these tumors for loss of heterozygosity in the p53 gene using PCR (15), screened for mutations in the p53 gene by single-stranded conformational polymorphism analysis of PCR-amplified DNA fragments (16), and confirmed the identity of these mutations by genomic DNA sequencing.

MATERIALS AND METHODS

Tissue Samples and DNA Extraction. Samples used in this study were obtained at biopsy or surgery on patients who were admitted into the Department of Obstetrics and Gynecology at the Osaka University Hospital. Surgically removed tissues were sampled for histopathological diagnosis, and remainders were frozen for the extraction of DNA. Histological classification of tumors was carried out according to the WHO histological typing system (17). Samples which contained more than 50% of tumor cells were used in this study. Tumors included 30 squamous cell carcinomas and 6 adenocarcinomas of the uterine cervix. The clinical stage of the disease was also established according to the International Federation of Gynecology and Obstetrics staging system (18).

High-molecular-weight genomic DNA of which was provided by Dr. U. Schlegel. The primers used were 5'-GATGCTGTCCGCGGACGATATT-3' (upstream) and 5'-CGTG-CAGTCAGACITTGTCG-3' (downstream) (15). PCR amplification was performed using 1 μl of each diluted sample as a template, and the amplified DNA fragments were processed for Southern blot hybridization analysis as described above.

Detection of HPV DNA. High-molecular-weight genomic DNAs (10 μg) extracted from aliquots of frozen tissue were digested with PstI, fractionated in 1% agarose gel, and transferred to a nitrocellulose filter by the method of Southern. Initially, hybridization and washing were performed under conditions of low stringency for the screening of HPV infection. Filters were prehybridized at 42°C with 20% formaldehyde, 5× SSC, 20× Denhardt's solution, 50 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer (pH 7), and 100 μg/ml of denatured salmon sperm DNA, and a 32P-labeled HPV-58 DNA probe (7 ng/filter, 1.2 x 106 cpm/ng) was added to the prehybridization buffer. The filters were washed once with 2× SSC, 0.1% SDS at room temperature for 5 min and three times with 2× SSC, 0.1% SDS at 48°C for 15 min and then autoradiographed. After initial screening for HPV infection, the same filters were used for typing HPV. Filters were stripped and then rehybridized with probes specific for HPV-16, -18, -31, -33, -52, -56, and -58, sequentially under stringent conditions for the screening of HPV infection. Filters were prehybridized at 42°C with 20% formaldehyde, 3× SSC, 10× Denhardt's solution, 50 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer (pH 7), and 100 μg/ml of denatured salmon sperm DNA. The filters were washed with 0.2× SSC, 0.1% SDS at 60°C for 1 h and autoradiographed.

The presence of HPVs was further examined using PCR to generate amplified DNA fragments within the open reading frames of E6 of both HPV-16 and -18 (20). The PCR mixture was prepared from a GeneAmp DNA amplification reagent kit (Perkin-Elmer Cetus Corp., Norwalk, CT) following the manufacturer's instructions. Primers used were 5'-ATTAGTGAGTATAGACATTA-3' (H1), 5'-GGCTTTTGACATACATAGTG-3' (H2), 5'-ATTAGTGAGTATAGACATTA-3' (H3), 5'-ATTGGAACAACTAGAAGACGACATTACAAACACCGTTGTG-3' or HPV-18 (H5): 5'-ATTGGAGACACATTGGAAAAACTAACTAACA-CTGGTATA-3' at 37°C for 16 h in a buffer containing 50% formamide, 4× SSC, 5× Denhardt's solution, 50 μg/ml of denatured salmon sperm DNA, and 0.2% SDS. After hybridization, filters were washed twice with 2× SSC and 0.1% SDS at room temperature for 15 min and once with 0.1× SSC and 0.1% SDS at 60°C for 30 min.

In an attempt to evaluate the sensitivity and specificity of our standard protocol to detect HPV DNA sequences, we performed the following dilution assay. A series of 2-fold dilutions was made of DNA derived from HeLa cells, which contain 10-50 copies of HPV-18/cell (21), and of DNA derived from Caski cells, which contain as many as 600 copies/cell of HPV-16 DNA sequences (21), by adding DNA derived from normal WBC. Five μg of each sample were processed for Southern blot analysis and hybridized with a probe specific for HPV-16 or -18. The sensitivity and specificity of PCR-Southern blot analysis were also evaluated. A series of 10-fold dilutions was made of the DNAs derived from HeLa and Caski cells. PCR amplification was performed using 1 μl of each diluted sample as a template, and the amplified DNA fragments were processed for Southern blot hybridization analysis as described above.

Detection of Loss of Heterozygosity in p53 Gene. The second position of codon 72 of the p53 gene is naturally polymorphic; CGC (Arg) as well as CCC (Pro) are frequently observed (22). This polymorphism can be detected by the restriction enzyme BstUI if the sequence CGCG is present (15). PCR was performed to generate amplified DNA fragments of 247 base pairs surrounding codon 72. The primers used were 5'-GATGCTGTCCGCGGACGATATT-3' (upstream) and 5'-CGTG-CAGTCAGACITTGTCG-3' (downstream) (15). One base in this upstream primer was altered to create an artificial invariant BstUI site for the internal control for BstUI digestion. DNA extracted from the tumors and WBC of the patients were used for the PCR template. The PCR incubation mixture was first heat-denatured for 10 min, then 2.5 units of Taq polymerase were added. One cycle of PCR consisted of 1 min at 95°C, 30 s at 50°C, and 30 s at 72°C, and a total of 30 cycles were performed. Twenty μl of amplified fragments were digested with 0.2 U of BstUI at 12 h at 60°C and then fractionated on a 12% polyacrylamide gel. DNA fragments were visualized by staining with ethidium bromide. The 247-base pair DNA fragments were cut into two fragments (236 and 11 base pairs) when no restriction site was present in codon 72, whereas three fragments (160, 76, and 11 base pairs) were yielded with the presence of the restriction site in that codon.

Detection of Point Mutations in p53 Gene by PCR-SSCP Analysis. Exons 5–8 of the p53 gene were amplified by PCR using the primers the sequences of which were provided by Dr. U. Schlegel. The primers used are as follows:

- exon 5: 5'-GTATCCTCCCCGCGCTCACA-3' (upstream), 5'-CTCACCATCGCTATCTGAGCA-3' (downstream)
- exon 6: 5'-TTGCTCTTATAGGTCTGGCCCC-3' (upstream), 5'-CAGACCTACCGGGCTCATA-3' (downstream)
- exon 7: 5'-TGGGTTGGCTCTGACGTGACCC-3' (upstream), 5'-TGACCTGGAGTCTCTCCAGGG-3' (downstream)
- exon 8: 5'-ATGGGTAATCTACTGGAGCC-3' (upstream), 5'-ACCTCGCTAGTGTCTCC-3' (downstream)

The PCR reaction mixture (total 3 μl) contained genomic DNA (0.1 μg), deoxynucleotide triphosphates (60 μM), 32P-end-labeled primer (0.1 μM each), MgCl2 (1.5 mM), Tris (pH 8.3) (10 mM), KCl (50 mM), and Taq polymerase (0.1 units; Perkin Elmer Cetus Corp., Norwalk, CT), and PCR amplification was performed for 30 cycles. The annealing temperatures were 58°C, 62°C, 60°C, and 60°C for exons 5, 6, 7, and 8, respectively. DNAs extracted from the WBC of each patient were also amplified as normal controls. SSCP analysis was conducted according to the previous report (16) with the following modifications. The PCR product (3 μl) was diluted 10-fold with stop solution (20 mM EDTA, 95% formamide, 0.05% bromphenol blue, 0.05% xylene cyanol) and heat-denatured at 98°C for 5 min. One μl of this mixture was immediately loaded into an 8% nondenaturing acrylamide gel (0.5% acrylamide: methylenebisacrylamide, 80:1) and run at constant voltage of 400 V at 4°C for 12–16 h. The gel was vacuum dried and exposed to Kodak X-Omat film at room temperature for 1–2 days.
DNA Direct Sequencing Analysis. PCR-amplified DNA fragments which showed bands with altered mobilities by SSCP analysis were further analyzed by direct sequencing. The PCR-amplified fragments were purified on a 2% low-melting-temperature agarose gel and used for asymmetric PCR to generate single-strand DNA fragments for sequencing. Asymmetric PCR amplification was performed with 50 pmol of downstream primer, 1 pmol of upstream primer, and 10 ng of template, and a total of 15 cycles were performed. The asymmetric PCR products were purified and concentrated by Centricon 30 ultrafiltration units (Amicon, Danvers, MA) and precipitated with ethanol. Sequencing was performed by the dideoxy method with 32P-end-labeled upstream primer in each exon using a Sequenase 2.0 kit (USB, Cleveland, OH). One pmol of DNA template and 1 pmol of 32P-end-labeled upstream primer were dissolved in 10 μl of 1X Sequenase reaction buffer, heat-denatured at 98°C for 2 min, annealed at 65°C for 10 min, and gradually cooled down to room temperature. Then, 2 units of Sequenase, 1.75 μl of enzyme dilution buffer, 1 μl of O.l M dithiothreitol, and 1 μl of H2O were added. This mixture was divided into four tubes containing 2.5 μl of dideoxy G, A, T, or C termination mixtures and incubated for 5 min at 37°C, after which stop solution (4 μl) was added. The samples were denatured at 80°C for 2 min and electrophoresed on an 8 M urea/8% polyacrylamide gel.

RESULTS

Detection of HPV in Cervical Cancer. DNAs from a total of 36 cervical carcinomas of human uterus, 30 squamous cell carcinomas, and 6 adenocarcinomas were examined in the present study. Southern blot analysis of genomic DNA with HPV probes showed that 19 of 36 tumors (53%), 16 squamous cell carcinomas, and 3 adenocarcinomas contained HPV DNAs (Fig. 1, A and B): 10 cases with type 16 (28%); 3 cases with type 18 (8%); 3 cases with type 58 (8%); 2 cases with type 56 (6%); and one case with type 52 (3%).

The presence of HPV-16 or -18 was further examined by Southern blot analysis of PCR-amplified DNA fragments (Fig. 1, C and D). Our standard protocol for the analysis of genomic DNA could detect HPV-16 DNA sequences as small as 1 copy/10 cells and HPV-18 DNA sequences as small as 1 copy/2-10 cells, which is consistent with the previous report (23). On the other hand, the evaluation of amplified PCR fragments significantly enhanced sensitivity, allowing the detection of 1 copy of HPV/10^5 cells (data not shown), which is also

Table 1 Status of HPV prevalence and p53 alterations in 36 cervical carcinomas

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* CIS, carcinoma in situ; SCC, squamous cell carcinoma; QI, questionable invasion; L, large cell type; K, keratinizing type; AD, adenocarcinoma; W, well-differentiated type; M, moderately differentiated type; P, poorly differentiated type; C, clear cell type; NT, not tested; -, not detected; wt, wild type.
P53 MUTATIONS AND HPV INFECTIONS IN CERVICAL CARCINOMAS

comparable to the results of the previous report (23). By using PCR, HPVs were detected in an additional 10 cases which initially yielded no detectable HPV sequences from genomic DNA: 9 cases with type 16 and one case with type 18. Mixed infections with both -16 and -18 were observed in 2 cases (cases 4 and 19). In 11 cases in which we detected HPV-16 DNA (cases 1, 11, 12, 13, 17, 18, 28, 29, and 32) or HPV-18 DNA (cases 16 and 35) by Southern blot hybridization analysis, we confirmed the presence of HPV-16 or -18 by Southern blot analysis of PCR fragments.

In summary, HPV sequences were detected in 29 of 36 cervical carcinomas (81%): type 16 in 18 cases; type 18 in 3 cases; type 58 in 3 cases; type 56 in 2 cases; type 52 in one case; and mixed infection with both types 16 and 18 in 2 cases (Table 1).

Loss of Heterozygosity in the p53 Gene in Primary Cervical Carcinoma. Of 28 cases in which DNA derived from WBC or normal tissues was available, 10 cases showed heterozygosity in codon 72 of the p53 gene by PCR-restriction fragment length polymorphism analysis. However, loss of heterozygosity was not observed in any of the 10 informative cases (Table 1).

Mutations in the p53 Gene in Primary Cervical Carcinoma. Mutations in exons 5 through 8, where the vast majority of point mutations are reported in human neoplasms, were screened by PCR-SSCP analysis. Altered mobilities of the PCR product, which suggest the existence of mutation, were observed in 2 of 36 tumors (6%): one in exon 5 (case 26) and another in exon 7 (case 36) (Fig. 2). In addition to the one or two bands with mobility shifts, two bands corresponding to the normal allele were observed in these two cases, whereas only the two normal allele bands were seen in the remaining 34 tumors. Mutations in p53 genes were also confirmed by direct sequencing. An ATG—CTG transversion in codon 133 in exon 5 in case 27, resulting in a Met—Leu substitution, and a CGG—TGG transition in codon 248 in exon 7 resulting in an Arg—Trp substitution, were detected (Fig. 3).

Fig. 3. Demonstration of point mutations in p53 gene by direct genomic sequencing. A, an ATG—CTG transversion in codon 133 in exon 5 in case 26 resulting in a Met—Leu substitution; B, CGG—TGG transition in codon 248 in exon 7 resulting in an Arg—Trp substitution.

DISCUSSION

A strong association between HPV-16 or -18 infection and cervical carcinoma has been reported from many geographic areas using a variety of techniques: Southern blot hybridization (24); in situ hybridization (25); or PCR (20). In Japan, HPV-16 (33%) was the predominant subtype detected in cervical carcinomas by Southern blot hybridization analysis. However, two tumors which contained point mutations in the p53 gene were among those in which a small copy number of HPV DNA was observed (1 copy/10^1 to 10^5 cells), so HPV DNA was detectable only by PCR. Moreover, 7 tumors in which we did not detect any HPV DNAs contained no point mutations in the p53 gene. Both tumors with point mutations in the p53 gene were in advanced stages (case 26 in stage II and case 36 in stage IV).
analysis of genomic DNA. The incidence of HPV infection in cervical carcinomas using PCR is 84% in France (29) and 85% in Japan (30), which is also comparable with our findings.

Our results show that inactivation of the p53 gene by allelic loss or by point mutation was infrequent in primary cervical carcinoma in the population of Japanese women in this study. Allelic loss was not observed in 10 informative cases, and the overall incidence of point mutations in p53 gene was 6% (2 of 36). No mutations in the p53 gene were observed in 19 tumors with HPV copy numbers large enough to be detected by Southern blot analysis (>1 copy/2-10 cells). The absence of point mutations in the p53 gene in these tumors is consistent with the observations in cervical carcinoma cell lines, in which no mutations were found in five HPV-positive cell lines (12). It is unclear, however, whether the p53 protein is sufficiently inactivated by complexing with E6 protein in any of the 19 cervical carcinomas with a large copy number of HPV DNAs and, thus, whether inactivation of p53 consequently plays a role in transformation in these tumors.

If the inactivation of the p53 gene is essential for cervical tumorigenesis, allelic loss or point mutations in p53 in the tumors negative for HPV infection would be predicted. However, we detected neither allelic loss nor point mutations in 7 HPV-negative tumors. Moreover, since all of the tumors we have analyzed contained more than 50% of tumor cells, it is unlikely that p53 is sufficiently inactivated by complexing with E6 in the tumors in which HPV could be detected only by PCR (therefore they contain 1 copy of HPV/10^4 to 10^6 cells). Nevertheless, we detected point mutations in p53 in only 2 of 10 tumors in which HPV could be detected only by PCR. Therefore, inactivation of the p53 gene may not be obligatory for tumorigenesis of uterine cervix. For primary human keratinocytes, it has been demonstrated that efficient immortalization of cells requires both the E6 and E7 genes (31, 32); however, E7 alone can less efficiently immortalize the cells (33). This suggests that the E7 gene contains the main transforming ability and that the E6 gene enhances the ability of the E7 gene to immortalize human keratinocytes. Since E7 may contain the principal transforming activity of HPV, inactivation of the RB gene, and not the p53 gene, may be the critical event. It may also be possible that there are new types of HPVs or related oncogenic viruses, which are undetectable by the present methodologies, that can inactivate p53 protein in a manner similar to that of E6 of HPV-16 or -18.

Two point mutations in the p53 gene were in the five domains highly conserved in mammals, amphibians, birds, and fish (34): one was in codon 133 in exon 5 of domain ii, the other was in codon 248 in exon 7 of domain iv. It is unlikely that mutations exist in exons that were not evaluated, since studies that included outlying exons suggest that mutations outside exons 5–8 are rare (35). It is also unlikely that we did not detect p53 alterations due to the technical failure, since we observed both allelic loss and point mutations in significantly higher incidence in endometrial carcinoma of uterine corpus using the same method (allelic loss, 11 of 19, 58%, P = 0.0022 by Fisher’s exact test; point mutations, 10 of 39, 26%, P = 0.018). The incidence of Ki-ras-2 mutations is also reported to be significantly lower in cervical carcinoma than in endometrial carcinoma (36). These observations may indicate an important difference in the tumorigenic pathway between these tumors. There are several reports which suggest that other cellular genes are involved in cervical tumorigenesis. By introducing normal human chromo-

some 11 via microcell fusion, tumorigenicities of HPV-18-positive HeLa cells and HPV-16-positive SiHa cells are completely suppressed (37, 38). Human embryonic fibroblasts with a large deletion in the short arm of one copy of chromosome 11 are susceptible to transformation by early HPV-16 DNA, whereas normal diploid human embryonic fibroblasts are not (39). These observations suggest the presence of a putative tumor-suppressor gene on chromosome 11 for HPV-mediated transformation. The gene located on chromosome 11 between 11p11 and 11p15 suppresses transformation induced by HPV-16, at least in part, by down-regulating the ability of the viral early promoter (40). By Southern blot analysis using polymorphic markers, genes located on chromosome 11q may be tumor suppressor genes for cervical carcinoma (41). Using the same approach, loss of heterozygosity at chromosome locus 3p14–21 was observed in 9 of 9 cervical carcinomas (42). Moreover, point mutations in codon 12 of H-ras were frequently detected in cervical carcinomas of advanced stages, but not in early stages (43). These observations may indicate that in addition to p53 and RB, other oncogenes or suppressor genes function in a significant manner during the tumorigenesis of the uterine cervix, a situation analogous to the tumorigenesis of colon epithelium.

The present findings that point mutations in p53 were detected in none of 7 HPV-negative cervical carcinomas or only 2 of 10 tumors with low copy numbers of HPV may suggest that there is an alternative pathway in the etiology of cervical carcinomas, one which is independent of p53 inactivation, especially in tumors which were negative for HPV infection. Riou et al. (29) reported that patients with no detectable HPV DNA had a 2–6 times higher risk of overall relapse and 4–5 times higher risk of distant metastasis. Differences in the genes involved in carcinogenesis may influence the clinical outcome in these tumors. Further studies will be necessary to identify the genetic changes including inactivation of RB genes that are involved in tumorigenesis of human uterine cervix.

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