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Short Communication

Prevalence of Human Papillomavirus DNA in Different Histological Subtypes of Cervical Adenocarcinoma

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The prevalence of human papilloma virus (HPV) DNA in different histological subtypes of cervical adenocarcinoma and related tumors was examined using formalin-fixed, paraffin-embedded tissue samples from 105 primary cervical adenocarcinomas and adenosquamous carcinomas. Broad-spectrum HPV DNA amplification and genotyping was performed with the SPF10 primer set and line probe assay (LiPA), respectively. HPV DNA was detected in 82 of 90 (91%) mucinous adenocarcinomas, encompassing endocervical, intestinal, and endometrioid histological subtypes, and in nine of nine adenosquamous tumors (100%). HPV DNA was not detected in any nonmucinous adenocarcinomas including clear cell, serous, and mesonephric carcinomas (0/6). The most common viral types detected in adenocarcinoma were HPV 16 (50%) and HPV 18 (40%), followed by HPV 45 (10%), HPV52 (2%), and HPV 35 (1%). Multiple HPV types were detected in 9.7% of the cases. In conclusion, mucinous adenocarcinomas and adenosquamous carcinomas of the cervix demonstrate a very high prevalence of HPV DNA, similar to that reported for cervical squamous cell carcinoma. Only rare histological variants of cervical adenocarcinoma seem unrelated to HPV infection. *(Am J Pathol 2000, 157:1055–1062)*

Adenocarcinoma of the cervix (AdCx) accounts for approximately 15% of cervical cancers and has been increasing in incidence during the last few decades, particularly in younger women.[1] The etiology of squamous cell carcinoma of the cervix, the most common type of cervical malignancy, is linked to infection with oncogenic types of human papillomavirus (HPV), but the pathogenesis of AdCx is less well understood. Although HPV DNA is consistently detected in >90% of squamous cell carcinomas of the cervix,[2] the reported prevalence of HPV DNA in AdCx varies significantly, from 32% to 100%, depending on the detection method used.[3–13]

A strong association between a sexually transmitted agent (HPV) and the risk of development of cervical squamous cell carcinoma has been clearly established, however, the relationship between HPV and cervical adenocarcinoma remains uncertain.[14,15] Only a few, small, epidemiological studies separately examining AdCx have been conducted and the statistical power to detect an association with HPV has been limited.[16] Epidemiological risk factors for cervical adenocarcinoma include those that correlate with the risk of acquiring HPV infection, such as early age at first sexual intercourse and multiple sexual partners.[14,15,17,18] In addition, AdCx was also found to be associated with obesity, a well-known endometrial cancer risk factor.[14,15,17,18] Some studies have reported an association of AdCx with the prolonged use of oral contraceptives.[14,15,18] However, the lack of a protective effect of barrier contraception could be a confounding factor in these studies, because the relationship between AdCx and oral contraceptives disappeared after accounting for HPV infection[19] and the use of a diaphragm was found to be inversely related to the risk for AdCx.[18]

Cervical adenocarcinomas include several different histological types. The majority of tumors are mucinous adenocarcinomas that resemble either endocervical, in-

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testinal-type, or endometrioid epithelium and are often associated with the presence of squamous intraepithelial lesions. The nonmucinous tumors include clear cell carcinomas and serous carcinomas that resemble the clear cell and serous tumors found in the endometrium and ovary. Many of the studies involving endocervical adenocarcinomas have not analyzed the different histological subtypes separately. This lack of separation of histological types may have confounded both the results of the HPV studies and the epidemiological findings.

To further investigate the relationship between HPV and cervical adenocarcinoma, we examined a large number of tumors encompassing a broad spectrum of morphological differentiation, including mucinous and nonmucinous adenocarcinomas and related tumors with adenosquamous differentiation. HPV DNA amplification was performed using a novel, sensitive, broad-spectrum HPV polymerase chain reaction (PCR) assay (SPF10 PCR), which permits general HPV DNA amplification of at least 43 known HPV types.[20] HPV genotyping was performed using a novel line probe assay (LiPA). This LiPA version enables simultaneous identification of 25 individual HPV genotypes, allowing efficient detection of single and/or multiple HPV infection.[21]

Materials and Methods

Clinical Specimens

Consecutive cases of *in situ* and invasive cervical adenocarcinomas and related tumors were retrieved from the archives of the Departments of Pathology at New York Presbyterian Hospital (1978 to 1998) and Lenox Hill Hospital (1990 to 1998), both in New York City; Kyto Diagnostics LLC, New City, NY (1997); and Laboratory of Reproductive Pathomorphology, Warsaw Medical School, Warsaw, Poland (1998). A total of 73 cases of invasive adenocarcinomas, 23 cases of adenocarcinoma *in situ* (AIS), two cases of adenoid basal carcinoma, and one case of glassy cell carcinoma were collected. Six cases of adenosquamous carcinoma were selected and included in the study as a positive control group. Nonprimary cervical carcinomas were excluded.

All cases were reviewed and diagnostic groups were assigned and graded according to standard histological criteria.[22,23] The presence of an associated squamous intraepithelial lesion was recorded. A representative tissue block from each case was selected for HPV analysis. Clinicopathological parameters were obtained from the pathology reports.

DNA Extraction

Three, 5-μm sections of formalin-fixed, paraffin-embedded tissue were placed on glass slides after cutting deep into the block. The microtome blade was changed after each case. The tissue sections were deparaffinized and stained with hematoxylin. Tumor tissue was carefully microdissected from the adjacent squamous epithelium and stroma using a sterile scalpel blade. Benign cervical stroma away from the tumor was separately scraped from the same slide and processed in parallel as a negative control. The samples were incubated with proteinase K (1 mg/ml) for 18 hours at 56°C and heat inactivated.

HPV DNA Detection and Typing

Broad-spectrum HPV DNA amplification was performed using the short PCR fragment (SPF10) primer set, as described previously.[20] The SPF10 primers amplify a 65-bp fragment from the L1 region of the HPV genome.[20,21] The PCR products were analyzed by both 3% agarose gel electrophoresis and HPV DNA enzyme immunoassay (DEIA), a microtiter plate-based hybridization assay (Innogenetics Inc., Alpharetta, GA), as previously described.[20] To ensure adequate DNA preparation, PCR amplification of β-globin was performed in a separate reaction using primers PC03 and PC04, resulting in a 96-bp product.[24]

Samples identified as positive for HPV DNA were genotyped with the INNO-LiPA HPV prototype research assay (LiPA; Innogenetics Inc.).[21] In this assay, the HPV PCR product is hybridized to the genotype-specific probes immobilized as parallel lines on a nitrocellulose strip. Twenty-five individual HPV genotypes (HPV 6, 11, 16, 18, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68, 70, and 74) can be identified simultaneously in a single assay. The hybridization and the color reaction, which results in a purple precipitate, were performed automatically in an AutoLiPA device. The results of hybridization were assessed visually by comparing to the standard grid.

Statistical Analysis

The differences of the means of the continuous variables were analyzed with the Student's *t*-test and the distribution of noncontinuous clinicopathological variables *versus* HPV status was analyzed with the chi-square test, using the SPSS software package (SPSS Inc., Chicago, IL). *P* values of <0.05 were used as the cut-off for statistical significance.

Results

Clinicopathological Characteristics

The clinicopathological characteristics are presented in Table 1. Patients with adenocarcinoma *in situ* were almost a decade younger than those with invasive adenocarcinoma (36.3 years *versus* 45.2 years, $P < 0.05$). The average age of patients with invasive adenocarcinoma and adenosquamous carcinoma was almost identical (45.2 years *versus* 45.8 years). Patients with adenoid basal carcinoma, clear cell, and minimal deviation adenocarcinoma were older than the other patients; however, the differences were not statistically significant.

AIS was identified in 52% of patients with invasive mucinous tumors. High-grade squamous intraepithelial lesions were identified in all cases of adenosquamous carcinoma. High-grade squamous intraepithelial lesions

Table 1. Clinicopathological Data

Diagnosis	*n*	Average age	Age range	HSIL %	Grade 1 %	Grade 2 %	Grade 3 %	Stage 1 %	Stage 2 %	Stage 3 %
Adenocarcinoma *in situ*	23	36.3	19–58	39.1	N/A	N/A	N/A	N/A	N/A	N/A
Endocervical-type AdCx	55	44.1	22–80	18.1	66.6	31.5	1.8	87.1	4.3	8.6
Intestinal-type AdCx	6	47.3	26–69	16.7	66.6	33.3		66.6		33.3
Endometrioid-type AdCx	4	50.0	33–69	0.0	75.0	25.0		100.0		
Minimal deviation AdCx	2	53.5	37–70	0.0	100.0			50.0		50.0
Clear cell AdCx	4	52.2	45–62	0.0			100.0	75.0		25.0
Serous AdCx	1	39.0	39	0.0			100.0	100.0		
Mesonephric AdCx	1	35.0	35	0.0		100.0				
All invasive adenocarcinomas	73	45.2	22–80	15.0	61.6	30.1	8.2	82.8*	2.8	14.2
Adenoid basal carcinoma	2	59.5	55–64	50.0		50.0	50.0	100.0		
Glassy cell carcinoma	1	36.0	36	0.0			100.0	unknown		
Adenosquamous carcinoma	6	43.0	31–60	100.0		66.6	33.3	100.0		
All adenosquamous carcinomas	9	45.8	31–64	77.7	0.0	55.5	45.5	100.0†		

AdCx, adenocarcinoma; HSIL, high-grade squamous intraepithelial lesion.
*Clinicopathological stage available in 35 cases.
†Clinicopathological stage available in six cases.

were also identified in 39.1% of the AIS cases and in 18.1% of mucinous adenocarcinomas. Patients with high-grade squamous intraepithelial lesions were younger than those with no identifiable squamous intraepithelial lesion (39.1 years *versus* 44.3 years, $P < 0.05$).

HPV DNA Detection and Typing

β-globin DNA was amplified in all cases and HPV DNA was amplified in 91 of 105 cases, some of which were stored in the paraffin blocks for as long as 20 years. In

Table 2. HPV DNA Detection and Typing

		HPV+		HPV16		HPV18		HPV45		Other HPV types*		Multiple HPV types†	
Diagnosis	*n*	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Adenocarcinoma *in situ*	23	23	100.0	10	43.4	6	26.0	1	4.3	1[A]	4.3	5[D]	21.7
Endocervical-type AdCx	55	50	90.7	22	44.0	21	42.0	3	6.0	1[B]	2.0	3[E]	6.0
Intestinal-type AdCx	6	5	83.3	0	0.0	1	20.0	4	80.0	0	0.0	0	0.0
Endometrioid-type AdCx	4	4	100.0	2	50.0	2	50.0	0	0.0	0	0.0	0	0.0
Minimal deviation AdCx	2	0	0.0										
All *in situ* and invasive mucinous adenocarcinomas	90	82	91.1	34	41.4	30	36.5	8	9.7	2	2.4	8	9.7
Clear cell AdCx	4	0	0.0										
Serous AdCx	1	0	0.0										
Mesonephric AdCx	1	0	0.0										
All nonmucinous adenocarcinomas	6	0	0.0										
Adenoid basal carcinoma	2	2	100.0	2	100.0	0	0.0	0	0.0	0	0.0	0	0.0
Glassy cell carcinoma	1	1	100.0	0	0.0	1	100.0	0	0.0	0	0.0	0	0.0
Adenosquamous carcinoma	6	6	100.0	2	33.3	2	33.3	1	16.6	1[C]	16.6	0	0.0
All adenosquamous carcinomas	9	9	100.0	4	44.4	3	33.3	1	11.1	1	11.1	0	0.0

AdCx, adenocarcinoma.
*Other HPV types: [A]HPV 35, [B]HPV 52, [C]HPV 31.
†Multiple HPV types: [D]HPVs: 16+18; 16+31; 16+53; 16+39+66; 16+11; [E]HPVs: 16+18; 16+33; 18+52.

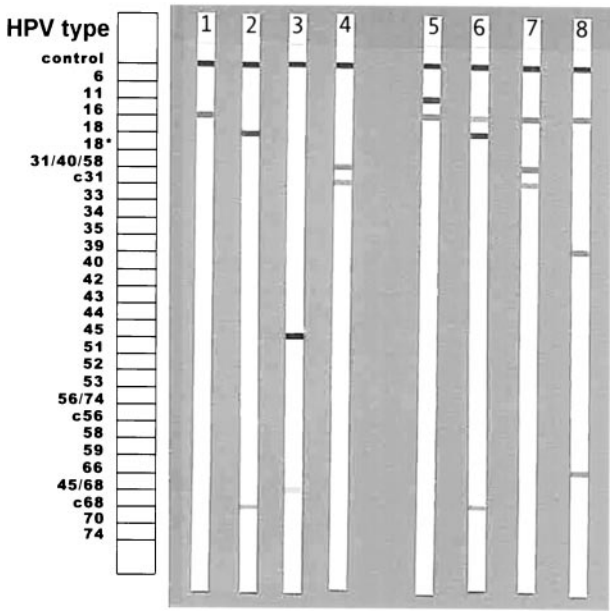

Figure 1. Identification of HPV genotypes using the LiPA. LiPA strips with hybridization bands indicating a single HPV type infection (**lane 1**, HPV 16; **lane 2**, HPV 18; **lane 3**, HPV 45; **lane 4**, HPV 31) and a multiple HPV type infection (**lane 5**, HPV 11 + 16; **lane 6**, HPV 16 + 18; **lane 7**, HPV 16 + 31; **lane 8**, HPV 16 + 39 + 66. Note: HPV 18 is reactive with two probes: 18 and c68.

cases with HPV DNA amplification, the presence of HPV-specific sequences was confirmed with the DNA enzyme immunoassay and the individual HPV genotypes were subsequently identified with the LiPA. The cases, in which HPV DNA was not detected, were of various storage ages.

The results of HPV DNA detection in different histological tumor subtypes are summarized in Table 2. The tumor subtypes were grouped as follows: 1) *in situ* and invasive mucinous adenocarcinomas; 2) nonmucinous adenocarcinomas; and 3) tumors with adenosquamous differentiation. We included the endometrioid type of adenocarcinoma in the mucinous group, as it expresses the same range of mucins as the tumors with endocervical-type histology (see Discussion).[25]

HPV DNA was detected in 82 of 90 *in situ* and invasive mucinous adenocarcinomas (91.1%), in none of the six nonmucinous adenocarcinomas (0%), and in all nine tumors with adenosquamous differentiation (100%) (Table 2). HPV 16 was the most common viral type identified and was detected in 50% of the HPV-positive adenocarcinomas. These included cases in which HPV 16 was found as the sole viral type (41.5%) and cases with multiple-type HPV infection (8.5%) (Figure 1). HPV 18 was almost equally prevalent and was detected in 40.2% of all HPV-positive adenocarcinomas—as a single HPV type in 36.5% and with other HPV types in 3.7%. HPV 45 was found in 9.7% of HPV-positive adenocarcinomas. HPVs 35 and 52 were identified in one tumor each as a single HPV-type infection. Carcinomas with adenosquamous differentiation had a similar HPV-type distribution.

Multiple HPV types were detected in 9.7% of the cases (Table 2 and Figure 1). Multiple-type infection was more frequent in AIS than in endocervical AdCx (21.7% *versus* 6.0%, $P < 0.05$). In all cases of multiple HPV infection, either HPV 16 or HPV 18 was always detected in addition to other high- or low-risk viral types, which included HPV 11, 31, 33, 39, 52, 53, and 66. (Table 2, footnote). After accounting for multiple-type infections, the ratio of HPV 16 to HPV 18 was essentially 1:1 in endocervical AdCx, endometrioid AdCx, and adenosquamous carcinomas. In AIS, HPV 16 was more than twice as common as HPV 18 (2.5:1).

The average age of the patients with HPV DNA-positive *versus* HPV DNA-negative tumors (42.8 years *versus* 44.1 years) was not significantly different ($P = 0.7$). There was no difference in the patients' average age when stratified by HPV type (HPV 16 *versus* 18 *versus* 45). However, patients with multiple HPV infection were significantly younger than patients with a single viral type (33.5 years *versus* 43.7 years, $P < 0.05$). No association between the tumor grade or stage and the presence of HPV DNA or a particular HPV type was detected.

Discussion

Our results demonstrate a very high prevalence of HPV DNA in cervical adenocarcinomas when compared to most previous reports[3–12] and similar to that reported for cervical squamous cell carcinoma.[2] The relative difficulty in detecting HPV DNA in adenocarcinomas, in contrast to squamous cell carcinomas, may be attributed to a lower viral load in glandular lesions as compared to squamous lesions. Premalignant and malignant squamous lesions, in particular those associated with HPV 16, contain a large number of episomal viral particles, in addition to integrated HPV sequences.[26] Glandular epithelium does not support productive viral infection and HPV DNA in endocervical neoplasms (notably HPV 18), is usually present in the integrated form.[27] As a result, detection of HPV DNA in adenocarcinomas requires a sensitive detection assay. Further, as the successful amplification of HPV DNA in a PCR assay depends on the presence of intact DNA target sequences, two additional factors may reduce the efficiency of HPV detection: 1) DNA fragmentation as a result of formalin fixation and storage in paraffin; and 2) loss of portions of the viral genome during integration. Integration of HPV DNA may result in deletion of the viral genome containing the sequences targeted in the PCR reaction. In such cases, the detection of HPV DNA in the assay will depend on the presence of intact episomal HPV copies. The absence of an episomal HPV genome in the majority of glandular tumors, as opposed to squamous tumors,[27] may result in a significant underestimation of HPV DNA prevalence in adenocarcinomas.

In this study, HPV DNA amplification was performed using a novel, sensitive, broad-spectrum HPV PCR assay (SPF 10) which allows for the detection of at least 43 known HPV types. The SPF 10 assay significantly diminishes the problems of HPV detection by amplifying only a 65-bp fragment located within the L1 region of the HPV genome. The amplification product is much shorter than the products obtained with other frequently used general

Table 3. Reference Summary of HPV DNA Detection in Cervical Adenocarcinomas and Related Tumors

Diagnosis	n	HPV DNA positive (%)	HPV 16:18 (%:%)	References
Adenocarcinomas				
Adenocarcinoma *in situ*	23	100	43:26	Pirog et al. (current study)
	21	76	37:63	Anciaux et al. 1997
	37	66	35:65	Duggan et al. 1994
	9	78	29:71	Stoler et al. 1994
	30	70	48:52	Leary et al. 1991
Mucinous Adenocarcinomas				
Endocervical-type adenocarcinoma	55	91	44:42	Pirog et al. (current study)
	12	92	not available	Ferguson et al. 1998
	88	97	50:50	Tenti et al. 1996
	47	64	50:36	Duggan et al. 1995
	16	81	38:62	Stoler et al. 1994
	15	80	46:54	Milde-Langosch et al. 1993
Intestinal-type adenocarcinoma	6	83	0:20	Pirog et al. (current study)
	7	71	43:57	Tenti et al. 1996
	1	100	0:100	Duggan et al. 1995
Endometrioid-type adenocarcinoma	4	100	50:50	Pirog et al. (current study)
	9	44	not available	Ferguson et al. 1998
	33	58	57:43	Tenti et al. 1996
	6	67	50:50	Duggan et al. 1995
	7	86	50:50	Stoler et al. 1994
	5	60	100:0	Milde-Langosch et al. 1993
Minimal deviation adenocarcinoma	2	0	0	Pirog et al. (current study)
	6	0	0	Toki et al. 1999
	3	0	0	Ferguson et al. 1998
Nonmucinous Adenocarcinomas				
Clear cell adenocarcinoma	4	0	0	Pirog et al. (current study)
	10	70	25:75	Tenti et al. 1996
	1	100	100:0	Duggan et al. 1995
	14	21	HPV 31 only	Waggoner et al. 1994
	4	50	0:100	Stoler et al. 1994
	1	0	0	Milde-Langosch et al. 1993
Serous adenocarcinoma	1	0	0	Pirog et al. (current study)
	2	100	0:100	Duggan et al. 1995
	1	0	0	Milde-Langosch et al. 1993
Carcinomas with adenosquamous differentiation				
Adenosquamous carcinoma	6	100	33:33	Pirog et al. (current study)
	19	100	74:16	van Muyden et al. 1999
	8	50	25:75	Anciaux et al. 1997
	17	76	46:31	Duggan et al. 1995
	21	91	52:48	Yamakawa et al. 1994
	13	77	50:50	Stoler et al. 1994
Adenoid basal carcinoma	2	100	100:0	Pirog et al. (current study)
	9	67	66:0	Grayson et al. 1997
Adenoid cystic carcinoma	11	73	85:0	Grayson et al. 1996
	1	0	0	Stoler et al. 1994
Glassy cell carcinoma	1	100	0:100	Pirog et al. (current study)
	18	28	20:80	Kenny et al. 1992
	3	66	0:100	Stoler et al. 1994
Clear cell adenosquamous carcinoma	11	100	0:100	Fujiwara et al. 1995

primer sets such as My11/09 (450 bp) or GP 5+/6+ (150 bp).[28,29] The kinetics of the PCR reaction favor amplification of shorter DNA sequences and consequently, the SPF assay has been shown to be more sensitive than amplification systems using My11/09 or GP 5+/6+ primers.[20] In addition, a short target sequence is statistically less likely to be affected by either DNA fragmentation or loss during viral integration.

HPV 16 and HPV 18 were the most common viral types identified and occurred with almost equal frequency. This result is similar to that reported by other investigators (Table 3) and highlights a difference from that found in squamous cell carcinomas where the frequency of HPV 16 is much greater than HPV 18.[2] Other less common HPV types identified were HPV 45, followed by HPVs 52 and 35. Multiple HPV types were detected in 9.7% of the cases and in each, either HPV 16 or 18 was always identified in addition to other viral types. Multiple HPV infection was more frequently present in adenocarcinoma *in situ* and correlated with a younger age. According to a recent study of a large number of cervical cancers in Morocco, the odds ratio for the development of cervical

cancer was higher with double HPV infection *versus* single HPV infection (odds ratio, 1.4 *versus* 1.0).[30] Further research is required to determine the importance of multiple HPV-type infection.

HPV DNA was identified in >90% of *in situ* and invasive mucinous adenocarcinomas, which encompass endocervical, intestinal, and endometrioid morphology and account for ~95% of all cervical adenocarcinomas. Several previous studies have also found a high HPV prevalence in cervical mucinous tumors, especially in those with endocervical morphology [5,11,12,31–35] (Table 3). Traditionally, endometrioid tumors of the cervix were classified separately from the mucinous cervical tumors. In this study only the tumors that did not show intracellular mucin with the standard hematoxylin and eosin staining were subclassified as endometrioid. Many of the cases, however, had mixed patterns of differentiation with areas of glands with abundant intracellular mucin, areas with modest amounts of mucin, as well as areas with mucin-depleted glands resembling endometrioid-type epithelium. All these cases were subclassified as endocervical subtype of adenocarcinoma. It is thought, however, that many of the tumors classified as endometrioid adenocarcinomas may in fact represent less-differentiated mucinous endocervical-type tumors that have decreased capacity to produce mucin.[23] The arguments in support of this opinion are the following: 1) many of the cases of cervical adenocarcinoma show a spectrum of differentiation from areas with abundant intracellular mucin to mucin-depleted areas resembling endometrioid-type tumors; 2) when examined with the histochemical stains, endometrioid-type adenocarcinomas express the same range of mucins, but in lesser quantities, as the mucinous tumors with endocervical-type histology.[25]

Minimal deviation adenocarcinoma (MDA) may be a special category among mucinous cervical tumors. MDA is a rare lesion, accounting for only 1 to 3% of cervical adenocarcinomas, and is occasionally associated with Peutz-Jeghers syndrome and synchronous ovarian mucinous tumors. In our series, only two cases of MDA were available for analysis and both were negative for HPV DNA. This result is consistent with previous reports in which a total of 9 cases were negative for HPV.[5,36] Recently, Lee et al[37] described loss of heterozygosity of the 19p13.3 chromosomal region in nine sporadic cases of MDA, suggesting the presence of a putative tumor suppressor gene in this area. The clinical association between MDA and Peutz-Jeghers syndrome along with the results of molecular genetic and HPV studies indicates that the pathogenesis of MDA may not be related to HPV infection.

Nonmucinous adenocarcinomas of the cervix are relatively rare neoplasms and only six tumors were available for analysis in this study. All six were negative for HPV DNA, including four clear cell carcinomas (CCCs). CCCs account for 2 to 7% of cervical adenocarcinomas and comprise a heterogeneous group of malignancies. CCCs presenting in young patients and involving the ectocervix are usually associated with diethylstilbestrol (DES) exposure *in utero*.[38] Other patients have no known risk factors and occur in an older age group. In the largest published series of CCCs, three of 14 tumors were positive for HPV 31[38] (Table 3). Other investigators have reported a highly variable prevalence of HPV DNA in CCCs[11,12,31,35] (Table 3). Of note, CCC of the cervix has to be differentiated from clear-cell squamous carcinoma and clear-cell adenosquamous carcinoma, as both of the latter tumors are associated with HPV.[39]

Serous AdCx is another rare tumor with distinct clinicopathological characteristics including a bimodal age distribution with one peak occurring before the age of 40 and the second peak after the age of 65, coinciding with the peak occurrence of uterine serous carcinoma.[40] The only patient in our series was a 39-year-old woman with a family history of ovarian and peritoneal serous carcinomas, and breast carcinoma.[41] The clinical history in this case suggests the presence of a germline BRCA-1 mutation responsible for the familial breast-ovary cancer syndrome. HPV DNA was not identified in this tumor. Of three cases of serous carcinoma reported previously in the literature, two were HPV DNA-positive and one was HPV-negative[11,35] (Table 3).

Mesonephric adenocarcinoma is another rare, nonmucinous cervical tumor, which is derived from the mesonephric ducts located deep in the lateral cervical stroma.[42] The single case analyzed in our series was negative for HPV DNA. To our knowledge, no previous reports of HPV DNA detection are available in these tumors.

Carcinomas with adenosquamous differentiation account for 5 to 25% of all cervical cancers. The histological subtypes include adenosquamous (not otherwise specified), adenoid basal, adenoid cystic, glassy cell, and clear-cell adenosquamous carcinoma. In this study and in previously published reports, these tumors have been found to be associated with HPV in a high percentage of cases [3,8,11,31,43–46] (Table 3). Another group of cervical tumors which display focal glandular and squamous differentiation are neuroendocrine carcinomas. These tumors also have a high prevalence of HPV DNA, ranging from 53 to 85%, and are associated with both HPV 16 and 18; however, HPV 18 seems to be the most predominant in the small-cell carcinoma histological subtype.[47–50]

Our results combined with data from epidemiological, clinicopathological, and molecular studies indicate that squamous cell carcinomas, adenosquamous carcinomas, mucinous adenocarcinomas, and neuroendocrine carcinomas of the cervix share a common pathogenesis that involves infection with oncogenic HPV types. Although little is known about the molecular genetic events involved in the pathogenesis of cervical adenocarcinoma after HPV infection, it is well-established that expression of the high-risk HPV E6 and E7 oncoproteins in keratinocytes (squamous cells) disrupts the function of the cell cycle-regulating proteins p53 and pRB, respectively.[51,52] It is assumed that the same mechanism of HPV-related carcinogenesis occurs in cervical glandular epithelium.

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