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ORIGINAL ARTICLE

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Differences in HPV 16- and HPV 18 E6/E7 oncogene expression between in situ and invasive adenocarcinomas of the cervix uteri

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Abstract To evaluate the importance of high-risk human papillomavirus (HPV) types in in situ and invasive adeno- and adenosquamous carcinomas (ACISs/ACs, and ASCISs/ASCs) of the cervix uteri, we analyzed HPV infection and HPV 16- and HPV 18 E6/E7 oncogene expression in different histologic subtypes. Using the polymerase chain reaction (PCR) technique, 29 of 33 (88%) ACISs, 2 of 2 (100%) ASCISs, 46 of 54 (85%) ACs, and 8 of 10 (80%) ASCs were found to be HPV 16- and/or HPV 18-positive. In 25 of 35 (71%), 10 of 35 (29%), and 4 of 35 (11%) ACISs/ASCISs, HPV 16, HPV 18, and HPV 16 and HPV 18 were detected, respectively. Invasive ACs/ASCs were more frequently infected with HPV 18 (36 of 64, 56%) than with HPV 16 (28 of 64, 44%). Ten (16%) of these cases were positive for HPV 16 and HPV 18. In ACISs/ASCISs, HPV 16 oncogene expression predominated (62%) relative to HPV 18 (25%) expression, whereas in invasive ACs/ASCs, only 21% of the cases expressed HPV 16, but 48% of the cases expressed HPV 18 oncogenes. Thus, detection of HPV 18 in ACISs/ASCISs might be associated with an increased risk of progression. HPV oncogene expression was not dependent on histologic subtype of in situ or invasive AC. Normal glandular epithelia and glandular dysplasias (GDs, *n*=4) were always negative concerning HPV oncogene expression. In HPV 16- and HPV 18-double-infected cases, HPV 18 oncogene expression was most frequently detected, and we did not find a coexpression of HPV 16- and HPV 18-specific oncogenes in purely glandular lesions or in cases with an additional CIN (cervical intraepithelial neoplasia) II or CIN III. HPV E6/E7 expression of the same HPV type in both in situ or invasive ACs and associated CIN II/III suggest that these lesions might be histogenetically related.

Keywords Adenocarcinoma · Adenocarcinoma in situ · Cervix uteri · HPV infection · HPV 16 and HPV 18 E6/E7 oncogene expression

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Introduction

Adenocarcinomas (ACs) of the cervix uteri account for approximately 10–30% of cervical carcinomas, and their incidence is increasing, especially among young women [35]. While improvements in polymerase chain reaction (PCR) methodology have demonstrated that up to 100% of squamous cell carcinomas (SCCs) of the cervix uteri are associated with high-risk human papillomavirus (HPV) types [27, 49], HPV infection rates in ACs differ extremely between 35% and 80%, probably depending on the detection method used [12, 14, 18, 24, 33, 41, 43, 46, 47]. Similar to cervical SCCs, the high-risk HPV types 16 and 18 are the most important types associated with cervical ACs. However, in contrast to SCC with predominance of HPV 16, HPV 18 infections are increased in ACs [18, 33, 36, 45]. Some authors even described a predominance of HPV 18 [24, 46, 53].

AC in situ (ACIS) of the cervix is a well-characterized and increasingly recognized pathologic entity, and some histologic subtypes are thought to be precursors of invasive ACs [3, 4, 7, 9, 23, 25, 51]. The fact that the high-risk HPV types 16 and 18 are already detectable in ACISs underlines the close relationship of ACISs to ACs. Similar to ACs, the data about the frequency of HPV infection in ACISs are varying [2, 20, 34, 44], and there are no studies about HPV infection in the histologic subtypes of ACISs.

The oncoproteins E6 and E7 of high-risk HPV types play an important role in the HPV-associated cervical cancerogenesis. A lot of functions leading to disruption of normal growth regulatory mechanisms were described for these proteins, including interactions with p53, pRB,

and other cell cycle-regulating proteins [1, 16, 29, 50, 52]. Additionally, the activity of the ribonucleoprotein telomerase is increased by the action of E6, leading to elevated immortalization and proliferation of the keratinocytes [28]. In contrast to cervical intraepithelial neoplasia (CIN) I, an expression of the oncogenes E6 and E7 of high-risk HPV could be observed in most of CIN II/III and SCCs [15, 21, 38, 42]. The increase in oncogene expression is realized via different ways, but most frequently, especially in case of HPV 18, by an integration of the virus into the host cell genome and thereby disruption of the gene encoding the negative regulatory protein E2 [40]. This is followed by a dysregulated expression of the oncogenes E6/E7, which are consistently found to be expressed in HPV-associated cancers [26, 48]. Compared with CINs and SCCs, less is known about the expression of these viral oncogenes in glandular lesions of the cervix uteri. Only some sparse data from early studies exist about HPV oncogene expression in ACISs or ACs [17, 21, 42]. Moreover, no studies concerning the viral oncogene expression in different histologic subtypes of both in situ and invasive ACs were accomplished.

Accordingly, the aim of the current study was to evaluate the frequency of HPV infection in relation to HPV 16- and HPV 18 oncogene expression in a larger series of ACISs and ACs and to analyze the histologic subtypes in order to identify differences in HPV infection and expression of the viral oncogenes.

Because the role of glandular dysplasias (GDs) as potential precursors of ACISs and ACs has been controversially discussed [19], some cases of GDs were included in this study. Furthermore, our interest was focused on the localization of oncogene-specific mRNA, either in atypical glandular cells, squamous epithelium, or both, because coexisting CIN II/III is a major problem in HPV detection of glandular lesions [2, 14].

Material and methods

Tissue samples

This report is based on a study of 54 ACs, 10 adenosquamous carcinomas (ASCs), 33 cases of ACIS, 2 adenosquamous in situ carcinomas (ASCISs), and 4 GDs of the cervix uteri selected following histologic review from the files of the Department of Gynecopathology, University of Hamburg, the Division of Women's and Perinatal Pathology at Brigham and Women's Hospital (Boston, Mass.), several referring institutions, and from the pathology archives of Columbia Presbyterian Medical Center (New York, N.Y.). All cases were re-classified by two pathologists, independently, according to the most recent World Health Organization (WHO) criteria (1994), and a histologic subtyping was performed. In situ and invasive ACs composed of more than one histo-logic subtype and containing more than 10% of a secondary component were defined as mixed-differentiated type [9].

Two in situ carcinomas (see above) exhibited an atypical squamous and an atypical glandular component in one lesion. In this study, similar to the invasive counterpart, these lesions were termed ASCISs. ACs with strong nuclear atypia, extensive solid growth pattern, and without specific differentiation were grouped as poorly differentiated ACs. Additionally, all specimens were evaluated for associated CINs. Cases without detectable surface epithelium in the transformation zone of the analyzed tissue sample were designated as non-informative. All tissues had been routinely fixed in 4% buffered formalin and were paraffin embedded.

Detection of HPV infection

Genomic DNA was extracted from paraffin-embedded specimens using a tissue DNA extraction kit (Machery and Nagel, Düren, Germany). HPV detection was performed by means of PCR using the consensus primers GP5+/GP6+ [11] from the L1 region of the HPV genome. PCR-positive cases were further characterized using PCR with HPV 16-specific primers derived from the upstream regulatory region (URR; forward 5′-GCAGCTCTGTGCATAAC-3′, reverse 5′-CTGCACATGGGTGTGTGC-3′), HPV 18-specific primers (E2-gene; forward 5′-GAATTCACTCTATGTGCAG-3′, reverse 5′-TAGTTGTTGCCTGTAGGTG-3′) and HPV 6/11-specific primers [10]. Identification of PCR products occurred in a 10% polyacryamide gel with silver nitrate staining [39]. The integrity of the extracted DNA was tested by amplifying a fragment of the human beta globin gene. Appropriate positive, negative, and contamination controls were included in the PCR assays to verify the specificity of the method.

HPV 16- and HPV 18 E6/E7 probes

The construction of the HPV 16 E6/E7-specific probe was described earlier [38]. Plasmid pBR322 containing the whole HPV 18 genome [6], which was kindly provided by Dr. E. M. de Villiers, DKFZ Heidelberg, served as a target for PCR with the following primers:

- Forward: 5′-CGCTCTAGATATGGCGCGCTTTGAGGAT-3′
- Reverse: 5′-GCCAAGCTTTTACTGCTGGGATGCACAC-3′

*Bam*HI/*Hind*III-restricted vector pBluescript-IIKS$^+$ and PCR product were ligated and transformed into *Escherichia coli* DH5α. Identity of the 790-bp insert that corresponds to the appropriate region of the unspliced transcript was confirmed using direct plasmid sequencing.

Generation of labeled riboprobes

Radioactively labeled antisense and sense RNA probes were generated by means of run-off transcription in a solution containing 1 µg linearized plasmid, either T3 polymerase or T7 polymerase, 60 µCi ^{35}S-uridine triphosphate (specific activity 1000 Ci/mmol; DuPont NEN, Brussel, Belgium), and 1 mM each of the other three precursors as a cold substrate. This procedure yields RNA with a specific activity of about 5×10^9 cpm/µg.

In situ hybridization

RNA-RNA in situ hybridization (ISH) was described previously [38]. Autoradiography was performed for 14–35 days at 4°C. Slides were counterstained with hematoxylin and eosin (H&E). For ISH with HPV 16- and HPV 18 oncogene-specific probes, only cases infected with the appropriate HPV type were selected. Cases double-infected with HPV 16 and HPV 18 were analyzed with both probes.

Microscopic semiquantitative evaluation

Evaluation of conventional histology, immunohistochemistry, and autoradiographic signals was performed on serial sections by two pathologists, independently. The intensity of autoradiographic signals was determined by counting the grains over 100 cells in representative fields of every section and calculating the mean value. The same procedure was carried out with the corresponding

serial control sections (sense probes), which had no or markedly decreased signals, to establish the intensity of background signals. After subtraction of background signals, results were grouped as faint (>5–15 grains per nucleus), moderate (>15–35 grains per nucleus), strong (>35 grains per nucleus), or absent expression (number of grains corresponds to background or <5 per nucleus).

Statistics

Statistical analysis was performed with the help of the generalized Fisher's test. Results with *P*-values less than 0.05 were considered statistically significant.

Results

Glandular dysplasias

In three of four cases of GDs, HPV DNA was detected. We found HPV 16 in two of four cases and HPV 18 in three of four cases. Two cases were double-infected with HPV 16 and HPV 18. All three HPV-infected cases showed adjacent CIN II or CIN III. HPV oncogene expression was only observed in these CINs, one of them with HPV 16-specific and the other one with HPV 18-specific hybridization signals. HPV oncogene expression was not detected in cells of GD.

In situ adeno- and adenosquamous carcinomas

Altogether, 29 of 33 (88%) ACISs and 2 of 2 (100%) ASCISs were infected with HPV 16 and/or HPV 18. In four ACISs/ASCISs, both HPV 16 and HPV 18 were detected. The distribution of HPV 16 and HPV 18 between the histologic subtypes analyzed in this study is demonstrated in Table 1. Low-risk HPV types 6/11 were detected exclusively in association with high-risk types in two endocervical and one endometrioid ACISs.

All 28 analyzed HPV 16- and/or HPV 18-infected cases (three HPV 16-infected ACISs were not suitable for ISH) demonstrated viral oncogene expression signals specific for the detected HPV type over atypical glandular cells. The intensity was most frequently strong or moderate (Table 1, Fig. 1 and Fig. 2).

Simultaneous expression of HPV 16- and HPV 18-specific oncogenes was not detected in any of the double-infected cases. Two cases demonstrated HPV 18 oncogene-specific signals, and two cases demonstrated HPV 16 oncogene-specific signals (Table 1). Altogether, 20 of 32 (62%) ACISs/ASCISs expressed HPV 16 oncogenes, while only 8 of 32 (25%) expressed HPV 18-specific mRNA (Table 1). In columnar cells adjacent to atypical cells, neither HPV 16- nor HPV 18 oncogene-specific hybridization signals could be detected (Fig. 1A).

HPV oncogene expression in CIN II/III adjacent to ACIS/ASCIS

Additional CIN II or CIN III were observed in 15 of 35 (43%) of the cases. No significant differences in the infection rates with HPV 16 and/or HPV 18 were found between cases with (14 of 15, 93%) or without (18 of 20, 90%) adjacent CIN II/III.

In the majority of ACISs/ASCISs, HPV 16-specific oncogenes were expressed. Eight cases with HPV oncogene expression in both ACISs/ASCISs and CIN II/III were found (Fig. 1B), while in four cases, only atypical glandular cells displayed HPV oncogene-specific signals. There were no cases with E6/E7-specific signals over CIN II/III exclusively (Table 2).

Invasive AC/ASC

Altogether, 46 of 54 (85%) ACs, and 8 of 10 (80%) ASCs were infected with HPV 16 and/or HPV 18 (Ta-

Table 1 Human papillomavirus (HPV 16)- and HPV 18 infections and HPV 16/18 E6/E7 oncogene expression in subtypes of adenocarcinomas in situ (ACISs) and in adenosquamous carcinomas in situ (ASCISs; %). *PCR* polymerase chain reaction; *ISH* in situ hybridization

HPV	Total	Subtypes of ACIS			ASCIS
Infection (PCR)		Endocervical	Endometrioid	Mixed-differentiated	
HPV 16[a]	21/35 (60)	5/9 (56)	13/20 (65)	2/4 (50)	1/2 (50)
HPV 18[a]	6/35 (17)	2/9 (22)	2/20 (10)	2/4 (50)	0/2
HPV 16 and HPV 18	4/35 (11)	0/9	3/20 (15)	0/4	1/2 (50)
Oncogene expression (ISH)					
HPV 16[b]	18/18 (100)	4/4 (100)	12/12(100)	1/1	1/1
HPV 18[c]	6/6 (100)	2/2 (100)	2/2 (100)	2/2 (100)	
HPV 16[d]	2/4 (50)		1/3 (33)		1/1
HPV 18[d]	2/4 (50)		2/3 (67)		0/1

[a] Without HPV 16- and HPV 18-double-infected cases
[b] In cases only infected with HPV 16
[c] In cases only infected with HPV 18
[d] In HPV16/18-double-infected cases

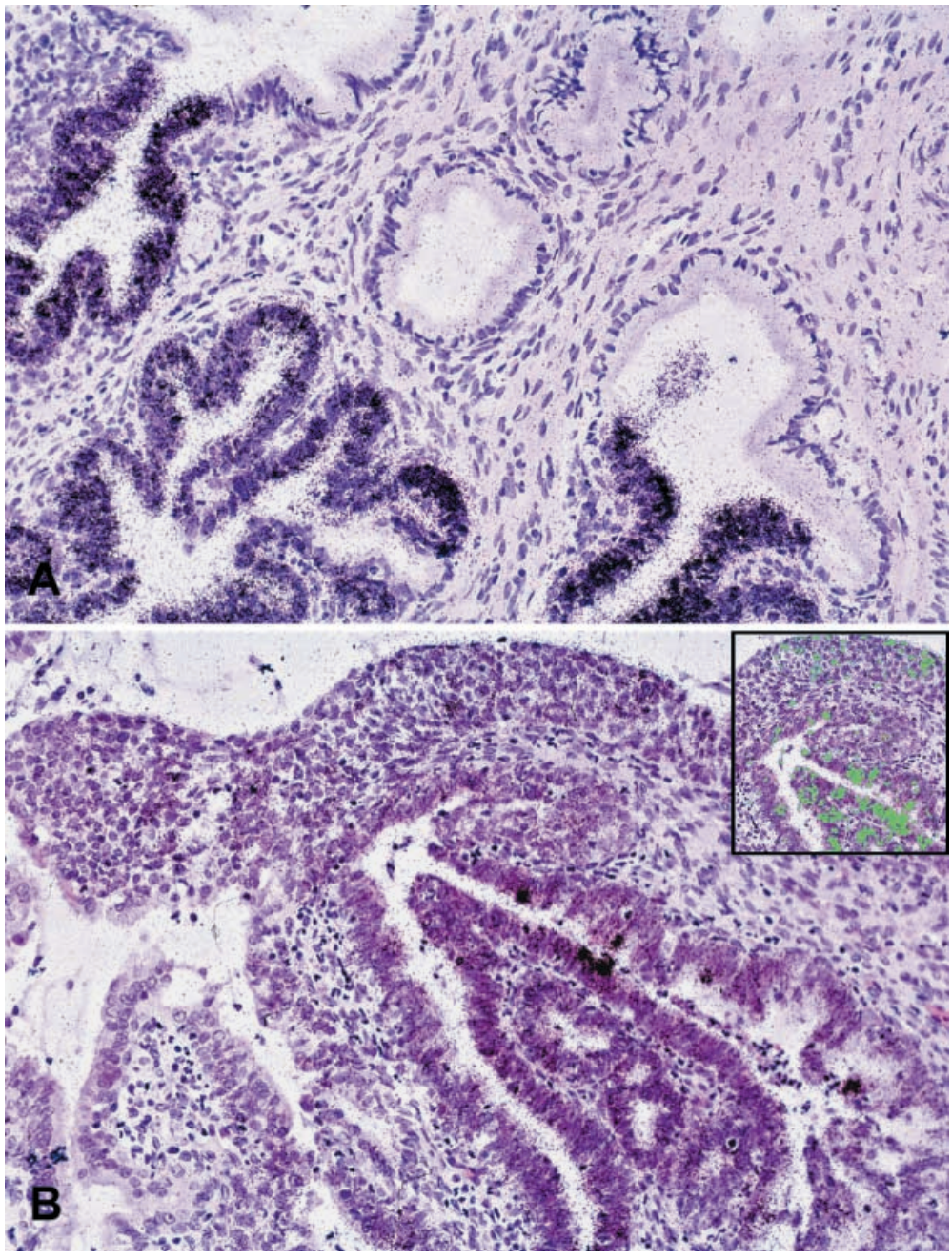

Fig. 1 In situ hybridization with ^{35}S-labeled human papillomavirus (HPV) 16 and HPV 18 oncogene-specific antisense RNA probes. **A** Strong black HPV 16 E6/E7-specific signals only over atypical glandular cells of adenocarcinoma in situ (ACIS); ×200. **B** Moderate HPV 18 E6/E7-specific signals over cervical intraepithelial neoplasia (CIN) III of surface epithelium and over ACIS; ×200. In the *inset*, hybridization signals (grains) were labeled by a *green color*

ble 3). Although failing to reach statistical significance, percentages of HPV-infected endocervical ACs and ASCs were lower than those of the other histologic subtypes (Table 3). Low-risk HPV types 6/11 were only detected, in addition to high-risk types, in one ASC.

Coexpression of HPV 16- and HPV 18-specific oncogenes was not observed in any of the HPV 16/18-double-infected cases. HPV 18 oncogene expression predominated in double-infected ACs/ASCs. Eight of them expressed HPV 18 oncogenes, but only two expressed HPV 16 oncogenes (Table 3).

Three ACs (two HPV 16- and one HPV 18-infected) could not be analyzed using ISH. Altogether, only 13 of 61 (21%) ACs/ASCs expressed HPV 16 oncogenes (Fig. 3B), while 29 of 61 (48%) demonstrated HPV 18-specific hybridization signals (Fig. 3A). HPV 16- and HPV 18 E6/E7-specific hybridization signals were evident not only over invasive foci, but also over atypical cells of adjacent ACISs/ASCISs when present in the same specimens. Most frequently, signals with strong intensity were observed.

No significant dependence of HPV oncogene expression on histologic subtype could be observed (Table 3). Poorly differentiated ACs expressed HPV 18 oncogenes exclusively. The single intestinal and serous carcinomas expressed HPV 16- and HPV 18-specific oncogenes, respectively, with strong intensity, whereas a clear-cell car-

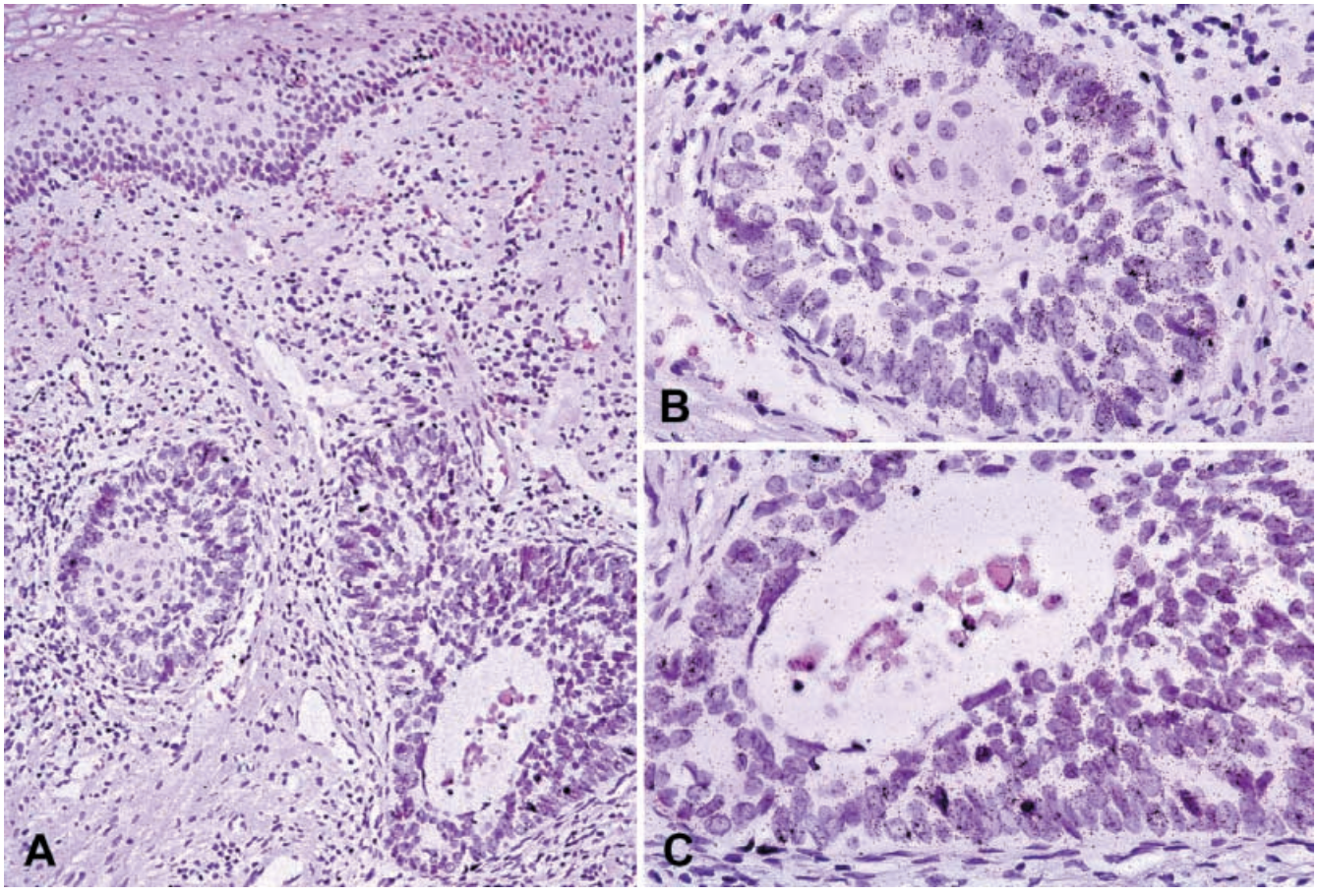

Fig. 2 In situ hybridization with a ^{35}S-labeled human papillomavirus (HPV) 16 oncogene-specific antisense RNA probe. Adenosquamous carcinoma in situ (A) with moderate hybridization signals over atypical squamous (B) and atypical glandular cells (C); ×400

Table 2 Number of human papillomavirus (HPV) 16/18 oncogene-expressing adenocarcinomas in situ/adenosquamous carcinomas in situ (ACISs/ASCISs) and adenocarcinomas/adenosquamous carcinomas (ACs/ASCs) per number of cases with additionally detected cervical intraepithelial neoplasia (CIN) II/III. *ISH* in situ hybridization

HPV infection (PCR)	Oncogene expression (ISH)			
	Only in CIN	Only in glandular atypical cells	In CIN and glandular atypical cells	No expression or not evaluable
ACIS/ASCIS				
HPV 16	0/11	2/11	7/11	2/11
HPV 18	0/1	0/1	1/1	0/1
HPV 16 and HPV 18	0/2	2/2 (HPV 18)	0/2	0/2
AC/ASC				
HPV 16	1/2	0/2	0/2	1/2
HPV 18	0/4	1/4	3/4	0/4
HPV 16 and HPV 18	0/2	1/2 (HPV 18)	1/2 (HPV 16)	0/2

cinoma, although infected with HPV 18, did not express the oncogenes. The only mesonephric carcinoma was HPV negative (other types, Table 3).

HPV oncogene expression in CIN II/III adjacent to AC/ASC

In 9 of 45 (20%) informative invasive ACs and ASCs, adjacent CIN II or CIN III were observed. There was no significant difference in HPV infection between cases with (8 of 9, 89%) or without CIN II/III (29 of 34, 85%). In two cases, HPV oncogene expression could only be observed in glandular tumor cells, while in four cases, HPV-specific signals were seen over both CIN II/III and AC/ASC (Table 2). There was only one mixed-differentiated AC with HPV 16 oncogene-specific hybridization signals over CIN III exclusively (Table 2).

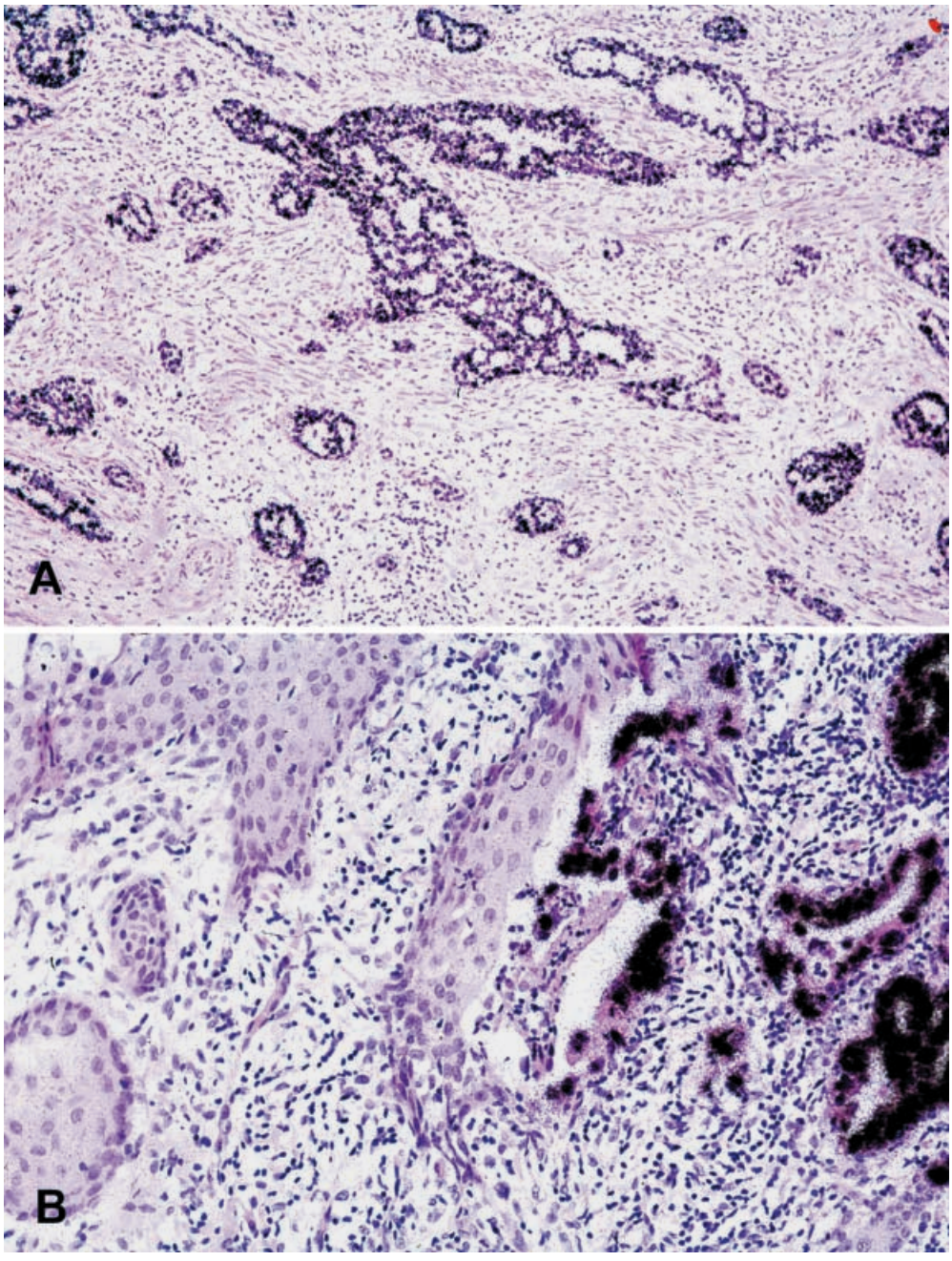

Fig. 3 In situ hybridization with ^{35}S-labeled human papillomavirus (HPV) 16 and HPV 18 oncogene-specific antisense RNA probes **A** Strong black HPV 18 oncogene-specific signals over atypical cells of adenocarcinoma (AC); ×50. **B** Strong HPV 16 oncogene-specific signals over atypical cells of a microinvasive AC. No expression in adjacent squamous metaplasia; ×200

Discussion

ACs of the cervix uteri have become more prevalent in recent years. In contrast to SCC, for which the development from CIN to invasive carcinoma in close relation to high-risk HPV infection has been well characterized comparably less is known about etiology and pathogenesis of cervical ACs.

Cervical AC comprises a morphologically heterogeneous disease, with approximately 70% of the endocervical type being most frequent [9]. Tumors of endocervical and intestinal type together form the group of mucinous ACs. The second most frequent type is the endometrioid AC. Furthermore, clear cell, serous, and mesonephric ACs belong to pure cervical ACs. Additionally, ASCs are diagnosed with a rate of 15–35% among all cervical carcinomas with a glandular component [9].

Numerous studies revealed that ACs are less frequently infected with high-risk HPV types than SCCs [18, 30, 47]. However, taken together, with the results of recent studies, especially those obtained using highly sensitive PCR rather than dot blot hybridization or DNA ISH, it becomes clear that 70–90% of ACs are infected with high-risk HPV types [24, 41, 46]. We detected HPV 16 and/or HPV 18 in 84% of all AC/ASCs. The infection rates in endocervical ACs and in ASCs were 70% and

Table 3 Human papillomavirus (HPV) 16- and HPV 18 infections and HPV 16/18 E6/E7 oncogene expression in different types of adenocarcinomas (ACs) and adenosquamous carcinomas (ASCs; %). *PCR* polymerase chain reaction, *ISH* in situ hybridization

HPV infection (PCR)	Total	Subtypes of AC					ASC
		Endocervical	Endometrioid	Mixed-differentiated	Poorly differentiated	Other types[e]	
HPV 16[a]	18/64 (28)	5/20 (25)	7/17 (41)	3/9 (33)	0/4	1/4 (25)	2/10 (20)
HPV 18[a]	26/64 (41)	7/20 (35)	5/17 (29)	6/9 (67)	4/4 (100)	2/4 (50)	2/10 (20)
HPV 16 and HPV 18	10/64 (16)	2/20 (10)	4/20 (20)	0/9	0/4	0/4	4/10 (40)
Oncogene expression (ISH)							
HPV 16 [b]	11/16 (69)	3/4 (75)	5/6 (83)	2/3 (67)		1/1	0/2
HPV 18 [c]	21/25 (84)	5/6 (83)	5/5 (100)	5/6 (83)	4/4 (100)	1/2 (50)	1/2(50)
HPV 16 [d]	2/10 (20)	0/2	1/4 (25)				1/4 (25)
HPV 18 [d]	8/10 (80)	2/2 (100)	3/4 (75)				3/4 (75)

[a] Without HPV 16- and 18-double-infected cases
[b] In cases only infected with HPV 16
[c] In cases only infected with HPV 18
[d] In HPV 16/18-douple-infected cases
[e] One of each intestinal, serous, clear cell, and mesonephric adenocarcinomas

80%, respectively. The vast majority of endometrioid, mixed- and poorly differentiated carcinomas (90% up to 100%), however, was infected with high-risk HPV types. Tenti et al. found higher HPV infection rates in mucinous than in non-mucinous ACs [45].

Whether HPV 16 or HPV 18 is more frequently associated with AC is still controversially discussed. In agreement with previous reports [24, 33, 36, 46], the percentage of HPV 18-infected ACs/ASCs in our study reached almost 50% or more, but was not significantly higher than that of HPV 16. With the exception of poorly differentiated ACs that were exclusively infected with HPV 18, we found both HPV 16 or HPV 18 in all analyzed histologic subtypes (Table 3). In contrast, Duggan et al. most frequently detected HPV 16 in a series of ACs after substitution of dot blot hybridization with a sensitive PCR technique [14]. Whether these differences were due to different histologic subtypes involved in the studies remains unclear.

Many authors consider ACISs/ASCISs as precursors of invasive ACs/ASCs. Proliferative activity and expression of genes encoding cell cycle-regulating proteins in ACISs are similar to that of invasive ACs [31, 32]. Besides characteristic morphologic features, such as crowded and hyperchromatic enlarged nuclei [25], the expression of CEA (carcinoembryonic antigen), p53 accumulation, and detection of proliferative activity by Ki-67 expression are used to distinguish ACISs/ASCISs from benign glandular proliferations [8]. ACISs/ASCISs have a similar epidemiologic profile relative to their invasive counterparts, with an average appearance 10 years earlier. In addition, reports about a transition of ACISs into invasive ACs were published [3, 5, 22, 30, 35, 37].

In contrast to other studies [13, 17, 21, 42], which revealed higher HPV 18 than HPV 16 infection rates in ACISs, ACISs/ASCISs were more frequently infected with HPV 16 than with HPV 18 in our study. These differences could be due to a different sensitivity of the methods to detect individual HPV types [14]. Interestingly, in our study, the relation of HPV 16- and HPV 18-infected cases significantly differed between ACISs/ASCISs and ACs/ASCs in that HPV 18 was increasingly detected with a higher grade of malignancy (Table 1 and Table 3, P=0.014).

HPV oncogene expression in ACIS/ASCIS and AC/ASC

Because a causal role of the HPV-encoded oncoproteins E6/E7 is also supposed for the development of ACs, we analyzed the expression of HPV 16- and HPV 18 E6/E7 oncogenes in ACISs and ACs by RNA/RNA ISH. Our results clearly show that atypical glandular cells express HPV 16- or HPV 18 E6/E7 oncogenes in most cases. This was true both for in situ and for invasive carcinomas (Table 1 and Table 3; Fig. 1, Fig. 2, and Fig. 3).

A small number of ACs/ASCs did not express HPV 16- or HPV 18 oncogenes, although they were infected with either HPV 16 or HPV 18 (Table 3). This discrepancy could be due to infections with other than detected HPV types, a down-regulation of the transcription of these oncogenes, or differences in the sensitivity of the methods.

We did not find a significant correlation of HPV oncogene expression and histologic subtypes. Interestingly, in one HPV 16-infected serous AC, HPV 16-specific hybridization signals could be detected, whereas one HPV 18-infected clear-cell carcinoma did not express the oncogenes. The HPV infection detected in this case could be caused by an additionally detected CIN I without oncogene expression. Because of the different origin of mesonephric carcinomas from other ACs, it is not unexpected that the single case in our study was HPV-negative.

Oncogene-specific hybridization signals have never been found over morphologically normal glandular cells. Distinct borders between negative normal glandular cells and positive cells of ACISs exhibiting the characteristic morphological features were marked by HPV oncogene expression. In almost all ACISs/ASCISs, HPV oncogene

expression was detected in the transformation zone either in squamous and atypical glandular or only in glandular cells. Furthermore, we observed a close relationship between ACISs/ASCISs and invasive ACs in that premalignant areas at the surface showed the same oncogene expression as the invasive carcinoma [17].

As already shown for HPV 16 and HPV 18, infection rates in in situ and invasive ACs/ASCs differed significantly in their relation of HPV 16- to HPV 18 oncogene expressing cases (P=0.0012). Whereas among ACISs/ASCISs the number of HPV 16-expressing cases was higher than that of HPV 18-expressing cases, this relation was opposite in invasive ACs/ASCs.

HPV oncogene expression in GD

GDs are distinguished from ACISs by the lack of epithelial stratification, a lesser degree of nuclear atypia and a paucity of mitotic activity. Their role as precursors of ACISs is controversially discussed. HPV infections in GDs were described, but were mostly due to an associated CIN of the same specimen [2]. Accordingly, in our study, a small number of specimens with GDs were also HPV 16- or HPV 18-infected. However, HPV oncogene expression was exclusively observed in the adjacent CIN II/III.

HPV oncogene expression in adjacent CIN II/III

Because between 30% and 60% of ACISs and about 20% of ACs have a concomitant CIN [2, 13, 14, 44], we analyzed the oncogene expression of associated CIN II/III to recognize whether the CINs are additional or single sources of the PCR-detected HPV infection. A possible developmental histogenetic relation of ACISs/ASCISs and ACs/ASCs to CIN II/III was suggested by similar HPV 16- or HPV 18 oncogene expression in squamous and glandular lesions of the same specimens in some cases [17]. Expression of HPV oncogenes of different types simultaneously in CINs and glandular lesions as shown by Higgins et al. [21] could not be observed in our study analyzing HPV 16 and HPV 18 double-infected cases. Our ISH results clearly confirm that with one exception, the HPV infection was not exclusively caused by associated CIN II/III in both ACISs/ASCISs and ACs/ASCs. Thus, our results confirm the data obtained by Duggan et al., who used microdissection to separate squamous and glandular lesions and showed that the HPV status of ACISs was not predictive of a copresence of CIN II/III with ACISs [13].

HPV oncogene expression in HPV 16/18 double-infected cases

HPV 16/18-double infected ACISs/ASCISs and ACs/ASCs mainly expressed HPV 18 oncogenes (Table 1 and Table 3). This is in contrast to HPV 16/18 double-infected cervical SCCs and CIN II/III that only showed HPV 16 expression (own unpublished results). We did not find coexpression of HPV 16 and HPV 18 mRNA in purely glandular lesions, squamous lesions, or in glandular lesions with adjacent CIN II/III. The mechanisms repressing E6/E7 oncogene transcription of one of the infecting HPV types are not known. A better knowledge of the physical state of the HPV genome, whether or not it is integrated into the host cell genome, could help to explain this problem.

Our results demonstrate a predominant role of HPV 18 in the development of ACs/ASCs from their potential precursors. Although both HPV 16 and HPV 18 oncogenes were expressed in ACISs/ASCISs, the detection of HPV 18 infection and oncogene expression in in situ lesions might be associated with a higher risk of progression. In further studies, these results have to be correlated with clinical data. It remains to be elucidated whether quantitative differences of HPV 16 and HPV 18 oncogene mRNA amounts or different regulatory functions of HPV 16 and HPV 18 oncoproteins account for a more aggressive behavior and a worse prognosis of HPV 18-infected ACISs/ASCISs relative to HPV 16-infected ACISs/ASCISs.

Detection of HPV infection and HPV oncogene expression are not helpful for differential diagnosis of histologic subtypes of ACISs and ACs. However, HPV 16 or HPV 18 oncogene expression is a good marker for recognizing atypical cells of ACISs/ASCISs or invasive ACs/ASCs, to differentiate ACISs and GDs, or to clarify metastases of unknown origin.

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