Clonal History of Papillomavirus-Induced Dysplasia in the Female Lower Genital Tract

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Background: **Dysplastic lesions of the vagina or the vulva often occur in women who have a previous history of cervical dysplasia. Most lesions in the female lower genital tract are induced by infections with high-risk oncogenic human papillomaviruses (HR-HPVs), including HPV16 and HPV18. HR-HPV genomes frequently integrate into host cell chromosomes at random sites. We analyzed viral integration sites in multiple metachronous lesions of the lower genital tract from women previously treated for HR-HPV–positive cervical dysplasia or cancer to determine whether the metachronous lesions emerged from a single common preexisting dysplastic cell clone or as consequence of independent HR-HPV infection events in the female lower genital tract.** ***Methods:*** **From among 1500 patients with anogenital lesions, seven patients with high-grade vaginal or vulvar lesions and with a previous history of cervical disease (five with prior high-grade cervical dysplasia and two with a history of cervical cancer) were included in this study. Integration sites of HPV16 or HPV18 in vaginal or vulvar lesions were mapped by an adaptor ligation polymerase chain reaction (PCR) method. The sequence information was used to design an integrate-specific PCR assay that was applied to DNA extracted from archival paraffin-embedded material derived from biopsy samples of cervical lesions.** ***Results:*** **Identical HPV DNA integration loci were found in vaginal or vulvar and cervical samples of all lesions available for four of the five patients with a prior history of high-grade cervical dysplasia and for both patients with a history of cervical cancer.** ***Conclusions:*** **These data indicate that high-grade dysplastic lesions in the female lower genital tract may emerge primarily as monoclonal lesions from a transformed cell population derived from the uterine cervix. [J Natl Cancer Inst 2005;97:1816–21]**

Dysplastic and neoplastic lesions of the cervix, vagina, vulva, and anus have similar risk factors. It is well accepted that persistent infection with high-risk (i.e., oncogenic) human papillomaviruses (HR-HPVs) are required for the development of most of these lesions *(1)*. HR-HPV infections are particularly common in young, sexually active women *(2)*. Results of cross-sectional studies have revealed that although up to 40% of such women are infected with an HR-HPV, fewer than 10% of infected women develop persistent HPV infections and only 28% of those who do will develop high-grade intraepithelial dysplasia of the cervix within 5–6 years of the initial infection *(3–7)*. Thus, almost all HR-HPV infections resolve spontaneously without causing clinically relevant dysplastic lesions in the genital tract *(8,9)*. These epidemiologic features suggest that, although neoplastic transformation of epithelial cells in the cervix depends on HR-HPV infections, other factors are required to permit progression of HR-HPV infections to high-grade lesions and invasive cancer.

The clinical manifestations of dysplastic lesions in the various epithelial regions of the female genital tract (cervix, vagina, and vulva) show remarkable differences. For example, the incidence of cervical intraepithelial neoplasia (CIN) is 10-fold higher than the incidence of dysplastic lesions of the vagina or vulva. Also, the incidence of cervical dysplasia and derived cervical carcinomas peaks approximately 10 years earlier than the incidence of vaginal cancer *(10,11)*. These observations suggest that, despite the high frequency of HPV infection events in the female lower genital tract, transformation of the vaginal or vulvar epithelium occurs substantially less often than that of the cervical epithelium.

Most vaginal and vulvar lesions occur in women who have a history of HR-HPV–induced cervical dysplasia or carcinomas *(12–14)*. These lesions may arise at the same time (synchronous lesions) or up to several years after the initial cervical lesion (metachronous lesions). Several studies *(15–17)* have demonstrated that women who had had a high-grade cervical lesion or invasive cervical cancer had an increased risk of developing a metachronous cancer within the lower genital tract compared with women in the general population. The existence of both synchronous and metachronous lesions suggests that several independent local infection events induce development of multiclonal dysplastic lesions at distinct anatomic sites. The frequent development of dysplastic lesions in women who have vaginal and vulvar lesions associated with previous cervical disease might reflect their predisposition for HR-HPV–related lesions compared with the general population. Alternatively, the multicentric lesions might all originate from a distinct epithelial cell

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population that was infected and transformed by HR-HPVs before being disseminated throughout the epithelium of the genital mucosa. In the latter scenario, all lesions would be expected to be derived from one initially transformed cell clone.

Transformation of epithelial cells by HR-HPVs is mediated by the expression of two viral oncoproteins, E6 and E7. These proteins are required to induce and maintain the transformed phenotype of epithelial cells *(18)*. Expression of these viral oncoproteins in epithelial stem cells of the anogenital mucosa also interferes with cell cycle control and with the function of the mitotic spindle apparatus, resulting in severe chromosomal instability and structural and numeric chromosomal aberrations *(19–22)*. As a consequence of such chromosomal instability, the HPV genome may become integrated into the host cell genome by nonhomologous recombination, a feature that characterizes many high-grade dysplastic lesions and most invasive carcinomas *(23–27)*. HPV DNA integration sites are distributed randomly throughout host-cell genomes *(27)*. Thus, the unique junction sequences that are created by the integration of an HPV genome into a host cell genome represent specific molecular markers for each HR-HPV–induced transformed cell clone *(28,29)*.

We analyzed viral integration sites in genomic DNA isolated from anatomically independent lesions of the female lower genital tract from individual patients who were previously treated for dysplastic cervical lesions. To examine the clonal origins and relationships among the lesions, we studied samples obtained from patients who fulfilled the following criteria: those with a previous history of cervical disease who had vaginal or vulvar lesions that featured integrated HPV16 or HPV18 genomes in the primary lesion and for whom archival material of the previous cervical lesions was available. The samples were obtained from women participating in an ongoing clinical study of more than 1500 patients on the frequency of integrated HPV genomes in anogenital lesions *(24)*. We identified seven patients who fulfilled these criteria. Six patients had lesions in the vagina and one patient had a lesion in the vulva. Five patients had a previous history of high-grade squamous intraepithelial lesions (cervical intraepithelial neoplasia grade 2 or higher [≥CIN2]) and two patients had a previous history of squamous cell carcinoma of the cervix.

Materials and Methods

Clinical Samples

Vulvar and vaginal biopsy samples were collected from patients with anogenital lesions who were treated in the Departments of Obstetrics and Gynecology at the Universities of Heidelberg and Leipzig and immediately snap frozen in liquid nitrogen. The patients provided written informed consent; this study was approved by the local ethical committees at Heidelberg and Leipzig University. Total genomic DNA was extracted from the fresh-frozen vaginal and vulvar biopsy samples and from archival paraffin-embedded clinical samples with the use of a DNeasy tissue kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. HPV typing was performed by using the $GP5^+$/$GP6^+$ polymerase chain reaction (PCR)–enzyme immunoassay method as described by Jacobs et al. *(30)*. DNA from frozen tumor samples that were positive for HPV16 or HPV18 was used for the detection of integrated papillomavirus sequences (DIPS) assay (see below). DNA from paraffin-embedded archival material was used to analyze HPV integration events (IS-PCR assay; see below). RNA derived from laser-microdissected tissue samples obtained using a MicroBeam laser system (PALM Microlaser Technologies AG, Bernried, Germany) was isolated using an RNeasy RNA isolation kit (Qiagen; Hilden, The Netherlands), and integrate-specific fusion transcripts were amplified using the amplification of papillomavirus oncogene transcripts (APOT) assay *(24)*.

DIPS Assay

DNA isolated from snap-frozen vaginal or vulvar biopsy samples from 21 patients with high-grade vaginal or vulvar lesions and a previous history of cervical disease was subjected to the DIPS assay to analyze whether the HPV16 or HPV18 genome was integrated into the host genome or existed as an episome in the vaginal–vulvar lesions and to determine the exact DNA sequences of the viral–cellular junction sites. The DIPS assay (outlined in Fig. 1) is an adaptor ligation–based PCR method designed specifically to detect integrated papillomavirus DNA sequences *(31)*.

Genomic DNA (0.6–1.2 mg) was digested with Sau3AI (10 U; New England Biolabs, Beverly, MA) in a volume of 20 μL in a thermal cycler (GeneAmp 2400; Perkin Elmer, Foster City, CA) at 37 °C overnight, followed by heat inactivation of the enzyme according to the manufacturer's instructions. Double-stranded (ds) adaptors that included recognition sequences for Sau3AI (50 pmol; sequences and formation of the ds adaptor given in Supplemental Table 1; available at http://jncicancerspectrum.oxfordjournals.org/jnci/content/vol97/issue24) were ligated to Sau3AI-digested genomic DNA by adding T4 DNA ligase (5 U; Roche Mannheim, Germany) and ATP and dithiothreitol (final concentrations of 2 m*M* each) to the ligation reaction in a total volume of 30 μL with incubation at 14 °C overnight.

We subjected an aliquot of the ligation products to a first round of linear PCR amplification in a total volume of 25 μL in a thermal cycler. First-round linear PCR (40 cycles) was carried out in 1× PCR buffer (Gibco BRL, Gaithersburg, MD) containing 1.5 m*M* $MgCl_2$, 200 μ*M* of each dNTP, 0.2 μ*M* viral primer I (HPV for I, see Supplemental Table 1), 1 U *Taq* polymerase (Gibco BRL), and 2 μL of the ligation product. PCR parameters were as follows: initial denaturation at 96 °C for 2 minutes, followed by 40 cycles of denaturation at 96 °C for 30 seconds, primer annealing at the optimal annealing temperature (T_a; see Supplemental Table 1) for 30 seconds, and primer extension at 72 °C for 3 minutes, followed by final extension at 72 °C for 7 minutes. For second-round exponential PCR amplification, 2 μL of the first-round PCR product was subjected to amplification as described above, except that the two primers used were the viral primer II (HPV for II) in Supplemental Table 1 and the adaptor-specific primer AP1 reverse, each at 0.4 μ*M*), and 30 cycles of PCR were carried out. PCR products were resolved on 2% agarose gels, and the gels were stained with ethidium bromide and viewed by transillumination. To control for DNA quality, we amplified a 1.4-kb genomic locus on chromosome 21 (GenBank accession number AP001068) using control primer 1L in the first linear PCR and control primer 2L with primer AP1 reverse in the second exponential PCR.

APOT Assay

Total RNA from laser-microdissected tumor sections (0.1–0.5 μg) was reverse transcribed using an $oligo(dT)_{17}$ primer coupled

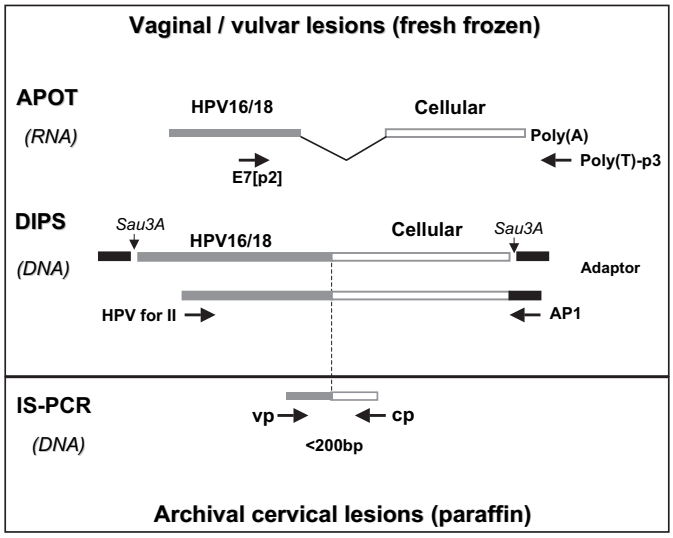

Fig. 1. Study design. The DIPS polymerase chain reaction (PCR) assay was used to detect papillomavirus sequences (**gray boxes**) that were integrated into genomic cellular sequences (**white boxes**) that was extracted from fresh-frozen vaginal and vulvar lesions. Digestion of genomic DNA with Sau3AI was followed by ligation to a double-stranded adaptor that included a recognition sequence for Sau3AI (**black boxes**). Exponential PCR was performed on the ligation product using nested viral primers specific for HPV16 or HPV18 (HPV for II, **arrow**) and an adaptor-specific primer (AP1, **arrow**). To determine whether the identified genomic integration loci were expressed in these lesions, we amplified transcripts encoding the viral E6 and E7 oncoproteins by using a previously described *(24)* reverse transcription-PCR–based amplification method for papillomavirus oncogene transcripts (APOT assay). RNA was reverse transcribed using an oligo$(dT)_{17}$ primer coupled to a linker sequence $(dT)_{17}$-p3. First-strand complementary DNAs encompassing the viral oncogene sequences were subsequently amplified by PCR using an HPV E7–specific primer (E7[p2], **arrow**) and poly(T)-p3 as the reverse primer. The sequence information obtained by DIPS was used to design integrate-specific PCR (IS-PCR) assays that could be applied to DNA extracted from archival paraffin-embedded material derived from biopsy samples of cervical lesions. IS-PCR was performed using a viral primer (vp, **arrow**) that targeted the HPV sequence and a second primer that corresponded to the adjacent cellular DNA sequences of the viral–cellular junction (cp, **arrow**). The expected size of the amplification product was less than 200 bp.

to a linker sequence $(dT)_{17}$-p3 *(24)* and 50 U of Moloney murine leukemia virus reverse transcriptase (SuperScript, Life Technologies) for 1 hour at 42 °C in a final volume of 20 μL. To control for RNA integrity and the quality of the first-strand complementary DNAs (cDNAs), total RNA from laser-microdissected tumor sections was also subjected to PCR amplification using glyceraldehyde-3-phosphate dehydrogenase–specific oligonucleotide primers, as previously described *(32)*. First-strand cDNAs encompassing viral oncogene sequences were subsequently amplified by PCR using an HPV E7-specific primer [p1] as the forward primer, p3 as the reverse primer, and 1.5 U of *Taq* DNA polymerase (Life Technologies) in a total volume of 50 μL. PCR parameters were as follows: initial denaturation at 94 °C for 2 minutes, followed by 30 cycles of denaturation at 94 °C for 30 seconds, primer annealing at T_a (see Supplemental Table 2; available at http://jncicancerspectrum.oxfordjournals.org/jnci/content/vol97/issue24) for 30 seconds and primer extension at 72 °C for 3 minutes, followed by final extension at 72 °C for 7 minutes. Primer sequences and optimal PCR T_as are listed in Supplemental Table 2. We used 5 μL of amplification product from this first round of PCR as template for the second nested PCR, under identical conditions except that the HPV E7–specific primer [p2] was used as the forward primer and $(dT)_{17}$-p3 was used as the reverse primer, and the annealing conditions used were those specified in Supplemental Table 2. Nested PCR products were resolved on 2% agarose gels, and the gels were stained with ethidium bromide and viewed by transillumination.

Sequence Analysis of Viral–Cellular Junction Fragments Obtained by DIPS and APOT

PCR products of interest were excised from agarose gels and extracted using a gel extraction kit (Qiagen, Hilden, Germany). The corresponding amplimers were either sequenced directly or cloned with the use of a TA cloning kit (Invitrogen, Carlsbad, CA), and the resulting plasmids were sequenced. Sequencing reactions were performed using a Big-Dye terminator DNA-sequencing kit (Perkin Elmer, Boston, MA) and a Prism 310 Genetic Analyzer (Applied Biosystems, Foster City, CA). Sequence data were analyzed using the BLASTN program (National Center for Biotechnology Information, Bethesda, MD).

Integrate-Specific PCR

Archival specimens of the cervical lesions were available for seven of the 21 patients. We used an integrate-specific PCR (IS-PCR) assay *(31)* to perform a retrospective analysis of specific HPV integration events in archival specimens for all seven patients. DNA from paraffin-embedded archival specimens is usually highly fragmented or damaged by preservation procedures, making amplification of long stretches of DNA (>200–300 bp) difficult. The IS-PCR assay overcomes this problem by using patient-specific oligonucleotide primers that are designed using DNA sequence information obtained from PCR products amplified in the DIPS assay from DNA extracted from fresh-frozen tissue samples from each patient. Specific PCR amplification of integrated HPV DNA sequences was performed using a viral primer (vp) that targeted the HPV DNA sequence and a cellular primer (cp) that targeted the adjacent host DNA of the viral–cellular junction (Fig. 1). The specificity of each IS-PCR assay was checked by performing parallel reactions that used DNA isolated from the archival samples of each of the patients and DNA isolated from human cervical cell lines (i.e., SiHa, CaSki, and HeLa cells) that contain HPV16 or HPV18 in their integrated forms as negative controls (data not shown). Also, all PCR amplicons were verified by direct sequencing of the viral–cellular junction. The expected amplicon size for the integrate-specific PCR assay ranged from 153 kb to 184 kb. All DNA samples extracted from archival samples were prescreened for DNA quality by PCR amplification using β-globin–specific primers designed to generate an amplified product of 205 bp *(33)*. All archival samples obtained from each patient were analyzed by IS-PCR for the presence of unique HPV integration events.

The PCR products were analyzed on 2% agarose gels, and their identities were confirmed by direct sequencing of the PCR products.

Results

Identification of Patients With Integrated HPV Genomes in Vaginal–Vulvar Lesions

To examine the clonal origins of anatomically distinct dysplastic lesions in the female lower genital tract, we identified

21 patients who had high-grade vaginal or vulvar lesions and a previous history of cervical lesions. Among these 21 patients, 11 had vaginal or vulvar lesions that were positive for integrated HPV16 or HPV18 genomes by the DIPS assay. Archival samples of the cervical lesions were available for only seven of these patients. Among these seven patients, six had vaginal lesions and one had vulvar lesions (Fig. 2).

DIPS analysis revealed that HPV had integrated at a distinct chromosomal locus (i.e., 16p12, Xp22, 1q42, chromosome 7, 17q23, 14q32, and 4q21) in vaginal–vulvar lesions from each of the seven patients. To demonstrate that the identified genomic integration loci were indeed expressed in these cells, we amplified E6/E7 containing transcripts using a previously described reverse transcription-PCR–based amplification of papillomavirus oncogene transcripts (APOT) *(24)*. By using the APOT assay, we confirmed that the identified integrated HPV DNA was actively transcribed in the vaginal–vulvar lesions (data not shown).

Patients' Clinical Histories

The clinical histories of the seven patients whose samples were analyzed in this study are schematically presented in Fig. 2. Five patients (Cases 1–5) had a history of high-grade lesions (≥CIN2) of the cervix, and two patients (Cases 6 and 7) had a history of squamous cell carcinoma of the cervix. Four of the five patients with high-grade lesions and the two patients with squamous cell carcinoma underwent hysterectomy, and one patient with a high-grade squamous intraepithelial lesion was treated by cold knife conization. In all cases, histologic evaluation of the surgical specimens showed disease-free R0 resection margins.

Among the five patients who had high-grade lesions of the cervix (three of whom had a grade 3 vaginal intraepithelial neoplasia [VAIN3] and two of whom had a squamous cell carcinoma of the vagina), the first diagnosis of vaginal lesions occurred 38–143 months after treatment for the cervical lesion. Three patients had several vaginal recurrences during the follow-up period. Among those patients, the period from the first diagnosis of a VAIN3 to progression to invasive vaginal cancer ranged from 53 to 204 months.

The remaining two patients each had a history of squamous cell carcinoma of the cervix (stages pT2b and pT2a, International Federation of Gynecology and Obstetrics [FIGO]). One patient developed a squamous cell carcinoma of the vulva, and the other patient was diagnosed with an invasive carcinoma of the vagina within 6 and 5 years, respectively, after they were treated for cervical carcinoma.

Clonal Relationship Between Multicentric Lesions

To examine the clonal origins of the vaginal and vulvar lesions with respect to the previous cervical lesions, we analyzed whether the cervical lesions had same HPV integration sites as the vaginal–vulvar lesions from the same patient. The sequence information obtained with DIPS was used to design IS-PCR assays to verify the

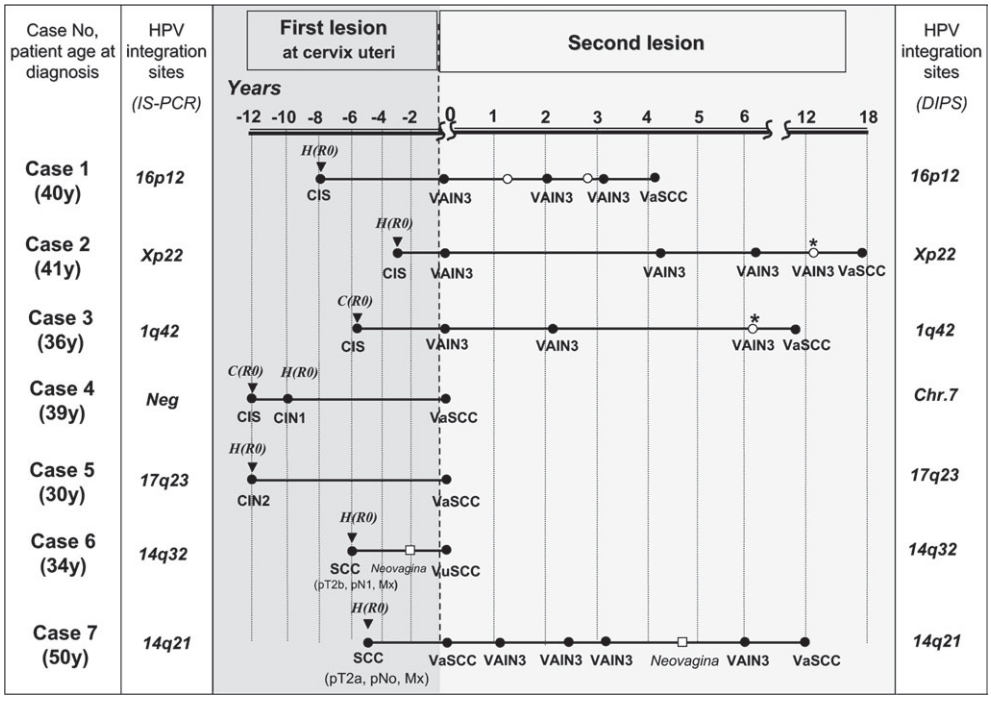

Fig. 2. Summary of the clinical histories of the seven patients with anatomically distinct metachronous lesions of the female low genital tract. Five patients (Cases 1–5) had a previous history of high-grade lesions, and two patients (Cases 6 and 7) had a previous history of squamous cell carcinoma (SCC) of the cervix. Six patients (Cases 1, 2, 4, 5, 6, and 7) underwent hysterectomy (H), and one patient (Case 3) was treated by cold knife conization (C). In all cases, specimens obtained from the surgical procedures showed disease-free R0 resection margins upon histologic evaluation. The first diagnosis of vaginal–vulvar lesions (time point 0, **dashed line**) ranged from 38 to 143 months after treatment of the cervical lesion. Four patients had several vaginal recurrences during the follow-up period and were subsequently treated (**filled circles**) or not (**opened circle marked with asterisk***) if patients refused therapy. One of two patients (Case 7) with a reconstructed vagina (**open squares**) presented with a recurrence located in the neovagina. In six of seven patients, identical HPV integration sites were found in the primary cervical lesions (IS-PCR) and the subsequently developed vaginal or vulvar carcinomas (detection of integrated papillomavirus sequences [DIPS]). In one patient (Case 4), the primary cervical lesion was negative for IS-PCR (Neg) but positive for the presence of HPV16 genome. For Case 1, during two follow-up visits after treatment, the biopsy samples were taken and histopathologically classified as normal (**open circle**).

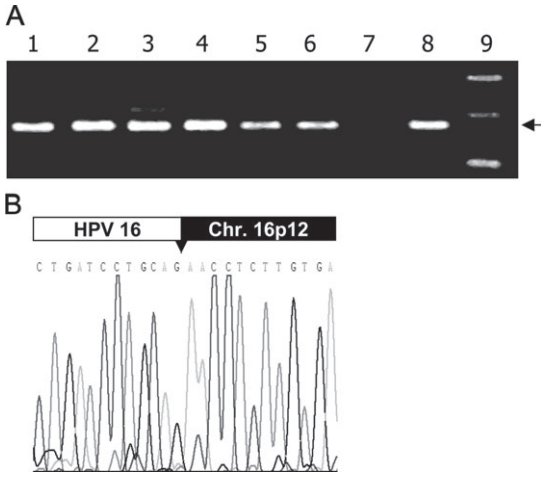

Fig. 3. Integrate-specific PCR amplification of an HPV DNA–host cell DNA junction in genomic DNA isolated from recurring vaginal lesions and prior high-grade lesion of the cervix (Case 1). **A**) Integrate-specific PCR (IS-PCR) was performed using genomic DNA isolated from the frozen and archival biopsy samples from a patient who was treated for carcinoma in situ of the cervix, and the PCR products were resolved on a 2.0% agarose gel. **Lane 1**, carcinoma in situ of the cervix; **lanes 2–4**, three distinct grade 3 vaginal intraepithelial neoplasia (VAIN3) lesions; **lanes 5 and 6**, two distinct biopsy samples histologically classified as normal; **lane 7**, CaSki cells (HPV16-positive cell line, negative control); **lane 8**, invasive squamous cell carcinoma of vagina; **lane 9**, 100-bp ladder. **Arrow** indicates the 184-bp IS-PCR amplification product. **B**) Results of IS-PCR were confirmed by direct sequencing of PCR products. Identical integration-specific fusion fragments were detected in all samples obtained from this patient.

presence of integrate-specific amplimers in DNA extracted from the archival cervical samples (data summarized in Fig. 2).

In four of the five patients who had a high-grade lesion of the cervix, each of the metachronous vaginal lesions that developed several years after primary therapy for the cervical lesion had an HPV integration site that was identical to that in the cervical lesion (Fig. 2). In one patient (Case 4), the cervical lesion was IS-PCR negative, even though the cervical lesion was positive for HPV16 DNA. Although the vaginal lesion in this patient may well have emerged from the initial cervical dysplasia, we cannot conclusively establish the clonal relationship between the vaginal lesion and the cervical lesion because of the lack of a specific marker for the initial cervical lesion. For another patient (Case 1), biopsy samples taken during two follow-up visits after treatment were histopathologically classified as normal, although both samples contained integrate-specific fragments suggestive of residual disease (Fig. 3). In that patient, the vaginal lesions became clinically apparent as VAIN 3 lesions 11 and 8 months later (Fig. 2).

For the two patients in the second group (Cases 6 and 7), we found identical HPV integration sites in the primary cervical carcinoma and the subsequently developed vaginal and vulvar carcinomas, respectively.

In summary, these data strongly suggest that all anatomically distinct lesions in the lower genital tract of at least six of seven patients had a common clonal origin with the cervical lesion that was presumably located at the transformation zone of the cervix uteri.

Discussion

We have previously found that most high-grade cervical lesions and invasive cervical carcinomas are monoclonal with respect to HPV DNA integration sites *(28,29)*. In this study, we analyzed the clonal relationships among anatomically distinct metachronous lesions by examining the sequences of specific HPV DNA integration sites in samples obtained from seven patients who had been diagnosed with vaginal or vulvar carcinoma after being treated for high-grade lesion or carcinoma of the cervix. We found that for six of the seven patients, the viral genome integration sites in metachronic, multifocal vaginal or vulvar lesions were the same as that in the preexisting cervical lesion, suggesting a common clonal origin. We presume that the vulvar and vaginal lesions were derived from quiescent dysplastic HPV-transformed cells that were not detected by the conventional histopathologic analysis because they were locally disseminated early in the neoplastic process. Although all resection margins were reported to be disease free (R0), in six of the seven cases we could detect the identical transformed cell clones in the vulvar–vaginal lesions and in the previous cervical lesions. These findings—together with the long intervals observed between the diagnosis of an initial lesion at the cervix and the manifestation of the extracervical lesions and the fact that vaginal lesions occur 10 times less frequently than cervical lesions and primarily in older women—suggest that lower genital tract lesions originate at the transformation zone of the cervix uteri. The data presented here do not allow us to define precisely how the dysplastic cells derived from the cervix reached the independent anatomical sites. Local continuous or discontinuous intraepithelial spread appear to be the most likely mechanisms.

Among the patients whose primary lesions were high-grade lesions of the cervix, the first recurrence in vagina was always a high-grade lesion (i.e., VAIN3) or an invasive carcinoma of the vagina. Minucci et al. *(34)* have also reported that the grade of the vaginal abnormality was equal to or more severe than that of the original lesion in the cervix. This finding further supports the concept that vaginal and vulvar lesions represent the local progression of preexisting cervical lesions.

Our study has several limitations. Because of the rarity of multicentric lower genital tract lesions and the lack of archival cervical material in many cases, only limited material was available for analysis. Thus, we cannot exclude the possibility that some vaginal or vulvar lesions that develop following cervical dysplasia or cancer represent independent primary lesions at these sites. However, given the high proportion of clonally related lesions in our samples, we believe that a frequent cause of multicentric high-grade dysplasia within the female genital tract is local spreading of a preexisting monoclonal dysplastic cell clone originating at the transformation zone of the cervix. It would be expected that distant spreading of malignant cells occurs only at invasive cancer stages; however, in our study, we showed spreading of transformed cell clones from preinvasive cervical lesions (in four of five CIN3 lesions) to distant sites in the lower genital tract. This early spreading of transformed cells, if supported by results of future studies of more patients, might influence the clinical follow-up management of patients who are treated for preinvasive lesions of the cervix.

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NOTES

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