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Detection of Human Papillomavirus DNA in Prostate Gland Tissue by Using the Polymerase Chain Reaction Amplification Assay

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Human papillomavirus (HPV) is associated with specific benign and malignant lesions of the epithelial and mucosal surfaces. Of the sexually transmitted types, HPV type 16 (HPV 16) and HPV 18 are commonly associated with severe dysplasia and carcinoma of the uterine cervix. In men, genital HPV infections which have been studied are manifest as external lesions usually involving types other than 16 and 18. The nature of HPV 16 and 18 infections in men has not been explored. Since the most common neoplasias of the male genital tract involve the prostate gland, we assayed benign hyperplastic and cancerous prostate tumors for the presence of HPV DNA, using type-specific primers in polymerase chain reaction amplifications. Normal prostatic tissue obtained at autopsy was included in the survey. Amplified sequences specific for HPV 16 were found in 14 of 15 benign prostatic hyperplasias and in all of four carcinomas tested. In contrast, HPV 18 was identified in only three benign hyperplasias, which also contained HPV 16 DNA. Four of five normal prostates demonstrated no HPV infection; one contained HPV 16 sequences. The presence of these oncogenic HPV types in prostate tissues suggests a reservoir for sexual transmission; a potential role for the virus in the etiology of prostatic neoplasia remains to be demonstrated.

There is compelling epidemiologic evidence linking human papillomavirus (HPV) infection with benign, premalignant, and cancerous lesions of the uterine cervix (1, 2, 4, 5, 7, 10). Sexual transmission is recognized as the most common route for dissemination of the virus, yet the prevalence of apparent HPV infection is lower in men than in women. Most studies of HPV infections in men have focused on epithelial lesions of the external genitalia and the urethral mucosa (9), including a recent study demonstrating HPV type 6 (HPV 6) and HPV 11 in urine sediments from male patients with condylomata acuminata in the meatus urethrae (11). The authors suggest that ejaculum containing HPV-infected urethral cells may be a mode of viral transmission. In another study, HPV genomic DNA was detected in seminal fluids of individuals with disseminated cutaneous HPV infections and no evidence of urinary tract infections (14). Urine specimens from these individuals did not contain HPV DNA implicating the male accessory sex glands as potential sites for HPV infection and possible reservoirs for HPV transmission. The most common genital neoplasias occurring in men are benign prostatic hyperplasia (BPH) and prostatic carcinoma (CaP), diseases of unknown etiology. Preliminary evidence obtained by Southern blot analysis of a small number of prostate tissues indicated that HPV DNA was present; however, the virus genotype was not determined (P. J. McNicol and J. G. Dodd, 6th International Papillomavirus Workshop, abstr. no. 49, 1987). To determine specifically whether HPV 16 and/or HPV 18 is associated with these genital tumors in the male, and to do so with the most sensitive diagnostic assay available, we used polymerase chain reaction (PCR) amplification (17) to identify each virus type. Here we describe the examination of hyperplastic, cancerous, and normal prostatic tissues for the presence of HPV DNA by using PCR amplification.

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MATERIALS AND METHODS

Tissue treatment. Prostatic tissues were obtained at random from patients undergoing surgical treatment for urinary obstruction. A sexual history was not taken. Tissues were placed directly into sterile, disposable plastic containers by the attending surgeon, with a portion reserved for histopathologic assessment. Tissues were classified as predominately BPH or CaP. Since papillomavirus-associated lesions have been identified in the male urethra (12), tissues obtained by transurethral resection or by suprapubic prostatectomy were examined in our study. Tissues obtained by the latter procedure should be free from possible contamination which might occur during the removal of tissue through an infected urethra. Normal prostatic tissues were obtained randomly at autopsy and handled as described above. Because of the natural history of prostatic neoplasia, normal specimens were available only from individuals younger than those receiving treatment. All tissues were held at −70°C until analysis.

DNA isolation. Genomic DNA from prostatic tissues and from the human lymphoma cell line IM9 was isolated by proteinase K digestion essentially as described previously (2). Genomic DNA was isolated by treating tissues with a solution of 0.1 M Tris (pH 7.5)–0.1 M NaCl–0.005 M EDTA–1% sodium dodecyl sulfate with proteinase K (100 μg/ml) at 37°C overnight. Specimens were extracted with buffered phenol and chloroform, and then nucleic acids were precipitated with ethanol.

PCR amplification. The PCR procedure was used to amplify HPV sequences infecting tissue samples. Synthetic oligonucleotide primers (Table 1) targeting sequences within the E6 gene of HPV 16 and 18 were used. The primers amplified 126- or 160-base-pair fragments for HPV 16 or 18, respectively. The type specificity of the amplified sequence was confirmed by hybridization of oligonucleotide probes (Table 1). The PCR reaction mixture contained 50 mM KCl; 10 mM Tris hydrochloride (pH 8.3); 1.5 mM $MgCl_2$; 200 μM each dATP, dGTP, dCTP, and TTP; and 3 μg of DNA for

TABLE 1. Sequences and locations of oligonucleotide primers in the HPV genomes

Primer or probe	Sequence (5′ to 3′)	E6 genome location (bases)	
		HPV 16	HPV 18
Primer	CAGGACCCACAGGAGCGACCC	110–130	
Primer	GTCATATACCTCACGTCGCAG	215–235	
Probe	CCACAGTTATGCACAGAGCTGC	140–161	
Primer	CAGTATACCCCATGCTGCATGCC		278–300
Primer	CGGTTTCTGGCACCGCAGGCACC		415–437
Probe	CAGACTCTGTGTATGGAGACAC		349–370

each tissue specimen or 55 pg of HPV plasmid DNA. Primers were added to a final concentration of 1 μM. The amount of the *Taq* polymerase added to each reaction mixture was 2.5 U. Forty cycles of amplification were done, beginning with DNA denaturation at 94°C for 1 min and followed by primer annealing and extension at 55°C for 5 min. Amplification products were concentrated by ethanol precipitation.

Analysis of amplified DNA. For each sample, the amplification products were separated by electrophoresis through 4% agarose gels (3% NuSieve agarose; 1% SeaKem agarose [FMC Corp., Marine Colloids Div.]). The molecular weights of the amplification products were determined by comparison with *Hae*III restriction fragments derived from pBR322 plasmid DNA. The gels were blotted, and membranes were hybridized to corresponding HPV 16 or HPV 18 oligonucleotide probes which were 5′ end labeled with ^{32}P to a specific activity of at least 10^8 cpm/μg. Filters were prehybridized for 3 h at 45°C in a solution containing 5× standard saline citrate (SSC) (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 5× Denhardt solution (50× Denhardt solution contains 1% Ficoll 400, 1% polyvinylpyrrolidone, and 1% bovine serum albumin fraction V), 0.5% sodium dodecyl sulfate, and 0.3 mg of sonicated salmon sperm DNA per ml. Probe was added to a final concentration of 2 ng/ml, and hybridization was carried out at 45°C for 1 h. Filters were washed for 20 min at 45°C in 6× SSC–0.1% sodium dodecyl sulfate and then for two 10-min washes in 2× SSC–0.1% sodium dodecyl sulfate. Autoradiography was carried out at −70°C with XAR-5 X-ray film (Eastman Kodak Co.) and intensifying screens (Du Pont, NEN Research Products) for 6 to 18 h.

Control reactions. The exquisite sensitivity of the PCR demands meticulous preparation of all reagents and materials in order to eliminate all possible sources of contamination. Positive-displacement pipettors with disposable pistons were used for all pipetting procedures. All reagents were assayed for the presence of contaminating HPV DNA, and all experiments were performed in parallel with positive- and negative-control DNA. Because the amplification products of one experiment can be a source of contamination for the next experiment, two physically separated laboratories and separate equipment were used for all experiments. Tissue storage, DNA isolation, reagent preparation, and the setup of PCRs (except cloned HPV genome-positive controls) were conducted in one laboratory (Department of Physiology, University of Manitoba), while the preparation of HPV-positive control reactions, PCR amplification, gel electrophoresis, and Southern hybridization were performed in the second laboratory (Cadham Provincial Laboratory). Negative controls included reaction mixtures lacking any DNA template and reaction mixtures with human DNA lacking HPV target sequences.

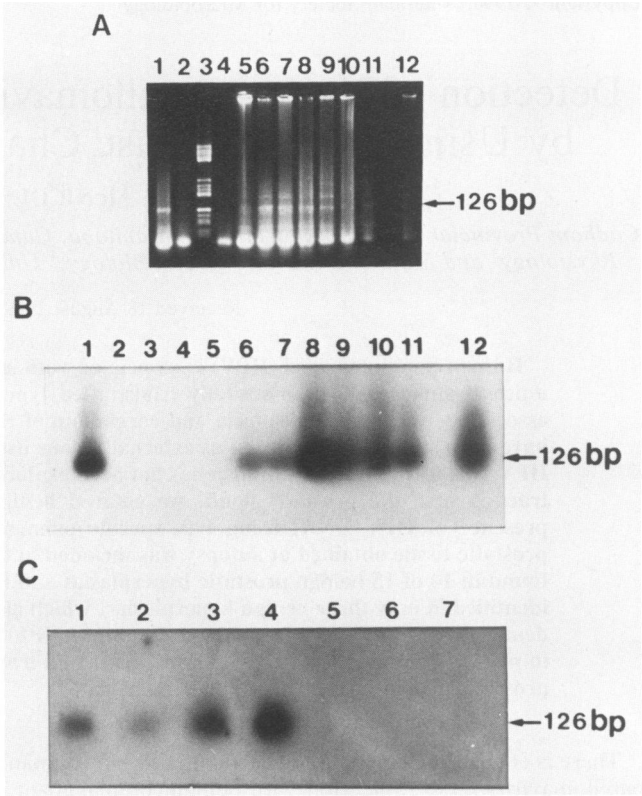

FIG. 1. Detection of HPV 16 sequences in human prostatic tissues by PCR. (A) Amplification products were resolved by agarose gel electrophoresis. The expected 126-base-pair (bp) fragment is evident by ethidium bromide staining in several lanes. Lanes: 1, HPV 16-carrying plasmid as a positive control; 2, HPV 18-carrying plasmid as a negative control; 3, pBR322 marker DNA restricted with *Hae*III; 4, reaction without DNA template as a negative control; 5 to 8, 11, and 12, BPH specimens; 9 to 10, CaP specimens. (B) A 16-h autoradiograph from blot of gel shown in panel A. Lanes: 1, HPV 16 positive control demonstrates a strong signal; 2 and 4, no signal is evident in negative controls; 5, BPH specimens fail to demonstrate a signal; 6 through 12, BPH and CaP specimens demonstrate the presence of HPV 16 DNA. (C) A 16-h autoradiograph from additional HPV 16 amplification reactions (gel not shown). Lanes: 1, 2, and 4, BPH specimens demonstrate the presence of HPV 16 DNA; 3, CaP specimen also harbors HPV 16 sequences; 5 to 7, normal prostate tissues fail to give an amplification product.

RESULTS

We examined hyperplastic, cancerous, and normal prostate tissues for the presence of HPV-specific DNA sequences by in vitro amplification, using *Taq* polymerase. Examples of the electrophoretic separation of amplification products and corresponding patterns of hybridization with HPV 16 and HPV 18 probes are presented in Fig. 1 and 2, respectively. The results are summarized in Table 2. Using primers to the E6 region of HPV 16 and an HPV 16-specific probe, we found that 14 of 15 BPH specimens (93%) and 4 of 4 CaP specimens harbored HPV 16 DNA. Using the HPV 18-specific primers and probe, we found only three BPH specimens harboring HPV 18 DNA. Each of these also contained HPV 16 DNA. No specimen harbored solely HPV 18 DNA. Five normal prostates were examined, and four had no detectable HPV 16 or 18; one contained HPV 16 only.

With respect to the surgical procedure used in tissue acquisition, HPV DNA was detected in 16 of 17 specimens

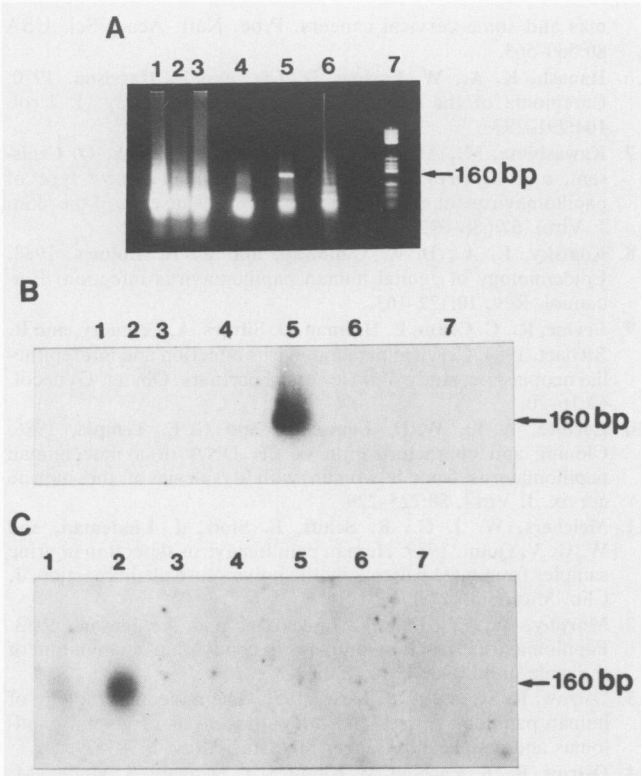

FIG. 2. Detection of HPV 18 sequences by PCR in human prostatic tissues. (A) Amplification products were resolved by agarose gel electrophoresis. The expected 160-base-pair (bp) fragment is evident by ethidium bromide staining in lane 5. Lanes: 1 to 3, BPH specimens; 4, HPV 16-carrying plasmid as a negative control; 5, HPV 18-carrying plasmid as a positive control; 6, human lymphoma cell line IM9 as a negative control; 7, pBR322 marker DNA restricted with *Hae*III. (B) A 16-h autoradiograph from blot of gel shown in panel A. Lanes: 1 to 3, BPH specimens free of HPV 18 sequences; 4, HPV 16-carrying plasmid does not demonstrate a signal; 5, HPV 18-carrying plasmid gives a strong positive signal; 6 and 7, human cell line and marker DNA give no hybridization signal. (C) A 16-h autoradiograph from additional HPV 18 amplification reactions (gel not shown). Lanes: 1 and 2, BPH specimens demonstrate the presence of HPV 18 DNA; 3 and 4, CaP and BPH specimens do not harbor HPV 18 sequences; 5 to 7, normal prostate tissues fail to give an amplification product.

(94%) obtained by transurethral resection and in both specimens obtained by suprapubic prostatectomy. Although it is possible that the tissues obtained by transurethral resection could include urethral epithelial cells which may harbor HPV, the suprapubic prostatectomy tissues should be free of urethral material. The presence of HPV sequences in these two samples indicates that the prostate is the site of infection.

DISCUSSION

The compelling evidence linking specific HPV types with vulvar and uterine cervical tumors led us to examine the most common tumors of the genitourinary tract in men, BPH and CaP, for the presence of the virus.

Preliminary studies, using genomic Southern blot analysis, demonstrated the presence of HPV DNA in human prostate DNA samples. However, further characterization of these samples, including virus typing, was hampered by the low copy number of the viral sequences, no doubt a function of

TABLE 2. Results from in vitro amplification of HPV DNA sequences in genomic DNA isolated from prostatic tissue

HPV type	No. of specimens positive for HPV obtained by:		
	Autopsy (normal)	Prostatectomy[a]	
		BPH	CaP
16 only	1	11 (1)	4
18 only	0	0	0
16 + 18	0	3 (1)	0
None	4	1	0

[a] Numbers in parentheses indicate specimens obtained by suprapubic prostatectomy. All others were obtained by transurethral resection.

the heterogeneous nature of the prostate specimen. Unlike the uterine cervix, from which dysplastic or frankly cancerous lesions can be biopsied directly, the prostatic specimen obtained at surgery is an admixture of normal and neoplastic tissue. Since a focus of HPV infection cannot be identified in the prostate, directed sampling is not possible. The development of PCR technology has facilitated detection of target sequences under these difficult conditions. Using this technology, we now have demonstrated HPV genomic sequences in prostate tissues. Although this does not confirm the presence of infectious virus particles, it does indicate that HPV infection in the male may be occult and may include internal sites in the reproductive tract.

Since papillomavirus-associated lesions have been identified in the male urethra (11, 12), tissues obtained by transurethral resection could include HPV-infected urethral epithelial cells. For this reason, tissues obtained by suprapubic prostatectomy were included in our study. Tissues obtained by the latter procedure would avoid this source of contamination. HPV was found in prostate specimens obtained by both procedures.

The E6 region of the HPV 16 and 18 genomes was targeted for amplification for several reasons. First, these sequences are consistently present in HPV-associated lesions, even when integration of the viral genome into host chromosomes has occurred (19). Second, expression of the E6 region appears to be an important component in the process of cellular transformation by these oncogenic HPV types (19, 20). Assays for the presence of E6 transcripts in prostatic tissues are under way.

We detected HPV 18 DNA in three BPH specimens that also contained HPV 16 DNA. None of the adenocarcinomas tested contained HPV 18 DNA. It has been suggested that HPV 18 is most frequently associated with adenocarcinoma of the uterine cervix (22); however, a study comparing cervical squamous-cell carcinoma and adenocarcinoma shows that HPV 18 was detected in 32 (11%) of 281 squamous-cell carcinomas and in only 6 (19%) of 31 adenocarcinomas (8), indicating that the exclusive association of type 18 with adenocarcinoma is not valid. Undoubtedly, the prevalence of the HPV type in any population influences the analytical outcome. The greater prevalence of HPV 16 infection in the prostate specimens in our study correlates with the prevalence of HPV types observed in studies of women in the same geographic area (P. McNicol, unpublished data). Detection of HPV 16 in one of five normal prostates is not surprising, since the virus can be sexually transmitted (13), and HPV has been detected in normal genital tissue (3).

To date, investigations of HPV infection in men have concentrated on the role of the virus in apparent penile disease, including condyloma and cancer (6, 15). Our data

show that the virus is associated with a very high proportion of prostatic neoplasia. However, as with other HPV-associated diseases, attributing a role to the virus in the etiology of benign and malignant prostatic diseases will require further detailed study.

Epidemiological studies have suggested a role for a sexually transmitted infectious agent in the etiology of prostate cancer (18, 21). Similar to the risk factors associated with the development of uterine cervical cancer, an increased risk for developing prostate cancer has been correlated with commencement of sexual activity at a young age, a history of sexually transmitted diseases, and multiple sex partners. While it is tempting to draw an analogy between prostate disease and uterine cervical disease, the similarities are limited. Both the prostate and the uterine cervix are steroid hormone target tissues with secretory epithelia. However, unlike cervical dysplasia and carcinoma, BPH and CaP are not regarded as different stages of progressive disease (16). At present they are regarded as two distinct diseases, although it is not uncommon to find manifestations of both in the same gland. A satisfactory explanation for the development of these diseases has yet to be elucidated. On the basis of our findings, we propose that the prostate gland is a potential reservoir for transmission of HPV types implicated in invasive cervical disease in women. However, attributing any role to the virus as an etiologic factor in prostatic neoplasia requires further study.

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LITERATURE CITED

1. **Beaudenon, S., T. Kremsdorf, O. Croissant, S. Jablonska, S. Wain-Hobson, and G. Orth.** 1986. A novel type of human papillomavirus associated with genital neoplasia. Nature (London) **321**:246–249.
2. **Boshart, M., L. Gissmann, H. Ikenberg, A. Kleinheinz, W. Scheurlen, and H. zur Hausen.** 1984. A new type of papillomavirus DNA, its presence in genital cancer biopsies and in cell lines derived from cervical cancer. EMBO J. **3**:1151–1157.
3. **de Villiers, E.-M., D. Wagner, A. Schneider, H. Wesch, H. Miklaw, J. Wahrendorf, U. Papendick, and H. zur Hausen.** 1987. Human papillomavirus infections in women with and without abnormal cervical cytology. Lancet **ii**:703–706.
4. **Durst, M., L. Gissmann, H. Ikenberg, and H. zur Hausen.** 1983. A papillomavirus DNA from a cervical carcinoma and its presence in cancer biopsy samples from different geographic regions. Proc. Natl. Acad. Sci. USA **80**:3812–3815.
5. **Gissmann, L., L. Wolnik, H. Ikenberg, U. Koldovsky, H. Schnurch, and H. zur Hausen.** 1983. Human papillomavirus types 6 and 11 DNA sequences in genital and laryngeal papillomas and some cervical cancers. Proc. Natl. Acad. Sci. USA **80**:560–563.
6. **Hanash, K. A., W. Furlow, D. Utz, and E. Harrison.** 1970. Carcinoma of the penis: a clinicopathologic study. J. Urol. **104**:291–297.
7. **Kawashima, M., A. Jablonska, M. Favre, S. Oblek, O. Croissant, and G. Orth.** 1986. Characterization of a new type of papillomavirus found in a lesion of Bowen's disease of the skin. J. Virol. **57**:688–692.
8. **Koutsky, L. A., D. A. Galloway, and K. K. Holmes.** 1988. Epidemiology of genital human papillomavirus infection. Epidemiol. Rev. **10**:122–163.
9. **Levine, R., C. Crum, E. Herman, D. Silvers, A. Ferenczy, and R. Richart.** 1984. Cervical papillomavirus infection and interepithelial neoplasia: a study of male sexual partners. Obstet. Gynecol. **64**:16–20.
10. **Lorincz, A. T., W. D. Lancaster, and G. F. Temple.** 1986. Cloning and characterization of the DNA of a new human papillomavirus from a woman with dysplasia of the uterine cervix. J. Virol. **58**:225–229.
11. **Melchers, W. J. G., R. Schift, E. Stolz, J. Lindeman, and W. G. V. Quint.** 1989. Human papillomavirus detection in urine samples from male patients by the polymerase chain reaction. J. Clin. Microbiol. **27**:1711–1714.
12. **Murphy, W., Y. Fu, W. Lancaster, and A. Jenson.** 1983. Papillomavirus structural antigens in condyloma acuminatum of the male urethra. J. Urol. **130**:84–87.
13. **Ostrow, R. S., and A. J. Faras.** 1987. The molecular biology of human papillomaviruses and the pathogenesis of genital papillomas and neoplasms. Cancer Metastasis Rev. **6**:383–395.
14. **Ostrow, R., K. Zachow, M. Niimura, T. Okagaki, S. Muller, M. Bender, and A. Faras.** 1986. Detection of papillomavirus in human semen. Science **231**:731–733.
15. **Rosenberg, S. K.** 1985. Subclinical papilloma viral infection of male genitalia. Urology **26**:554–557.
16. **Rotkin, I.** 1983. Origins, distribution and risk of benign prostatic hyperplasia, p. 10–21. *In* F. Hinman (ed.), Benign prostatic hypertrophy. Springer-Verlag, New York.
17. **Saiki, R. K., S. Scharf, F. Faloona, K. B. Mullis, G. T. Horn, H. A. Erlich, and N. Arnheim.** 1985. Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. Science **230**:1350–1354.
18. **Schuman, L., J. Mandel, C. Blackard, H. Bauer, J. Scarlett, and R. McHugh.** 1977. Epidemiologic study of prostatic cancer: preliminary report. Cancer Treat. Rep. **61**:181–186.
19. **Schwarz, E., A. Schneider-Gadicke, and H. zur Hausen.** 1987. Human papillomavirus type-18 transcription in cervical carcinoma cell lines and in human cell hybrids, p. 47–53. *In* B. M. Steinberg, J. L. Brandsma, and L. A. Taichman (ed.), Cancer cells 5: papillomaviruses. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
20. **Smotkin, D., and F. O. Wettstein.** 1986. Transcription of human papillomavirus type 16 early genes in a cervical cancer and a cancer-derived cell line and identification of the E7 protein. Proc. Natl. Acad. Sci. USA **83**:4680–4684.
21. **Steele, R., R. Lees, A. Kraus, and C. Rao.** 1971. Sexual factors in the epidemiology of cancer of the prostate. J. Chronic Dis. **24**:29–37.
22. **Tase, T., T. Okagaki, B. A. Clark, D. A. Manias, R. S. Ostrow, L. B. Twiggs, and A. J. Faras.** 1988. Human papillomavirus types and localization in adenocarcinoma and adenosquamous carcinoma of the uterine cervix: a study by in situ DNA hybridization. Cancer Res. **48**:993–998.