Virchows Archiv A Pathol Anat (1991) 419:479–485

Virchows Archiv A
Pathological Anatomy and Histopathology

Human papillomavirus and c-*myc*/c-*erb*B2 in uterine and vulvar lesions

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Received June 3, 1991 / Received after revision July 19, 1991 / Accepted July 22, 1991

Summary. The aim of this study was to investigate papillomavirus (HPV)-DNA in precancer and cancer of the cervix, vulva, and endometrium by in situ/dot blot/Southern blot hybridization and polymerase chain reaction (PCR). *Myc*/*erb*B-2 expression was examined by Northern blot analysis. PCR was the most sensitive HPV detection method, demonstrating HPV-DNA in all pre-invasive and invasive cervical lesions ($n=21$) and most (3 of 4) vulvar carcinomas in contrast to an overall rate of 60% with other techniques. Particular phenotypes (adenoid cystic/basal cell carcinoma of the vulva, cervical adenocarcinoma) were found to contain HPV. Endometrium harboured HPV not only in two cases of cervical cancer, but also in 3 of 8 primary endometrial carcinomas and 3 of 8 non-malignant conditions. *Myc* activation was confined to three squamous cell carcinomas, most markedly in one HPV-6-positive verrucous variant. *Erb*B-2 over-expression was only seen in one HPV-18 infected advanced endometrial tumour. Our findings point to a range of HPV-infected lesions broader than previously supposed and possible contributions of HPV-independent molecular events to carcinogenesis in this field.

Key words: Papillomaviruses – Oncogenes – Uterus – Vulva – Polymerase chain reaction

Introduction

Many studies have shown that human papillomaviruses (HPV), especially HPV-16, -18, -31, -33 and some less frequent HPV types, play an important role in squamous cell carcinogenesis of the cervix and vulva. Neoplastic growth of these tumours is usually associated with integration of HPV-DNA into the cell genome, thereby disrupting viral repressor functions and activating the transcription of the viral *E6* and *E7* genes (McCance 1988; Arends et al. 1990). In HPV-16, -18, and other high-risk papillomavirus types, the products of these genes are oncoproteins acting, at least partly, by binding to the tumour suppressor proteins p53 and pRB, respectively (Banks et al. 1990; Scheffner et al. 1990). In addition to these events, several observations suggest that other factors play a role in the pathogenesis of these tumours. Firstly, only a small percentage of cervical HPV-16/18 infections result in the development of malignant tumours; secondly, HPV-DNA is not found in 100%, but in only 80–90% of the tumours and pre-malignant cervical lesions if Southern blot or in situ hybridization techniques are used (zur Hausen 1987; Arends et al. 1990; Richart and Nuovo 1990). Thirdly, HPV-DNA is not always integrated into the cell genome in cervical carcinomas (Arends et al. 1990) and, finally, some squamous cell carcinomas (SCC) harbour the "benign" HPV-6 and -11 types which are otherwise found in condylomatous lesions or mild dysplasia (Gissmann et al. 1983; Okagaki et al. 1984; Rando et al. 1986; Tomita et al. 1986).

One possible co-factor in the transformation of squamous cells to neoplastic growth may be the activation of cellular oncogenes. In vitro studies have shown that the HPV-16 *E7* gene can cooperate with activated *ras* oncogenes in the transformation of baby rat kidney cells or human keratinocytes (Crook et al. 1989; DiPaolo et al. 1989). Yet, oncogene activation has seldom been found in cervical carcinoma tissue. Riou (1988) found c-*myc* and Ha-*ras* amplifications in 24% and 49% of stage III/IV cervical carcinomas, respectively, but these results were not reproduced by other authors (DiLuca et al. 1989; Yokota et al. 1989). To date, the role of cellular oncogenes in squamous cell carcinogenesis is far from clear.

In addition to SCC, HPV-16 and -18 DNA has been found in adenocarcinomas of the endocervix both in vivo and in vitro, with a relatively high frequency of HPV-18 in this tumour type (Lorincz et al. 1987a; Wilczynski et al. 1988). While the significance of these find-

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ings is not doubted, there are contradictory reports on the prevalence of HPV-DNA in endometrial tissue (De Villiers et al. 1986; Bergeron et al. 1988). Although there is a close anatomical relationship of the endocervical epithelium with the endometrial mucosa, the latter should be protected against viral infections by the endocervical mucus. In addition, epidemiological studies do not point to an association between HPV infection and endometrial neoplasia, as they do for cervical cancer (Kessler 1981).

In our study, 45 tissue samples of the cervix, vulva, and endometrium, including benign lesions, pre-malignant and malignant lesions and normal endometrial tissue, were analysed for the presence of HPV-DNA by dot blot and Southern blot hybridization, by the highly sensitive polymerase chain reaction (PCR), and partly by in situ hybridization. In addition, c-*erb*B-2 (HER2/neu) and c-*myc* oncogenes were studied in most cases and correlated with the results of HPV detection.

Materials and methods

The study included biopsies or surgical specimens from 7 cervical carcinomas (among them 1 verrucous carcinoma and 1 adenocarcinoma), 14 cervical intraepithelial neoplasias (CIN) including carcinoma in situ (CIS), 4 vulvar carcinomas (among them 2 SCC, 1 basal cell carcinoma and 1 adenoid cystic carcinoma), 3 condylomata acuminata (1 each of the cervical, vulvar, and perianal region), 8 adenocarcinomas of the endometrium, and 8 benign lesions or normal tissue from the uterus (Table 1). Tissue specimens were divided into two pieces: one was paraffin-embedded, while the other was frozen in liquid nitrogen and used for immediate diagnosis on cryostat sections. The rest of the latter piece was used for DNA/RNA extraction according to Chirgwin et al. (1979), as described previously (Rivière et al. 1990).

For dot blot hybridization, three aliquots each containing 1 µg DNA in water, were heat-denatured and adsorbed to three nylon membranes using a dot-blot apparatus. Pre-hybridization, hybridization, and detection of HPV-DNA were then performed using the ViraType HPV-DNA typing kit (Life Technologies, Gaithersburg, Md., USA), including ^{32}P-labelled RNA probe sets of HPV-6/11, HPV-16/18, and HPV-31/33/35.

For Southern blot hybridization, 10 µg DNA was digested with *Pst*I, followed by separation of the fragments in a 1% agarose gel in TRIS-acetate buffer (Maniatis et al. 1982). After blotting to a Biodyne B membrane (Pall, Dreieich, FRG), hybridization was performed under stringent conditions (T_m–25° C) with ^{32}P-labelled HPV-11, -16, -18 and -31 probes. For analysis of oncogene amplification, 10 µg DNA was treated with *Bam*H1, electrophoresed and blotted as above, and hybridized under stringent conditions with ^{32}P-labelled RNA probes.

In situ hybridization on paraffin sections of the cervical and vulvar lesions was performed using the ViraType in situ HPV detection kit (Life Technologies, Gaithersburg, Md., USA).

In the PCR, amplification reactions were performed in 50 µl containing 50 mM potassium chloride, 1.5 mM magnesium chloride, 10 mM TRIS-HCl, pH 8.3, 0.01% gelatin, 100–200 µM of each nucleotide (dATP, DTTP, dCTP, dGTP), 0.5–1 µM of each primer (see below) and 1 unit *Taq* DNA polymerase (Perkin Elmer Cetus, Überlingen, FRG). After addition of 5 µl water containing 1 µg DNA, the mixture was overlaid by 50 µl mineral oil and incubated for 5 min at 94° C for DNA denaturation. Amplification was done using an automated thermcycler (Biometra, Göttingen, FRG) in 35 cycles, each including 1 min 94° C for denaturation, 2 min 40° C (or 55° C) for annealing, and 3 min 72° C for chain elongation. For HPV detection in paraffin material, 8 µm sections were cut with sterile microtome blades and transferred to 1.5 ml tubes. After addition of 100–200 µl water and approx. 5 µg chelating resin (Sigma Deisenhofen, FRG), they were heated in a boiling water bath for 15 min, then centrifuged at room temperature and 13000 rpm for 5 min. Five microlitres of the aqueous phase was then used for PCR.

The primers used in our study were: (a) consensus primers MY09 and MY11 (Perkin Elmer Cetus; Manos et al. 1989); (b) general primers GP5 and GP6 (Snijders et al. 1990); (c) HPV-6/11 and HPV-16/33 primers from the *E6* region (Dallas et al. 1989); (d) HPV-18 primers H1 and H3 from the *E6* region (Genset, Paris; Shibata et al. 1988); and (e) HPV-31 primers corresponding to nt 111–130 and 411–430 (complementary strand) of the *E6* region of the published sequence (Goldsborough et al. 1989). For initial screening, general primer pairs (a) and (b) were used followed by analysis of the positive cases with all specific primers for HPV typing.

As positive controls, plasmids containing HPV-11, -16, -18, and -31 DNA or 1 µg DNA from the CasKi cell line containing HPV-16 DNA were used in the experiments. As negative control, DNA from tissues unlikely to contain HPV-DNA (such as breast tissue) and water were used. PCR mixtures were prepared and amplifications were performed under sterile conditions in a room separate from the following analytical work.

In order to analyse the PCR products, following the amplification, 15 µl of the reaction mixture was examined by electrophoresis in a 4% agarose gel (3% NuSieve GTG agarose; FMC, Rockland, Maine, USA; 1% standard low-M_r-agarose, Bio Rad, Munich, FRG) in TRIS-acetate buffer containing 10 µg/ml ethidium bromide. In experiments with type-specific primers, the gel was incubated for 30 min in 0.5 M sodium hydroxide, 1.5 M sodium chloride and the DNA was transferred to a nylon membrane (Biodyne B, Pall) by Southern blotting with 20 × SSC. After pre-hybridization for 1 h at 50° C in 5 × SSC, 5 × Denhardts' solution, 0.1% SDS, and 100 µg/ml denatured salmon sperm DNA, hybridization was performed overnight at 50° C in the same solution containing 1 pmol/ml oligonucleotide probe end-labelled with digoxigenin-dUTP (Boehringer, Mannheim, FRG) by terminal transferase (TdT, Gibco-BRL, Eggenstein, FRG). The nucleotide sequences of the probes were:

ATAGACCAGTTGTGCAAGACGTTTAATCTA/$_T$TCTA/$_T$TGCA (nt 137–175) for HPV-6/11, TATTCTAAAATTAGTGAGTATAGACATTATTGTTATAGTT (nt 311–351) for HPV-16, ATGGAGACACATTGGAAAAACTAACTAACACTGGGTTATA (nt 361–400) for HPV-18, AGATTTAACAATAGTATATAGGGACGACACACCACACGG (nt 251–290) for HPV-31, and TTTGGAATATGTAAAGTGTGTTTGCGGTTCTTATCTAAAA (nt 286–325) for HPV-33.

Hybrids were visualized by the digoxigenin nucleic acid detection kit (Boehringer, Mannheim, FRG).

The northern blot analysis of *erb*B-2 and c-*myc* expression was performed according to Rivière et al. (1990) using 15 µg total RNA per sample, as determined spectrophotometrically. The autoradiographic signals were assigned to one of the following groups: −, no detectable expression; +/−, faint signal after extended exposure time; +, low signal in the range of that observed for corresponding normal tissues; + +, over-expression; + + +, high over-expression reaching the level observed for cell lines MKN7 and Colon 320 (positive controls for *erb*B-2 and c-*myc*, respectively).

Results

In Table 1, the HPV detection results obtained by dot blot and Southern blot hybridization and PCR are summarized and related to the grading and staging of the tumours. In addition, the c-*myc* and c-*erb*B-2 expression, as analysed by Northern blot hybridization, is shown. In situ hybridization gave positive results in all condylomas and some of the cervical and vulvar carcinomas

Table 1. Papillomavirus infection and c-*myc*/c-*erb*B-2 oncogene expression in various cervical, vulvar, and endometrial lesions

No.	Age	Dignosis	G	S	*erb*B-2	*myc*	HPV dot blot[e]	HPV southern	HPV PCR
1	30	Cervical SCC	m	TII	ND	ND	16/18	16	16
2	39	Cervical SCC	p	TIb	+	+	–	–	X[a]
3	43	Cervical SCC	h	TI	+/–	+/–	16/18	16	16
		Endometrium			+	+	–	ND	16
4	46	Cervical SCC	m	TIa	+	++	16/18	–	16
5	53	Cervical adenocarcinoma	h	TIb	ND	ND	16/18	–	16
6	44	Cervical SCC	p	TIb	+/–	+	16/18	–	16+18
7	68	Verrucous cervical SCC	h	TIb	ND	ND	6/11	ND	6/11
		Endometrium			+	++/+++	6/11	6a	6/11
8	34	CIN 1			ND	ND	–	ND	16
9	25	CIN 1–2			ND	ND	–	–	6/11
10	32	CIN 1–2			+	+	16/18	–	16
11	26	CIN 2			–	–	16/18	16	16
12	56	CIN 2–3			+	ND	–	–	6/11
13	36	CIN 2–3			–	+	–	–	X[a]
14	48	CIN 3			–	+	6/11	6	6/11
15	58	CIN 3–CIS			+	ND	–	–	X[a]
16	33	CIN 3–CIS			+	+	31/33/35	–	6/11+16
17	40	CIN 3–CIS			ND	ND	16/18	16	16
18	41	CIS			+	+	–	X[b]	6/11
19	71	CIS			+	+	16/18	16	31
20	35	CIS			+	+	16/18	16	16
21	35	CIS			+	+	–	X[b]	X[a]
22	59	Vulvar SCC	h	TII	ND	ND	–	–	16
23	86	Vulvar SCC	h	TII	+	++	–	–	–
24	46	Vulvar BCC		TII	+	–	16/18	16	16
25	86	Vulvar ACC		TI	ND	ND	–	–	18
26	19	Cond. ac. portio			+	+	6/11	6	6/11
27	61	Cond. ac. vulva			+	+	6/11	ND	6/11
28	27	Cond. ac. perianal			+	+	6/11	6	6/11
29	63	Endometrial ca.	G1	TIa	ND	ND	ND	X[b]	16[c]
30	63	Endometrial ca.	G1	TIa	ND	ND	ND	–	–
31	61	Endometrial ca.	G1	TIA	ND	ND	ND	–	–
32	84	Endometrial ca.	G1	TIb	+	+	–	ND	–
33	60	Endometrial ca.	G1	TIa	+	+/–	–	ND	–
34	71	Endometrial ca.	G2	TIb	+	+/–	–	–	18
35	78	Endometrial ca.	G2	TIII	+	+	–	ND	–
36	71	Endometrial ca. mixed type	G2	TIb	++	+	–	ND	18[d]
37	40	Normal endometrium			+	ND	–	ND	–
38	48	Normal endometrium			+/–	ND	–	ND	–
39	62	Normal endometrium			+	+	–	ND	–
40	50	Normal endometrium			+	+/–	–	ND	–
41	49	Normal endometrium			+	+	–	ND	–
42	51	Endometrial hyperplasia			+	ND	16/18	–	16[c]
43	47	Endometrial hyperplasia			+	+/–	–	–	16+18[c]
44	50	Adenomyosis			+	+/–	–	–	18

[a] Positive results with both pairs of general primers, but not with specific primers
[b] Restriction fragments hybridizing with HPV-16 DNA
[c] Paraffin sections positive for HPV-16
[d] Paraffin sections positive for HPV-18
[e] HPV-16/18 = HPV-16 and/or HPV-18 (see Methods)
ND, Not done; SCC, squamous cell carcinoma; BCC, basal cell carcinoma; ACC, adenoid cystic carcinoma; ca., carcinoma; cond. ac., condylomata acuminata; G, grading or grade of differentiation (h, highly differentiated; m, moderately differentiated; p, poorly differentiated); S, staging (FIGO classification)

or dysplasias (Figs. 1, 2), but was negative or not interpretable in more tham 50% of the lesions (not included in Table 1). Endometrial samples were not analysed by this method.

All 7 cervical carcinomas revealed HPV-DNA after PCR analysis. In 5 cases, among them 1 adenocarcinoma and 4 SCC, high-risk HPV types-16 and -18 are found, 1 SCC harboured DNA of an unidentified HPV type, and 1 verrucous carcinoma was positive for HPV-6a (Figs. 1, 3). In 2 cases (nos. 3 and 7) endometrial tissue samples were also examined: the specimen from case 3 was histologically normal, whereas the endometrium corresponding to the verrucous carcinoma (case 7) was highly infiltrated by the tumour. In both cases the same HPV type was found in the tumour and in the endometrial tissue sample.

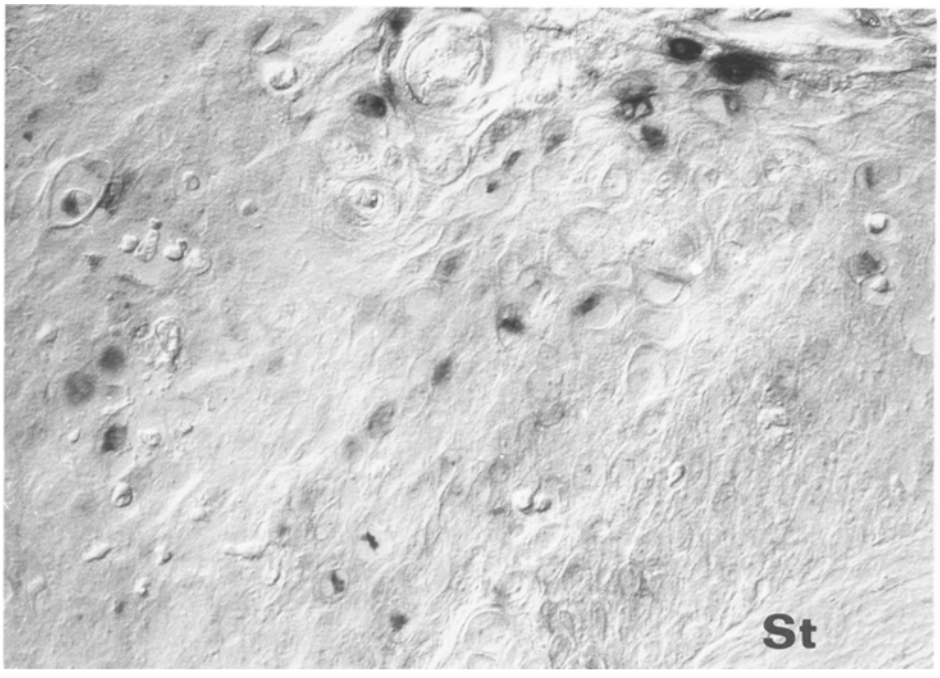

Fig. 1. In situ hybridization of verrucous carcinoma of cervix positive for HPV-6 (case 7). Hybridizing nuclei in highly keratinized tumour areas. *St*, Stroma, ×400

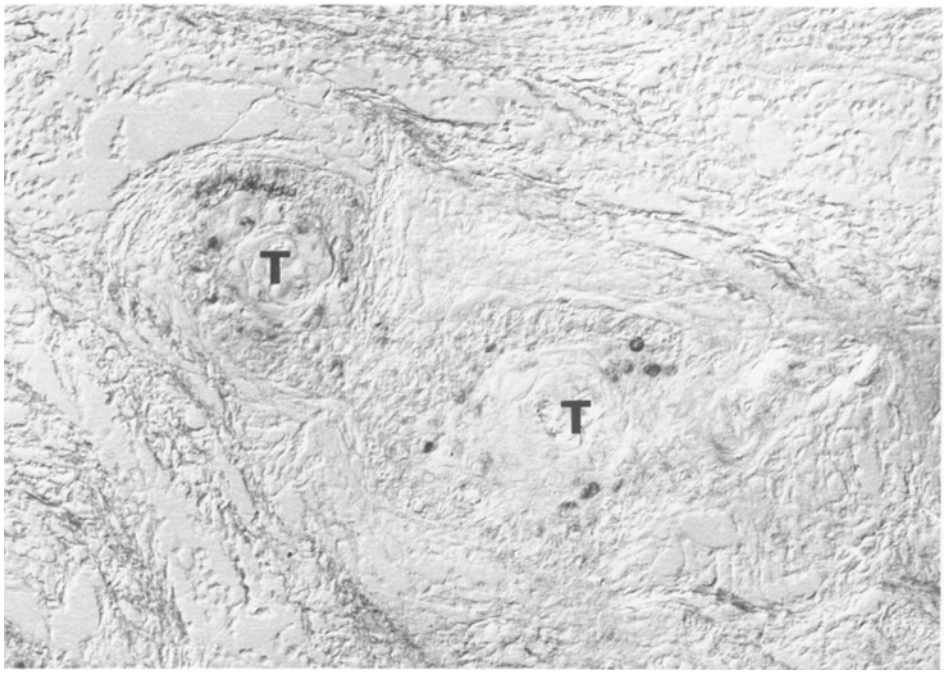

Fig. 2. In situ hybridization of vulvar basal cell carcinoma positive for HPV-16 (case 24). Sparsely distributed positive cells in invasive tumour cords. T, Tumour; ×250

In the infiltrated endometrium of the verrucous carcinoma and in one other SCC, a clear over-expression of the c-*myc* oncogene was detected. In these cases, Southern blot hybridization for detection of c-*myc* amplification was performed. In both cases over-expressing c-*myc*, a clear amplification (>10-fold) of this oncogene was observed (not shown).

No correlation between HPV infection or c-*myc* activation and the grading and staging of the tumours was observed.

By PCR analysis, all of the 14 CIN/CIS lesions (100%) were positive for HPV-DNA. HPV-16 was found in 5 cases, HPV-6/11 in 4 lesions, 1 case harboured both HPV-16 and -6/11 DNA, and in 3 cases unidentified HPV types (HPV X) were present. One specimen that was HPV-16/18-positive by dot blot and Southern blot hybridization yielded a positive result for the closely related HPV-31 by PCR. It is possible that alterations in the *E6* regions where the primers annealed led to these contradictory results. All 3 condylomata, of different origins, were positive for HPV-6. None of the cases showed a detectable oncogene activation.

HPV-DNA was detected in 3 of the 4 vulvar carcinomas analysed in this study. In 1 SCC and 1 basal cell carcinoma, HPV-16 DNA was identified (Fig. 2), whereas HPV-18 was found in 1 adenoid cystic carcinoma.

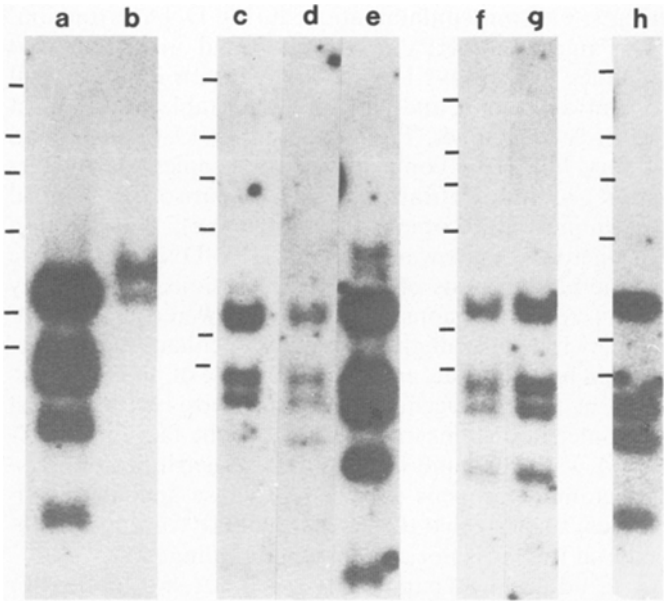

Fig. 3a–h. Southern blot hybridization of *Pst*1 digested DNA with a ^{32}P-labelled HPV-16 probe. DNA was extracted from the following samples: **a** Caski cells; **b** case 29: endometrial carcinoma; **c** case 1: cervical squamous cell carcinoma (SCC); **d** case 19: carcinoma in situ (CIS); **e** case 24: vulvar basal cell carcinoma; **f** case 17: cervical intraepithelial neoplasia (CIN)3-CIS; **g** case 11; CIN2; **h** Case 3: cervical SCC. *Bars* indicate the position of the molecular size markers (*Hin*dIII digested lambda DNA)

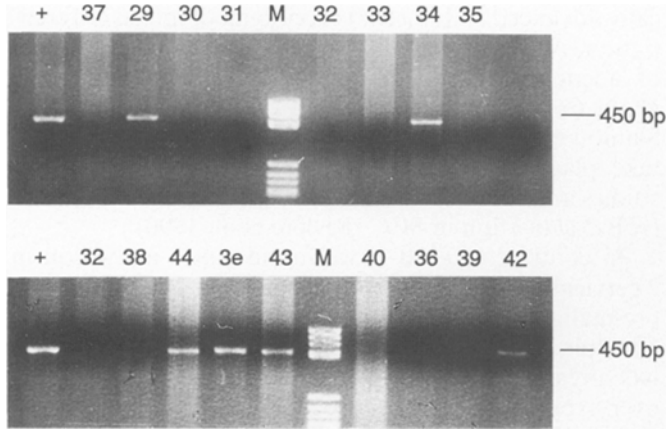

Fig. 4. PCR analysis of DNA extracted from endometrial tissues with MY09/MY11 consensus primers. Electrophoresis in a 4% agarose gel stained with ethidium bromide. The numbers correspond to the case numbers in Table 1. +, Positive control (CasKi DNA); −, negative control (no DNA); *M*, molecular size markers (*Hae*III digested pBR322 DNA). A weak band for case 36 cannot be seen

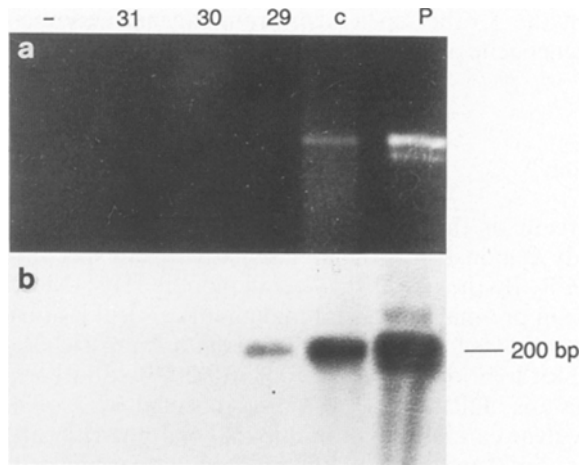

Fig. 5a, b. Polymer chain reaction (PCR) analysis of paraffin sections from endometrial carcinomas with HPV-16-specific primers. **a** Electrophoresis on a 4% agarose gel; **b** Southern blot hybridization with a digoxigenin-labelled, HPV-16-specific oligonucleotide probe. The numbers correspond to the case numbers in Table 1. −, Negative control (no DNA); +, positive control (CasKi DNA); P, positive control (plasmid)

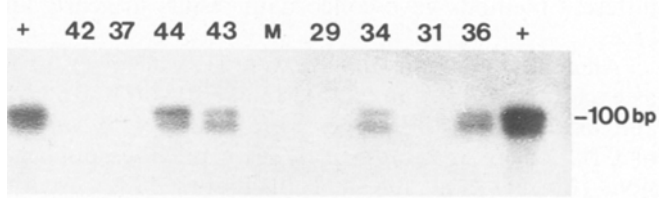

Fig. 6. PCR analysis of DNA extracted from endometrial tissues with HPV-18-specific primers. Southern blot hybridization with a digoxigenin-labelled, HPV-18-specific oligonucleotide probe. The numbers correspond to the case numbers in Table 1. +, Positive control (plasmid containing HPV-18 DNA). M, Molecular weight markers

One SCC which was negative for HPV-DNA exhibited c-*myc* over-expression.

Of the 8 endometrial tumours we studied, 7 were ordinary endometrial adenocarcinomas, whereas 1 carcinoma was of mixed type (endometroid with clear cell components). Squamous differentiation was seen in none. Three carcinomas were HPV-positive after PCR analysis with general and type-specific primers. One G1 tumour was positive for an HPV-16-related HPV type by PCR and Southern blot analysis. In 2 additional cases (1 adenocarcinoma, 1 tumour of mixed differentiation) HPV-18 DNA was found by PCR analysis. In order to rule out the possibility that the HPV-DNA found in endometrial lesions results from contamination during DNA extraction, the corresponding paraffin material was also analysed by PCR. In 2 carcinomas (cases 29 and 36) the same HPV type was also found in paraffin sections. In case 34, no HPV-DNA was detected in paraffin material.

In 1 tumour of mixed differentiation (case 36), *erb*B-2 over-expression was found, whereas no oncogene expression exceeding baseline level was detected in the other tumours. There was no correlation between *erb*B-2 expression or HPV infection and the grading and staging of the tumours.

Five pieces of normal endometrium taken after the hysterectomy (for uterine prolapse and fibroids), 2 cases of adenomatous hyperplasia, and 1 of adenomyosis – all without any signs of squamous differentiation – were studied. HPV-16 DNA was detected by PCR in both cases of hyperplasia in isolated DNA and in paraffin sections. In addition, HPV-18 was found in DNA from 1 of the hyperplasias and 1 adenomyosis, but not in paraffin sections of these cases. HPV-DNA was not

found in the 5 other specimens from normal endometrium. Oncogene activation was detected in none of the cases.

Discussion

Comparison of the HPV detection systems applied in this study demonstrated the high sensitivity and specificity of PCR. By the use of this technique, the HPV detection rate in pre-malignant and malignant cervical lesions increased to 100% when compared with 62% (13/21) by dot blot hybridization and 50% (10/20) by Southern blot analysis. HPV-DNA was also detected in 3 of 4 (75%) vulvar carcinomas, 3 of 8 (37%) endometrial carcinomas, 2 of 2 endometrial hyperplasias, 1 adenomyosis and 2 tissue samples from endometrium of patients with cervical SCC. These high numbers underline the importance of papillomaviruses as aetiological factors, at least in the pathogenesis of cervical and vulvar lesions. The existence of false-positive results can be ruled out because negative controls included in the study were always negative for HPV-DNA, and because HPV typing by different methods gave concordant results in nearly all cases.

All condylomata acuminata were HPV-6/11-positive, as expected, whereas in CIN lesions HPV-6/11, -16, -31 and unidentified HPV types were found. This variety of CIN lesions is in agreement with previous publications (Lorincz et al. 1987b; Hallam et al. 1989). As for malignant cervical and vulvar tumours, HPV-DNA was not only found in typical SCC but also in tumours of different morphological phenotype: HPV-16 was detected in 1 cervical adenocarcinoma and, to our surprise, in 1 vulvar basal cell carcinoma. HPV-6 DNA was found in 1 cervical verrucous carcinoma infiltrating the endometrium (case 7), and HPV-18 DNA was found in 1 adenoid cystic carcinoma (case 25). Papillomaviruses were already described in 2 adenoid cystic carcinomas of the cervix (HPV-16, -18; King et al. 1989), but to out knowledge never in adenoid cystic tumours of the vulvar region.

The detection of HPV-DNA in 3 endometrial adenocarcinomas and 3 benign endometrial lesions is in contrast to the findings of Bergeron et al. (1988), who did not find viral DNA in 28 tissue samples from normal or hyperplastic endometrium or adenocarcinomas by Southern blot hybridization. Yet in other studies, HPV-16 DNA was found in endometrial biopsies, especially in those from patients with cervical carcinomas (De Villiers et al. 1986; Macnab et al. 1986; Tsunokawa et al. 1986). In most endometrial lesions, we found HPV-DNA only by PCR and not by the less sensitive dot blot and Southern blot techniques. Therefore, we used paraffin material from the same lesions as control and found HPV-DNA in 4 of 7 HPV-positive cases (2 carcinomas and 2 hyperplasias). The positive HPV detection results with isolated DNA from the other 3 cases, including the unexpected HPV detection in 1 adenomyosis, could result from differences in the two tissue specimens used for DNA extraction and paraffin embedding, from differences in the amount of DNA used for both experiments, or from contamination during DNA extraction. The amount of HPV-DNA we found in endometrial samples was always low, compared with most cervical or vulvar lesions, and was only detectable by the most sensitive techniques. Tsunokawa et al. (1986) also found a very low HPV copy number (0.2 copies/haploid genome) in endometrial tissue in comparison to cervical carcinomas (100 copies/haploid genome). The question of whether these low amounts of HPV-DNA play a role in the pathogenesis of endometrial lesions such as they do in cervical lesions cannot be answered at present. While viral genes might contribute to enhanced cell division in hyperplasias, an aetiological role of papillomaviruses in the pathogenesis of adenomyosis with normal endometrium seems improbable, but the fact that HPV-DNA was not found in normal endometrium from hysterectomy specimens (cases of prolapse and uterine fibroids) suggests that the detection of HPV in some endometrial lesions is not an accidental finding.

In addition to papillomavirus DNA, we looked for possible activation of the oncogenes c-*myc* and *erb*B-2 as possible co-factors in the carcinogenesis of genital tumours. It should be noted that slight variations in signal intensity were not detected by our semi-quantitative method, and that only strong signals compared to the normal level were regarded as "over-expression". In addition, precise quantitation is not possible because we do not know the ratio of tumour/non-tumour cells in the sample.

*Erb*B-2 (HER2/neu) was only over-expressed in 1 locally advanced endometrial carcinoma of mixed differentiation. Activation of this oncogene is a common aspect of adenocarcinomas of the breast, ovary or salivary gland (Yokota et al. 1986, Van den Vijver et al. 1988; Slamon et al. 1989). Our results indicate that it can also take place in endometrial adenocarcinomas. Yet, our studies and previous publications have all failed to show *erb*B-2 activation in SCC (Rivière et al. 1990).

In contrast to *erb*B-2, we found c-*myc* activation in 2 cervical and 1 vulvar carcinomas, but in none of the pre-malignant or benign cervical lesions and tissues with glandular differentiation. Activation of this oncogene has already been described by Riou (1988) who found over-expression of this oncogene in 37% of stage I or II and 75% of stage III and IV tumours. An interesting aspect of our results is that 2 of 3 tumours exhibiting c-*myc* over-expression were negative for the high-risk HPV types -16, -18, -33 etc. One verrucous carcinoma harboured HPV-6 DNA (case 7), whereas 1 vulvar SCC was HPV-negative even by PCR (case 23). While these two tumours do not contain potent viral oncogenes, there is an activation of the c-*myc* oncogene. By analogy, Crook et al. (1991) recently found mutations within the *p53* tumour suppressor gene in 2 HPV negative cervical carcinoma cell lines. Obviously, the function of viral transforming genes is substituted by alterations of the cellular *p53* gene in these cases. Similarly, in our cases c-*myc* over-expression might either substitute the function of viral oncogenes or co-opperate with the *E6/E7* genes of low-risk HPV types in vivo, which are otherwise of very low transforming potential. Additional studies will be necessary to test this hypothesis.

Acknowledgements. The study was supported by the Hamburger Krebsgesellschaft e.V. (I 417) and the Hamburger Stiftung zur Förderung der Krebsbekämpfung.

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