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Detection of human papillomavirus (HPV) DNA in archival specimens of benign prostatic hyperplasia and prostatic cancer using a highly sensitive nested PCR method

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Abstract Human papillomavirus is thought to be an etiological factor for urological tumors such as penile cancer. However, there is much conflicting data surrounding prostatic cancer. We recently established a highly sensitive nested PCR method with consensus human papillomavirus (HPV) primers for the detection of many high-risk HPV types. HPV DNA from the long-control region (LCR) to E7 open reading frame was amplified with first primer pairs and subsequently amplified with second internal E6–E7 primers. Our nested PCR method could detect HPV16, 18, 31, 33, 35, 52, 58 and some undetermined HPV DNAs. Using this method, we investigated the existence of HPV DNA in formalin-fixed paraffin-embedded tissue of the prostate. We found HPV DNA in three of 71 specimens of benign prostatic hyperplasia (BPH) and in none of 38 prostatic carcinomas. These three samples were infected with HPV 16. These results suggest that HPV is not a causal factor for prostatic cancer and BPH.

Key words Human papillomavirus · Nested PCR · Archival specimen · Prostatic cancer · Benign prostatic hyperplasia

Introduction

Human papillomavirus (HPV) is a type of papovavirus, containing double-stranded, circular DNA about 8000 kilobases in length. An etiological role of HPV in uterine cervical cancer has been reported by many investigators. More than 80 HPV types have been isolated, and some HPV types are carcinogenic [2, 32]. HPV types 16, 18, 31, 33, 35, 45, 51, 52, 56, 58 and 59, often found in cervical carcinoma [4] and its precursor lesions [16], are thought to be high-risk types, whereas HPV 6, 11, 44 and 55 are the low-risk types found in benign lesions such as condyloma acuminatum [15]. Recent studies have demonstrated that over 80% of cervical carcinomas contained the high-risk HPV DNA [4, 25]. Since E6 and E7 genes of the high-risk HPV types were transcribed in cervical cancer tissues, the E6 and E7 genes are thought to have a prominent role in carcinogenesis [32]. An in vitro experimental study demonstrated that E6 and E7 proteins form a inactivation complex with p53 [26] and retinoblastoma(Rb) [20] proteins, respectively. Loss of tumor suppressor functions of the p53 and Rb gene product is considered to be a contributing factor in carcinogenesis.

Many epidemiological studies indicate that sexual activity of women is well correlated with the risk of HPV infection, suggesting that sexual contact is the most common route for HPV transmission [1, 14]. On the other hand, an association between the development of anal carcinoma and HPV infection is reported in homosexual men [28, 31]. Some recent reports have shown an association between HPV infection and the urinary tract diseases. HPV has been detected in penile cancers [5, 9, 17, 24] and bladder cancers [8, 11, 21]. Other reports have shown a high prevalence of HPV infection in prostatic cancers. However, the detection rate of HPV DNA in prostatic cancer varies from 0 to 75% [6, 18, 19, 24, 29, 30]. The detection rate of HPV DNA in benign prostatic hyperplasia (BPH) is similar to that in prostatic cancers. Since there are many conflicting explanations for the existence of HPV DNA in prostatic tissues, the etiological role of HPV infection for prostatic cancer remains to be established.

To elucidate the role of HPV infection for the development of prostatic cancer and BPH, we examined 109 formalin-fixed specimens of 38 prostatic cancers and

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71 cases of BPH for the existence of HPV DNA, using highly sensitive nested polymerase chain reaction (PCR) methods.

Materials and methods

Cases

We obtained tissue specimens of 38 prostatic cancers and 71 cases of BPH from patients who underwent surgical treatment. The operative method employed was subcapsular prostatectomy in 10 patients and transurethral resection of the prostate (TURP) in 61 patients with BPH. Total prostatectomy was performed on 38 patients with prostatic cancer.

Sample preparation

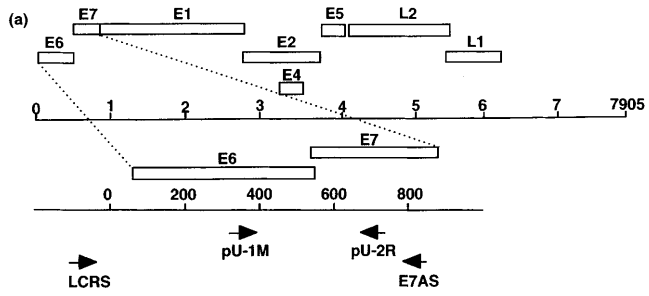
The tissue was fixed with formalin and embedded in paraffin. The paraffin-embedded tissue sections were sliced with a microtome knife in order to obtain three sections 5–10 μm thick for subsequent analysis. One tissue section was stained with hematoxylin-eosin and subjected to histopathological evaluation. The other two sections were deparaffinized by the addition of xylene and digested with proteinase K for cell lysis.

Amplification of HPV DNA by PCR

We established a new nested PCR method for the detection of HPV DNA. The procedure was as follows: Primer pairs between the long control region (LCR) and E7 open reading frame were designed to amplify a first PCR product. The location of the consensus primers on the HPV 16 genome and the nucleotide sequences of the primers are demonstrated in Fig. 1. LCRS and E7AS that were designed by ourselves were used as the outer primers, and pU-1M and pU-2R as reported by Fujinaga et al. [7] were used as the internal primers. The length of the first PCR product was about 650 base pairs (bp) and the next PCR product was about 250 bp. All DNA samples were tested to check whether they were suitable for use as template DNAs for PCR by an amplification of the internal β-actin gene. The oligonucleotide primers of β-actin gene were as follows: 5′-ATGGATGACGATATCGCTG-3′ and 5′-ATGAGGTAGTCT GTCAGGT-3′. The first PCR amplification of HPV was performed in 10 μl of solution containing 50 ng of genomic DNA and a reaction mixture containing 2 mM TRIS-HCl, pH 8.0, 10 mM KCl, 2.5 mM of each deoxynucleotide triphosphate (dATP, dCTP, dGTP and dTTP), 1.25 units of *Taq* polymerase (Takara) with 100 pmol of LCR and E7 primers. The second PCR reaction was subsequently performed, adding 1 μl of the first PCR product to the PCR reaction mixture containing internal E6–E7 primers. The PCR cycles were performed as described previously [25] and details of the method were as follows: five cycles of denaturation at 94 °C for 1 min, annealing at 45 °C for 1 min and extension at 72 °C for 2 min, 5 cycles of denaturation at 94 °C for 1 min, annealing at 48 °C for 1 min and extension at 72 °C for 1 min; 20 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min and extension at 72 °C for 1 min; and another cycle of extension at 72 °C for 5 min.

The PCR-amplified products were electrophoresed on 2% Seakem agarose (FMC Bioproducts) and stained with ethidium bromide. The DNA bands were visualized by a UV-monitor camera (Epi-Light UV FA1100, AIC, Japan).

Restriction fragment length polymorphism (RFLP) was used for HPV typing. HPV types were classified by particular DNA cleavage patterns with appropriate restriction enzyme. The details of HPV typing by RFLP are shown in Fig. 2. Our PCR method could detect HPV 16, 18, 31, 33, 35, 52 and 58. Each PCR product was first digested with *Rsa*I. HPV 31 was determined by cutting patterns with *Rsa*I. The other HPV types could not be cut with *Rsa*I, while they were digested with *Ava*II. HPV 16, 33 and 18 could be typed by cutting patterns with *Ava*II, whereas HPV-35, 52 and 58 were not cut with *Rsa*I and *Ava*II. HPV 52 was cut with *Sau*3AI and HPV 58 was cut with *Acc*I. The last HPV type, which could not be cut with any of these enzymes, was HPV 35.



(b)

Sense primer LCRS (5'-AAGGGAGTAACCGAAAACGGT-3')

	5'	3'	Mismatches
HPV16	26 AAGGGCGTAACCGAAATCGGT	46	1
HPV18	33 AAGGGAGTAACCGAAAACGGT	53	0
HPV31	29 TAGGGAGTGACCGAAAGTGGT	49	4
HPV33	30 TAGGGTGTAACCGAAAGCGGT	50	3
HPV52	29 TAGGGTGTAACCGAAAACGGT	49	2
HPV58	31 TAGGGTGTAACCGAAAACGGT	51	2

Antisense primer E7AS (5'-TCATCCTCCTCCTCTGAG-3')

	5'	3'	Mismatches
HPV16	671 TCATCCTCCTCCTCTGAG	655	0
HPV18	705 TCGTTTTCTTCCTCTGAG	688	4
HPV31	669 ACATCCTCCTCATCTGAG	652	2
HPV33	682 CCTTCATCCTCATCTGAG	665	4
HPV52	662 GTATCCTCCTCATCTGAG	645	3
HPV58	683 ATTTCATCCTCGTCTGAG	666	5

Sense primer pU-1M (5'-TGTCAAAAACCGTTGTGTCC-3')

	5'	3'	Mismatches
HPV16	419 TGTCAAAAGCCACTGTGTCC	438	3
HPV18	426 TGCCAGAAACCGTTGAATCC	445	4
HPV31	423 TGTCAAAGACCGTTGTGTCC	442	1
HPV33	424 TGTCAAAAACCTTTGTGTCC	443	2
HPV52	418 TGTCAAACGCCATTATGTCC	437	4
HPV58	425 TGTCAAAAACCATTGTGTCC	444	2

Antisense primer pU-2R (5' -GAGCTGTCGCTTAATTGCTC-3')

	5'	3'	Mismatches
HPV16	675 GAGCTGTCATCTAATTGCTC	656	3
HPV18	693 TCTGAGTCGCTTAATTGCTC	674	5
HPV31	654 GAGCTGTCGGGTAATTGCTC	635	2
HPV33	667 GAGCTGTCACTTAATTGCTC	648	1
HPV52	647 GAGCTGTCACCTAATTGCTC	628	2
HPV58	668 GAGCTGTCACATAATTGCTC	649	2

Fig. 1 a Location of human papillomavirus (HPV) consensus primers. *Open boxes* indicate open reading frames. The position of each primer is represented by an *arrowhead.* **b** Alignment of the corresponding sequences of several HPV types and primer pairs. The 5′ and 3′ nucleotide position and the numbers of mismatches are demonstrated

Southern blot analysis

Southern blot hybridization was performed at Tm-40 °C using HPV 58 probe. The details of the procedure have been described previously [16]. First, the filter was incubated with 8 ml of hybridization buffer for 2–16 h in a 42 °C water bath with shaking. The buffer consisted of 5 × SSC, 50 mM HEPES, 0.2% each of polyvinylpyrrolidone and Ficoll, and 20% formamide. Then, the filter was incubated with 6 ml of hybridization buffer and a heat-denatured probe at 42 °C for 12–16 h, and washed three times for 15 min with 200 ml of 2 × SSC, 0.1% SDS at 48 °C. In preparing the probe, HPV DNA was labelled with [α–^{32}P]dCTP using random oligonucleotide primers. The filter was exposed to X-ray film (Eastman Kodak, Rochester, N.Y.) with intensifying screens (du Pont de Nemours, Wilmington, Del.) at −80 °C for 4–16 h.

Results

We tested the sensitivity of our PCR assay for the detection of HPV DNA. Serial diluted plasmid DNA containing HPV 16 and 18 genome was used as a template. As previously reported [7], the PCR with primers pU-1M and pU-2R could detect 0.1 copies of HPV 16 DNA per cell. The present nested PCR method could detect 0.0001 copies of HPV 16 DNA and 0.001 copies of HPV 18 DNA (1 ng of the plasmid HPV DNA was equivalent to 250 copies of HPV genome per cell). Next we compared the detection rates of different HPV types on fresh cell samples from 26 women in Southern blot analysis using HPV 58 probe [16], ordinal E6–E7 PCR and the nested PCR. The results are summarized in Table 1. No samples that were negative by the nested PCR were positive with Southern blot and E6–E7 PCR. The nested PCR method appeared to be the most sensitive and the Southern blot analysis the most insensitive in detection of HPV 16, 18, 31, 52 and 58.

We examined 109 prostate tissue samples for the presence of HPV DNA using the nested PCR method. We found HPV DNA in three of 71 BPH specimens, while HPV DNA was detected in none of 38 prostatic cancers (Table 2). The PCR products of the three cases are 238 bp in size and were found to be HPV 16 by RFLP analysis, since all became 158 and 79 bp fragments by cutting with *Ava*II, and 105, 97 and 35 bp fragments by cutting with *Sau*3AI, but could not be cut with *Rsa*I (Fig. 3).

The detection rate of HPV DNA between BPH and prostatic cancer was not statistically different using the chi-square test ($P = 0.19$). From these findings, the etiological role of HPV in prostatic cancer and BPH is questionable because of the very low detection rate of HPV DNA in these prostatic samples.

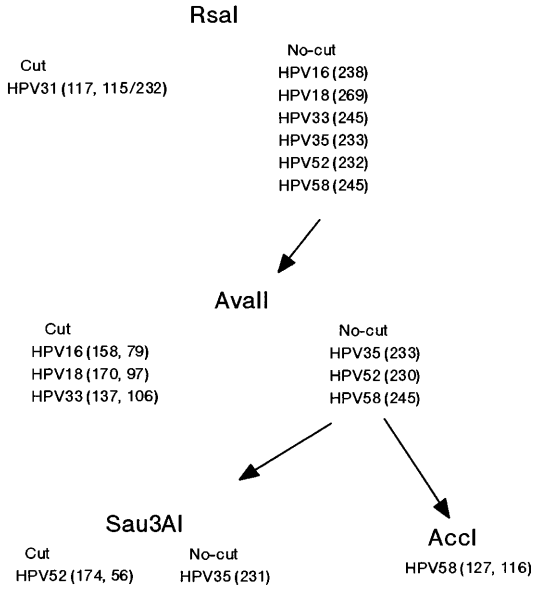


Fig. 2 Restriction fragment length polymorphism (RFLP) for HPV typing. Sizes of DNA fragments are shown in *parentheses*.

Table 1 Detection rates of human papillomavirus (HPV) DNA in Southern blot analysis, E6–E7 polymerase chain reaction (PCR) and nested PCR

HPV type	No. of cases[a]	Southern blot	E6–E7 PCR
HPV 16	9	4 (44%)[b]	7 (78%)
HPV 18	4	0	1 (25%)
HPV 31	3	1 (33%)	1 (33%)
HPV 52	6	5 (83%)	6 (100%)
HPV 58	4	1 (25%)	3 (75%)
Total	26	11 (42%)	18 (69%)

[a] The number of cases in which HPV DNA was detected by a nested PCR

[b] Detection rates are shown as percentages that were calculated by dividing the number of cases positive for HPV DNA by each detection method by the total number of cases positive for each HPV DNA

Discussion

The association of HPV and a variety of malignant urological tumors has been reported. Some recent re-

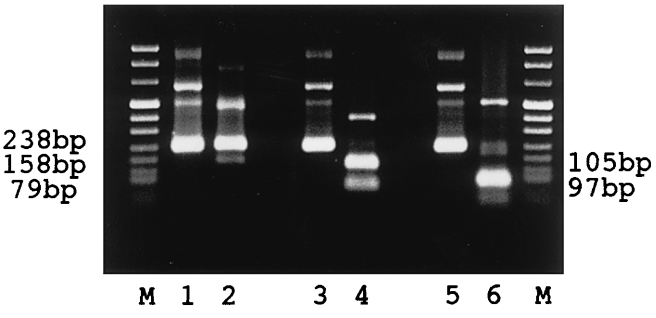


Fig. 3 Cutting pattern of polymerase chain reaction (PCR) products with appropriate restriction enzyme. *Lane M* DNA marker: pUCBM21 digested with *Hpa*II, *Dra*I and *Hind*III. *Lanes 1, 3, 5* a PCR product in one of three positive cases; *lane 2* digested with *Rsa*I; *lane 4* digested with *Ava*II; *lane 6* digested with *Sau*3AI

Table 2 Detection of HPV DNA in tissues from prostatic cancer and benign prostatic hyperplasia (BPH)

	Positive	Negative	HPV types	Total
Prostatic cancer	0	38 (100%)		38
BPH	3 (4.2%)	68 (96%)	HPV 16 (3), others (0)	71 P 0.19[a]
Total	3 (2.8%)	106 (97%)		109

[a] Difference in detection rates between prostatic cancer and BPH determined by chi-square test

ports suggested a role for HPV in carcinogenesis of the penis, because of a high prevalence of HPV DNA in penile cancer tissues. McCance et al. [17] reported that HPV 16 DNA was detected in 26 of 53 (49%) penile cancers and HPV 18 DNA was detected in five of 53 (9%) penile cancers. Scinicariello et al. [17] showed the existence of HPV DNA in the nucleus of cervical and penile cancers, also using an in situ hybridization method. Gregoire et al. [9] reported that HPV DNA was present in 26 of 117 penile cancers (22.2%). Cupp et al. [5] reported HPV type 16 DNA was detected in 23 of 42 invasive squamous cell carcinomas of the penis (55%).

The HPV detection rate in bladder cancers has varied from 0 to 100%. Knowles et al. [11] found no HPV DNA and Goldman et al. [8] reported a 100% detection rate of HPV DNA in transitional cell carcinoma of the bladder. The two groups of investigators used the same PCR method for detection.

Prostatic cancer is one of the most common urogenital cancers. The death rate for prostatic cancer is about 4000 people per year in Japan. Epidemiological research has been done on hormonal, familial, dietary, infectious and genetic factors; however, there is no clear association of certain factors with prostatic cancer. Several recent studies examined oncogenic HPV types in prostatic cancer tissue. McNicol et al. [18] found HPV 16 DNA in 34 of 56 (61%) benign prostatic lesions and in 14 of 27 (52%) prostatic cancers, hypothesizing that HPV infection has an etiological role in prostatic neoplasia. Moyret-Lalle et al. [19] also reported that HPV 16 DNA was detectable in the frozen tissues of prostatic cancer and BPH, in nine of 16 (53%) and seven of 22 (32%) samples, respectively. In contrast, Effert et al. [6] found no HPV DNA in 30 prostatic cancer tissues, suggesting that HPV has no causal role in carcinogenesis of the prostate. Wideroff et al. [30] found HPV DNA in the formalin-fixed paraffin-embedded tissue of prostatic cancer and BPH in seven of 56 (12.5%) and in four of 42 (15.2%) samples, respectively. However, this report suggested that HPV did not increase the risk of prostatic cancer, because the HPV detection rate was very low in these tissues and, besides, HPV was less frequently found in blacks (7.7%) than whites (15.2%) despite the incidence rate of prostatic cancer for blacks being higher than that for whites in the United States. Suzuki et al. [29] found HPV infection eight of 51 (16%) prostatic cancer patients. Thus the etiological role of HPV infection in prostatic lesions is controversial.

HPV types 16 and 18 are frequently found in premalignant and malignant cervical lesions, suggesting that they are high-risk types. In recent studies, some other HPV types such as HPV 31, 33, 35, 39, 45, 52, 56, 58 and 59 are often detected in invasive carcinomas and are considered high-risk types [2, 4]. Our nested PCR method can detect most of these high-risk HPV types (Fig. 1) and is very sensitive compared with both the ordinal E6–E7 PCR method and Southern blot analysis (Table 1). In the present study, HPV 16 DNA was detected in 90% (9/10) of the formalin-fixed paraffin-embedded samples of cervical cancer, while HPV was detected in 10 of 10 (100%) fresh frozen specimens derived from the same samples by the nested PCR method (data not shown). Nawa et al. [23] suggested that formalin fixation markedly reduces the level of sensitivity, but their nested PCR method could detect 10–50 copies of HPV DNA in 10^4 formalin-fixed cells. All these findings may suggest that our nested PCR may be sensitive enough in the detection of HPV DNA in formalin-fixed samples.

When we tested for HPV DNA in archival tissue samples, no HPV DNA was found in any of the prostatic cancers and only three patients with BPH were positive with HPV 16 DNA in the present study. Unknown or novel HPV types might be involved in the development of prostatic cancer and BPH, since our nested PCR method could detect only a limited selection of high-risk HPV types. However, this possibility seems unlikely because no unique HPV types have been identified in prostatic tissues in previous reports but rather the types that are very common in the female genital tract such as HPV 16 and 18. Therefore, the present results suggest that HPV may not have a carcinogenic effect on the prostate, and the etiological role of HPV in BPH is also questionable because of such a low detection rate of HPV DNA in BPH tissues.

A large-scale epidemiological study demonstrated a strong association between the sexual behavior of men and the risk of cervical cancer in their wives [3]. We have previously demonstrated the existence of HPV DNA in the semen of four of the male partners of 12 HPV-16-positive women [10] and indicated the possibility of dissemination through ejaculation [12, 13, 22]. These findings may support the possibility of an HPV transmission route from the prostate to the cervix, although we could not investigate in the present study whether the wives of these HPV-positive males were infected with HPVs or had HPV-related lesions. Further studies should be carried out to clarify the role of prostate as a reservoir of HPV in males.

References

1. Bauer HM, Ting Y, Greer CE, Chambers JC, Tashiro CJ, Chimera J, Reingold A, Manos MM (1991) Genital human papillomavirus infection in female university students as determined by a PCR-based method. JAMA 265:472
2. Bernard HU, Chan SY, Manos MM (1994) Identification and assessment of known and novel human papillomaviruses by polymerase chain reaction amplification, restriction fragment length polymorphisms, nucleotide sequence, and phylogenetic algorithms. J Infect Dis 170:1077
3. Bosch FX, Castellsague X, Munoz N, de Sanjose S, Gaffari AM, Gonzalez LC, Gili M, Izarzugaza I, Viladiu P, Navarro C, Vergara A, Ascunce N, Guerrero E, Shah KV (1996) Male sexual behavior and human papillomavirus DNA: key risk factors for cervical cancer in Spain. J Natl Cancer Inst 88:1060
4. Bosch FX, Manos M, Munoz N, Sherman M, Jansen AM, Peto J, Schiffman MH, Moreno V, Kurman R, Shah KV (1995) International Biological Study on Cervical Cancer (IBSCC) study group. Prevalence of human papillomavirus in

cervical cancer: a worldwide perspective. J Natl Cancer Inst 87:796
5. Cupp NR, Malek RS, Goellner JR, Smith TF, Epsy MJ (1995) The detection of human papillomavirus deoxyribonucleic acid in intraepithelial, in-situ, verrucous and invasive carcinoma of the penis. J Urol 154:1024
6. Effert PJ, Frye RA, Neubauer A, Liu ET, Walther PJ (1992) Human papillomavirus types 16 and 18 are not involved in human prostate carcinogenesis: analysis of archival human prostate cancer specimens by differential polymerase chain reaction. J Urol 147:192
7. Fujinaga Y, Shimada M, Okazawa K, Fukushima M, Kato I, Fujinaga K (1991) Simultaneous detection and typing of genital human papillomavirus DNA using the polymerase chain reaction. J Gen Virol 72:1039
8. Goldman PM, Bracken RB, Tricoli JV (1991) Human papillomavirus types 16 and 18 is associated with bladder tumor. J Urol 145:311A
9. Gregoire L, Cubilla AL, Reiter VE, Haas GP, Lancaster WD (1995) Preferential association of human papillomavirus with high-grade histological variants of penile-invasive squamous cell carcinoma. J Natl Cancer Inst 87:22
10. Inoue M, Nakazawa A, Fujii M, Tanizawa O (1992) Human papillomavirus (HPV) type 16 in semen of partners of women with HPV infection. Lancet 339:1114
11. Knowles MA (1992) Human papillomavirus sequences are not detectable by Southern blotting or general-primer mediated polymerase chain reaction in transitional cell tumours of the bladder. Urol Res 20:297
12. Kyo S, Inoue M, Kayama M, Fujita M, Tanizawa O, Hakura A (1994) Detection of high-risk human papillomavirus in the cervix and semen of sex partners. J Infect Dis 170:682
13. Lai YM, Yang FP, Pao CC (1996) Human papillomavirus deoxyribonucleic acid and ribonucleic acid in seminal plasma and sperm cells. Fertil Steril 65:1026
14. Ley C, Bauer HM, Reingold A, Schiffman MH, Chamber JC, Tashiro CJ, Manos MM (1991) Determinant of genital human papillomavirus infection in young women. J Natl Cancer Inst 83:997
15. Lorincz AT, Reid R, Jenson AB, Greenberg MD, Lancaster W, Kurman RJ (1992) Human papillomavirus infection of the cervix: relative risk associations of fifteen common anogenital types. Obstet Gynecol 79:328
16. Matsukura T, Sugase M (1995) Identification of genital human papillomaviruses in cervical biopsy specimens: segregation of specific virus types in specific clinicopathological lesions. Int J Cancer 61:13
17. McCance DJ, Kalache A, Ashdown K, Andrade L, Menezes F, Smith P, Doll R (1986) Human papillomavirus types 16 and 18 in carcinomas of the penis from Brazil. Int J Cancer 37:55
18. McNicol PJ, Dodd JD (1991) High prevalence of human papillomavirus in prostate tissue. J Urol 145:850
19. Moyret-Lalle C, Marcais C, Jacquemier J, Moles J, Daver A, Soret J, Jeanteur P, Ozturk M, Theillet C (1996) Ras, p53 and HPV status in benign and malignant prostate tumors. Prostate 28:318
20. Munger K, Scheffner M, Huibregtse JM, Howley PM (1992) Interactions of HPV E6 and E7 oncoproteins with tumour suppressor gene products. Cancer Surv 12:197
21. Mvula M, Iwasaka T, Iguchi A, Nakamura S, Masaki Z, Sugimori H (1996) Do human papillomavirus have a role in the pathogenesis of bladder carcinoma? J Urol 155:471
22. Nakazawa A, Inoue M, Fujita M, Tanizawa O, Hakura A (1991) Detection of human papillomavirus type 16 in sexual partners of patients having cervical cancer by polymerase chain reaction. Jpn J Cancer Res 82:1187
23. Nawa A, Nishiyama Y, Kikkawa F, Kawai M, Mano H, Goto S, Suganuma N, Tomoda Y, Nakashima N (1993) Detection of human papillomavirus from histologically normal lymph nodes of Japanese cervical cancer patients by nested polymerase chain-reaction assay. Int J Cancer 53:932
24. Rotola A, Monini P, Luca DD, Savioli A, Simone R, Secchiro P, Reggiani A, Cassai E (1992) Presence and physical state of HPV DNA in prostate and urinary-tract tissues. Int J Cancer 52:359
25. Sasagawa T, Dong Y, Saijoh K, Satake S, Tateno M, Inoue M (1997) Human papillomavirus infection and risk determinants for squamous intraepithelial lesion and cervical cancer in Japan. Jpn Cancer Res 88:376
26. Scheffner M, Takahashi T, Huibregtse JM, Minna JD, Howley PM (1992) Interaction of the human papillomavirus type 16 E6 oncoprotein with wild-type and mutant human p53 proteins. J Virol 66:5100
27. Scinicariello F, Rady P, Saltzstein D, Orihuela E, Tyring SK (1992) Human papillomavirus 16 exhibits a similar integration pattern in primary squamous cell carcinoma of the penis and in its metastasis. Cancer 70:2143
28. Surawicz CM, Gitchlow C, Sajer J, Hurt C, Hanes S, Kirby P, Goldbaum G, Kiviat N (1995) High grade anal dysplasia in visually normal mucosa in homosexual men: seven cases. J Natl Cancer Inst 90:1776
29. Suzuki S, Komiya A, Aida S, Ito H, Yatani R, Shimazaki J (1996) Detection of papillomavirus DNA and p53 gene mutation in human prostatic cancer. Prostate 28:318
30. Wideroff L, Schottenfold D, Carey TE, Beals T, Fu G, Sakr W, Sarkar F, Schork A, Grossman HB, Show MW (1996) Human papillomavirus DNA in malignant and hyperplastic prostate tissue of black and white males. Prostate 28:117
31. Zaki SR, Judd R, Coffield LM, Greer P, Rolston F, Evatt BL (1992) Human papillomavirus infection and anal carcinoma. Am J Pathol 140:1345
32. zur Hausen H (1989) Human papillomavirus in anogenital cancer as a model to understand the role of viruses in human cancer. Cancer Res 49:4677