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ORIGINAL PAPER

Association between human papillomavirus and endometrial adenocarcinoma

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Abstract Several studies have suggested a possible role of human papillomavirus (HPV) in the pathogenesis of endometrial carcinoma. The aim of the study was to investigate the presence of HPV DNA in endometrium cancers and nonneoplastic endometrium. Sixty endometrial adenocarcinomas with and without squamous differentiation and the nonneoplastic endometrium tissue of fifty-six of the same patients were analyzed for the presence of family 16 and family 6 HPV DNA by using chromogenic in situ hybridization technique on formalin-fixed and paraffin-embedded archival samples, and the results were confirmed by polymerase chain reaction method. HPV DNA was not detected either in the endometrial adenocarcinoma with or without squamous differentiation, or in the nonneoplastic endometrium tissue. It appears that HPV does not play any role in the pathogenesis of endometrial carcinoma, since endometrium may not to be a suitable host for HPV replication.

Keywords Endometrial carcinoma · Carcinogenesis · HPV DNA · In situ hybridization

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Background

Endometrial carcinoma is the most common neoplasm of the female genital tract and the fourth most frequent cancer in women (3.9 %) [1, 2]. The strong association between hyperestrogenism and the development of endometrial cancer was established. Obesity and diabetes are also well-defined risk factors for endometrial cancer. Other factors that have been associated with an increased risk of endometrial cancer include early age of menarche, later age of menopause and nulliparity. Endometrial cancer risk is correlated with dietary factors, decreased energy expenditure and decreased physical exercise [2, 3].

Worldwide, approximately 15–20 % of human cancers are associated with viral infections [4–6]. Among them, HPV has emerged as one of the most important risk factors for human cancer and is recognized as an etiologic agent in virtually all cases of cervical cancers. Furthermore, HPV is also linked to other anogenital cancers as well as to a subset of head and neck cancers [4, 5]. However, the relationship between HPV and other malignancies including upper genital tract, respiratory tract, digestive tract and breast carcinomas is not clear [7–11].

In this study, we analyzed tumor samples and nontumoral endometrium tissues to detect HPV DNA in order to clarify the controversial role of HPV in endometrium carcinomas.

Methods

Patients and tumor samples

A total of 60 endometrial carcinomas were obtained from the archives of Dr. Lutfi Kirdar Research and Training

Hospital, Pathology Department. The samples comprised hysterectomy specimens that were surgically removed in 2011–2012. All tumor specimens were fixed in 10 % buffered formalin, processed routinely and embedded in paraffin. All the available hematoxylin–eosin-stained sections were reviewed in order to evaluate the histological type, the myometrial invasion status, the cervical extension of endometrial carcinoma and the presence of cervical intraepithelial neoplasia. Two representative tissue blocks from each case were chosen and CISH was applied. One of the tissue blocks had neoplastic tissue, and the other block had residual nonneoplastic endometrium. Out of 60 endometrial carcinomas, 4 cases had no residual nonneoplastic endometrium tissue. Therefore, we had 60 paraffin blocks containing adenocarcinoma and 56 blocks containing nonneoplastic endometrium to investigate the presence of HPV DNA by CISH method. In order to confirm the sensitivity of CISH method, we analyzed the presence of HPV DNA in 30 paraffin blocks containing adenocarcinoma (50 % of all endometrium adenocarcinoma) and 25 blocks containing nonneoplastic endometrium tissue (44.6 % of all nonneoplastic endometrium tissue).

For control group, 6 samples with high-grade cervical intraepithelial lesion and 2 samples with vulvar condyloma accuminatum were chosen.

Chromogenic in situ hybridization

Chromogenic in situ hybridization (CISH) was applied on formalin-fixed and paraffin-embedded tumor samples; 4-μm-thick sections were obtained and each section was deparaffinized in the incubator for 60 min at 80 °C. Following the deparaffinization HPV III family 16 and HPV II family 6, probes were used in conjunction with ISH/VIEW Blue Plus Detection Kit and other reagents to stain the paraffin-embedded tissue on the Benchmark Series automated slide-stainers. Family 16 probe cocktail has an affinity to HPV genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 66, and family 6 probe cocktail has an affinity to HPV genotypes 6 and 11. The specificity of the DNA probes was checked by using positive control sections of high-grade squamous intraepithelial lesion in cervical tissues for family 16 group and vulvar condyloma accuminatum for family 6 group.

Polymerase chain reaction (PCR) method

DNA was extracted from paraffin blocks and amplification was made by using a "Rotor-Gene Q" (Qiagen, Germany). After DNA was amplified, screening was performed by melting curve analysis, and genotyping was performed by pyrosequencing. In the kit, HPV/β-globin primers amplified a conserved genomic region of HPV with the human β-globin gene which should always be present in the extracted DNA, and for that matter was used as internal control. By using the "PyroMark^{TM} Q96 ID system" (Qiagen), genotype-specific and 30-base pair log sequences were obtained with sequencing primers HPV 1 seq primer, HPV 2 seq primer, HPV 3 seq primer and HPV 4 seq primer. Alignment of sample sequences against the HPV library's genotype-specific sequences was performed with the "PyroMark^{TM} IdentiFire software 1.0" (Qiagen).

Results

Sixty endometrial carcinomas were reviewed from our files. In the study group, the patients' age varied from 37 to 83 with a mean age of 58.3 years. They included 4 (6.6 %) papillary serous carcinomas and 56 (93.4 %) endometrioid adenocarcinomas. Squamous differentiation was present in 16 (26.6 %) of endometrioid adenocarcinomas. We did not identify any koilocyte-like changes in the squamous differentiation areas. Tumors were evaluated for grading and staging in accordance with the International Federation of Gynaecologic Oncology (FIGO), 1989 [2]. Twenty-three (38.3 %) cases were grade I, 27 (45 %) grade II and 10 (16.7 %) grade III tumors. Five (8.3 %) tumors were confined to the endometrium (stage IA), 43 (71.7 %) involved the inner half of the myometrium (stage IB) and the remaining 12 (20 %) involved more than half of the uterine wall (stage IC). Cervical extension of endometrial adenocarcinoma was shown in 8 (13.3 %) cases. In only one case, low-grade cervical intraepithelial lesion coexisted.

Endometrial hyperplasia was present in 23 (38.3 %), atrophy in 19 (34 %), normal endometrium in 11(19.6 %) and endometrial polyp in 3 (5.3 %) cases out of 56 residual nonneoplastic endometrium tissues.

By using CISH method, out of 60 samples with endometrial carcinomas, papillary serous carcinomas and endometrioid adenocarcinomas regardless of the squamous differentiation were found negative for both HPV DNA family 16 and family 6 (Fig. 1). Fifty-six samples of residual nonneoplastic endometrium tissues were also negative.

Polymerase chain reaction (PCR)-based detection of HPV DNA was also negative in all 30 samples of endometrium adenocarcinomas and 25 samples of nonneoplastic endometrium tissue.

On the other hand, all cervical tissues with high-grade intraepithelial lesions were positive for HPV DNA family 16, and vulvar tissues with condyloma accuminatum were positive for HPV DNA family 6 in the control group (Fig. 2).

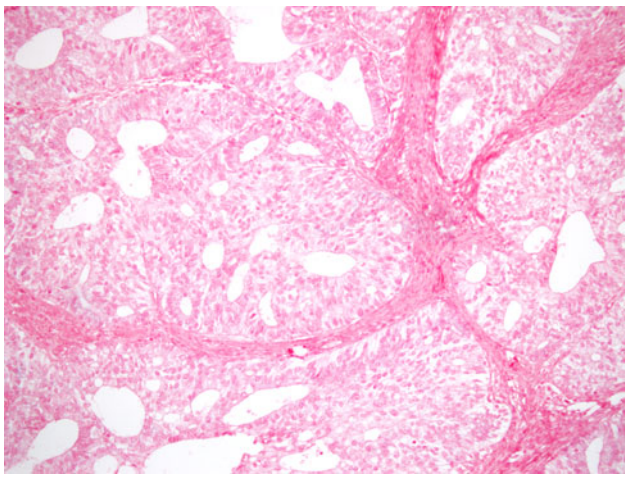

Fig. 1 Endometrioid adenocarcinoma, negative for HPV DNA (CISH ×200)

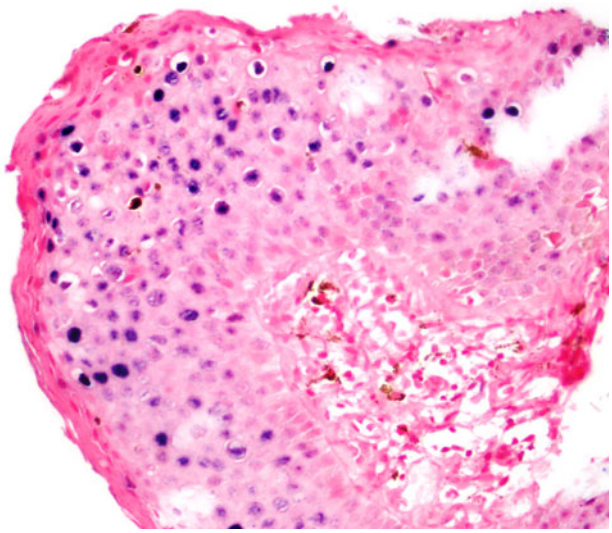

Fig. 2 Cervical intraepithelial lesion, positive for family 16 HPV DNA (CISH ×200)

Discussion

Human papillomavirus (HPV) was thought as being site- and tissue-specific, infecting the stratified squamous epithelium of the lower female genital tract, such as vulva, vagina and ectocervix [11, 12]. Multiple molecular studies have reported that HPV-mediated carcinogenesis is mainly due to the oncogenic activities of the viral early proteins E6 and E7. The E6 and E7 oncoproteins contribute to tumor initiation and also play an important role in the malignant progression through the induction of genomic instability and other mechanisms. The major cellular targets of the E6 and E7 oncoproteins, the p53, and the pRB tumor suppressor proteins are dysfunctional at some level in the tumor-suppressing pathway in almost every human solid tumor, and these aberrations are well-accepted to represent almost all universal driver mutations [3, 12, 13].

Today, it is well established that HPVs are very important human carcinogens causing not only the vast majority of cervical cancers but also a substantial proportion of other anogenital, head and neck cancers [5]. The role of HPV in the pathogenesis of other types of carcinomas such as breast, bladder and colorectal carcinomas has also been extensively investigated [7, 9, 10, 14–17].

HPV was known to be associated with squamous cell carcinoma, but it has also been found to be associated with adenocarcinoma. Although earlier reports suggested a minor etiopathological role of the virus, recent investigations supported the importance of the virus in the development of adenocarcinoma in cervix [3, 18–22]. Because of the vicinity of the endometrial cavity to the mucosal surfaces of the genital tract, HPV may play a role in the carcinogenesis of endometrial adenocarcinomas. Until now, the role of HPV in endometrial carcinomas has been investigated by contradictory results [11, 19, 21–27]. The studies showed that the presence of HPV DNA in endometrium cancers differed in a range from 9.1 to 24 %. Most of the HPVs were defined as the high-risk type. HPV DNA, mostly the 16 and 18 subtypes, was more intensively present in squamous differentiation areas [11, 23–26, 28].

Apart from the studies suggesting positive correlation, there are also many other published studies made on this subject showing negative correlation in which HPV either could be detected weakly only in a few cases or could not be detected at all in any endometrium carcinomas [11, 18, 19, 21, 22, 28–30].

O'Leary et al [24] in 63 cancers of series showed the segregation of the low-risk HPV type (HPV 6 and HPV 11) with benign squamous metaplastic epithelium and the high-risk type (HPV 33) with malignant squamous epithelium in endometrium adenocarcinomas. In this study, low-risk type (HPV 6) and high-risk type (HPV 33) were determined together in one case as a mixed infection.

Konidaris et al. investigated the frequency of HPV by in situ hybridization in 68 women with cervical cancers, 43 women with endometrial cancers and 84 women with benign gynecological diseases. The HPV detection rate was highest in the cervical cancer group (74.8 %) and the most common subtypes were 16 and 18. In the endometrial carcinoma group, HPV was present in 27.9 % of the cases and the most common subtypes were 31, 33 and 51. In the specimens with benign gynecological lesions, HPV was shown in 45.2 % of the cases and the most common subtypes were 6 and 11 [31]. The association of HPV with malign and benign lesions of the uterine endometrium has also been investigated by additional studies [11, 25].

Giardano et al. [32] detected HPV DNA as weak positive in the upper genital tract of all the 9 hysterectomy

specimens comprising HPV-positive invasive cervical carcinomas. They suggested that HPV detected in the upper genital tract could be a sign of a latent HPV infection, as well as a sign of the existence of micrometastases containing HPV DNA.

In our study, we used CISH method for the detection of HPV DNA family 16 and family 6 in 60 cases of endometrium carcinomas and 56 nonneoplastic endometrium tissues. However, no HPV DNA was detected in any of the endometrium adenocarcinomas with or without squamous differentiation, as well as in any of the nonneoplastic endometrium tissues. In one of the hysterectomy specimens, there was a cervical intraepithelial lesion which was infected by HPV DNA family 16 and an endometrial adenocarcinoma which was not affected by HPV at all. On the other hand, HPV DNA family 16 was detected in all cervical tissues with high-grade intraepithelial lesion, and HPV DNA family 6 was detected in vulvar condyloma accuminatum tissues in the control groups. In order to analyze the sensitivity of CISH method, we also confirmed the negative results by PCR method in approximately 50 % of the cases, which is extremely sensitive and specific for the detection of HPV DNA. PCR-based detection of HPV DNA was again negative in all endometrium adenocarcinomas and in nonneoplastic endometrium tissues.

In our study, no relation between HPV and endometrial adenocarcinoma was observed contrary to many other published data. According to us, the reason of this difference may be hidden in the rate of the general HPV prevalence of the Turkish population. Although a current data on the exact prevalence is not available, there is a survey made on the Turkish population which reported the HPV prevalence rate as 2.14 % [33].

Conclusions

In the study we have made, HPV DNA was not present in any of the neoplastic and nonneoplastic endometrium tissues. Although we suggest that HPV infection is unlikely to have any role in the etiology or the pathogenesis of the endometrial adenocarcinomas due to the fact that we could not detect any HPV DNA in our series, we believe that it is necessary to make further research on this area in order to clarify the issue.

Conflict of interest We declare that we have no conflict of interest.

References

1. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin. 2005;55:74–108.
2. Hedrick Ellenson L, Ronnett BM, Soslow RA, Zaino RJ, Kurman RJ. Endometrial carcinoma. In: Kurman RJ, Hedrick Ellenson L, Ronnett BM, editors. Blaustein's pathology of the female genital tract. 6th ed. New York: Springer; 2011. p. 394–456.
3. Silverberg SG, Kurman RJ, Nogales F, Mutter GL, Kubik-Huch RA, Tavassoli FA. Epithelial tumours and related lesion. In: Tavassoli FA, Devilee P, editors. World health organization classification of tumours pathology and genetics tumours of the breast and female genital organs. Lyon: IARC Press; 2003. p. 217–61.
4. Javier RT, Butel JS. The history of tumor virology. Cancer Res. 2008;68(19):7693–706.
5. Zur Hause H. Papillomaviruses in the causation of human cancers-a brief historical account. Virology. 2009;384(2):260–5.
6. Wong M, Pagano JS, Schiller JT, Tevethia SS, Raab-Traub N, Gruber j. New associations of human papillomavirus, simian virus 40, and Ebstein-Barr virus with human cancer. J Natl Cancer Inst. 2002;94(24):1832–6.
7. Yavuzer D, Karadayi N, Salepci T, Baloglu H, Dabak R, Bayramicli OU. Investigation of human papillomavirus DNA in colorectal carcinomas and adenomas. Med Oncol. 2011;28(1):127–32.
8. Hoory T, Monie A, Gravitt P, Wu TC. Molecular epidemiology of human papillomavirus. J Formos Med Assoc. 2008;107(3):198–217.
9. Giuliani L, Ronci C, Bonifacio D, Di Bonito L, Favalli C, Perno CF, Syrjänen K, Ciotti M. Detection of oncogenic DNA viruses in colorectal cancer. Anticancer Res. 2008;28:1405–10.
10. Yavuzer D, Salepci T, Karadayi N, Baloglu H, Kucukodaci Z. Human papillomavirus is not associated with breast carcinoma. Breast Cancer Res Treat. 2010;122(3):899–900.
11. Giatromanolaki A, Sivridis E, Papazoglou D, Koukourakis MI, Maltezos E. Human papillomavirus in endometrial adenocarcinomas: infectious agent or a mere "passenger"? Infect Dis Obstet Gynecol. 2007; doi:10.1155/2007/60549.
12. Buitrago-Pérez A, Garaulet G, Vázquez-Carballo A, Paramio JM, García-Escudero R. Molecular signature of HPV-induced carcinogenesis: pRb, p53 and gene expression profiling. Curr Genomics. 2009;10:26–34.
13. McLaughlin-Drubin ME, Münger K. Oncogenic activities of human papillomaviruses. Virus Res. 2009;143:195–208.
14. Aggarwal S, Arora VK, Gupta S, Singh N, Bhatia A. Koilocytosis. correlations with high-risk HPV and its comparison on tissue sections and cytology, urothelial carcinoma. Diagn Cytopathol. 2009;37(3):1747.
15. Frega A, Lorenzon L, Bononi M, De Cesare A, Ciardi A, Lombardi D, Assorgi C, Gentile M, Moscarini M, Torrisi MR, French D. Evaluation of E6 and E7 mRNA expression in HPV DNA positive breast cancer. Eur J Gynaecol Oncol. 2012;33(2):164–7.
16. Gumus M, Yumuk PF, Salepci T, Aliustaoglu M, Dane F, Ekenel M, Basaran G, Kaya H, Barisik N, Turhal NS. HPV DNA frequency and subset analysis in human breast cancer patients normal and tumoral tissue samples. J Exp Clin Cancer Res. 2006;25(4):515–21.
17. Perez LO, Abba NC, Laguens RM, Golijow CD. Analysis of adenocarcinoma of the colon and rectum: detection of human papillomavirus (HPV) DNA by polymerase chain reaction. Colorectal Dis. 2005;7:492–5.
18. Staebler A, Sherman ME, Zaino RJ, et al. Hormone receptor immunoreactivity and human papillomavirus in situ hybridization are useful for distinguishing endocervical and endometrial adenocarcinomas. Am J Surg Pathol. 2002;26(8):998–1006.
19. Jiang L, Malpica A, Deavers MT, Guo M, Villa LL, Nuovo G, Merino MJ, Silva EG. Endometrial endometrioid adenocarcinoma of the uterine corpus involving the cervix: some cases

probably represent independent primaries. Int J Gynecol Pathol. 2010;29(2):146–56.
20. Hording U, Daugaard S, Visfeldt J. Adenocarcinoma of the cervix and adenocarcinoma of the endometrium: distinction with PCR-mediated detection of HPV DNA. APMIS. 1997;105(4):313–6.
21. Ansari-Lari MA, Staebler A, Zaino RJ, Shah KV, Ronnett BM. Distinction of endocervical and endometrial adenocarcinomas: immunohistochemical p16 expression correlated with human papillomavirus (HPV) DNA detection. Am J Surg Pathol. 2004;28(2):160–7.
22. Plunkett M, Brestovac B, Thompson J, Sterrett G, Filion P, Smith D, Frost F. The value of HPV DNA typing in the distinction between adenocarcinoma of endocervical and endometrial origin. Pathology. 2003;35(5):397–401.
23. Fujita M, Shroyer KR, Markham NE, Inoue M, Iwamoto S, Kyo S, Enomoto T. Association of human papillomavirus with malignant and premalignant lesions of the uterine endometrium. Hum Pathol. 1995;26(6):650–8.
24. O'Leary JJ, Landers RJ, Crowley M, Healy I, O'Donovan M, Healy V, Kealy WF, Hogan J, Doyle CT. Human papillomavirus and mixed epithelial tumors of the endometrium. Hum Pathol. 1998;29(4):383–9.
25. Chinen K, Kamiyama K, Kinjo T, Arasaki A, Ihama Y, Hamada T, Iwamasa T. Morules in endometrial carcinoma and benign endometrial lesions differ from squamous differentiation tissue and are not infected with human papillomavirus. J Clin Pathol. 2004;57(9):918–26.
26. Ip SM, Wong LC, Xu CM, Cheung AN, Tsang PC, Ngan HY. Detection of human papillomavirus DNA in malignant lesions from Chinese women with carcinomas of the upper genital tract. Gynecol Oncol. 2002;87(1):104–11.
27. Jones MW, Onisko A, Dabbs DJ, Elishaev E, Chiosea S, Bhargava R. Immunohistochemistry and HPV in situ hybridization in pathologic distinction between endocervical and endometrial adenocarcinoma. A comparative tissue microarray study of 76 tumors. Int J Gynecol Cancer. 2013;23(2):380–4.
28. Brewster WR, Monk BJ, Burger RA, Bergen S, Wilczynski SP. Does human papillomavirus have a role in cancers of the uterine corpus? Gynecol Oncol. 1999;75(1):51–4.
29. Czerwenka K, Lu Y, Heuss F, Manavi M, Kubista E. Human papillomavirus detection of endometrioid carcinoma with squamous differentiation of the uterine corpus. Gynecol Oncol. 1996; 61(2):210–4.
30. Wong WS, Wong YF, Tam OS, Tam JS. Detection of human papilloma virus (HPV) infection in paraffin-embedded tissues of endometrial carcinoma. Aust N Z J Obstet Gynaecol. 1993;33(2): 180–2.
31. Konidaris S, Kouskouni EE, Panoskaltsis T, Kreatsas G, Patsouris ES, Sarivalassis A, Nonni A, Lazaris AC. Human papillomavirus infection in malignant and benign gynaecological conditions: a study in Greek women. Health Care Women Int. 2007;28(2): 182–91.
32. Giordano G, D'Adda T, Gnetti L, Froio E, Merisio C, Melpignano M. Detection of human papillomavirus in organs of upper genital tract in women with cervical cancer. Int J Gynecol Cancer. 2006;16(4):1601–7.
33. Inal MM, Köse S, Yildirim Y, Ozdemir Y, Töz E, Ertopçu K, Ozelmas I, Tinar S. The relationship between human papillomavirus infection and cervical intraepithelial neoplasia in Turkish women. Int J Gynecol Cancer. 2007;17:1266–70.