

The Etiologic Role of HPV in Vulvar Squamous Cell Carcinoma Fine Tuned

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Abstract

Purpose: High-risk human papilloma virus (HPV) plays a role in the development of a subset of vulvar squamous cell carcinomas. Uncertainty exists about the true impact of HPV in this tumor type because conflicting reports have been published with diverging prevalence rates. This study was done to fine tune the role of high-risk HPV infection in vulvar squamous cell carcinoma development in relation to clinical prognosis.

Experimental Design: 130 vulvar squamous cell carcinomas of patients with known survival data were analyzed for histology of the adjacent lesion (differentiated or HPV-associated usual vulvar intraepithelial neoplasia), in relation to p16^{INK4A} expression as marker of HPV activity, and presence and integration of high-risk HPV DNA.

Results: Usual vulvar intraepithelial neoplasia was present adjacent to vulvar squamous cell carcinoma in 25 of 130 cases. Usual vulvar intraepithelial neoplasia-associated squamous cell carcinomas had high p16^{INK4A} expression, and 24 of 25 squamous cell carcinomas contained integrated high-risk HPV DNA. Differentiated vulvar intraepithelial neoplasia was found adjacent to 105 of 130 vulvar squamous cell carcinomas. High-risk

HPV was detected in 11 (10.5%) differentiated vulvar intraepithelial neoplasia-associated vulvar squamous cell carcinoma but correlated with high p16^{INK4A} expression in only one case. Integration of viral DNA was never observed in differentiated vulvar intraepithelial neoplasia-associated squamous cell carcinomas, which suggests that a causal relationship of high-risk HPV in differentiated vulvar intraepithelial neoplasia-associated tumors is highly unlikely. The disease-specific survival of the differentiated vulvar intraepithelial neoplasia-associated vulvar squamous cell carcinoma patients was significantly worse compared with patients with a usual vulvar intraepithelial neoplasia-associated tumor.

Conclusions: High-risk HPV is causally associated with the development of usual vulvar intraepithelial neoplasia associated squamous cell carcinomas, which comprise 19% of all vulvar squamous cell carcinomas, but not with differentiated vulvar intraepithelial neoplasia-associated vulvar squamous cell carcinomas. Differentiated vulvar intraepithelial neoplasia-associated vulvar squamous cell carcinomas have a significantly worse prognosis. (Cancer Epidemiol Biomarkers Prev 2009;18(7):2061–7)

Introduction

The association between human papilloma virus (HPV) and vulvar squamous cell carcinoma oncogenesis is less straightforward than for cervical carcinomas, which are causally associated with HPV in nearly 100% of the cases (1). In previous work, we have shown that vulvar squamous cell carcinomas may develop via two different pathways, with their own specific premalignant lesions (2, 3). The first and most common pathway occurs in elderly women and mainly leads to keratinizing squamous cell carcinoma, often in a background of lichen sclerosis and differentiated vulvar intraepithelial neoplasia. The second pathway of vulvar squamous cell carcinoma occurs in younger women. This type of carcinoma is associated with warty or basaloid vulvar

intraepithelial neoplasia, currently referred to as usual vulvar intraepithelial neoplasia.

Human papilloma virus has been found in a subset of vulvar squamous cell carcinomas, suggesting a causal relationship. The exact impact of HPV in this tumor type is uncertain because diverging prevalence rates have been published. Sixteen studies comprising 1181 vulvar squamous cell carcinoma patients showed on average 35% HPV positivity and 25% positivity for HPV 16 and/or 18 (2, 4–18). In the United States, a higher percentage HPV positivity was found (63.2%) than in European countries (34.7%; ref. 19). However, HPV DNA presence alone does not per se indicate viral involvement in the carcinogenic process (20). Infection with HPV is an early event in the multistep process of vulvar carcinogenesis, and HPV integration into host cell genome seems to be related to the progression of vulvar dysplasia (21). Viral integration generally disrupts the E2 region, resulting in enhanced expression of E6 and E7. E6 and E7 have the ability to bind and inactivate p53 and pRb, which promotes rapid progression through the cell cycle without a p53-mediated control of DNA integrity. High

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p16^{INK4A} expression is caused by the functional inactivation of pRb and may be used as a read out for active HPV (22, 23).

With the aim to fine tune the etiologic role of HPV in vulvar squamous cell carcinoma oncogenesis, we used the histology of the adjacent vulvar intraepithelial neoplasia lesion in relation to p16^{INK4A} expression and HPV integration. As a consequence, the number of vulvar squamous cell carcinomas that may be prevented by the current HPV vaccines can be indicated more accurately, and it can be determined whether the two different pathways of vulvar squamous cell carcinoma lead to different survival patterns.

Materials and Methods

Patients. Between 1988 and 2005, 167 patients were treated with a curative intent for primary vulvar squamous cell carcinoma at Radboud University Nijmegen Medical Centre, the Netherlands. Tissue samples of the primary tumors of all patients were available for assessment of the adjacent vulvar intraepithelial neoplasia lesion (that is, into usual vulvar intraepithelial neoplasia, which is >90% HPV related, and differentiated vulvar intraepithelial neoplasia, which is not HPV related; ref. 24), p16^{INK4A} staining as molecular marker for HPV activity (13), and the presence of HPV by short fragment PCR and DNA *in situ* hybridization to show the physical status of HPV because HPV integration is a key event in carcinogenesis (25).

In 28 specimens, amplification of the housekeeping β -globin gene by PCR failed, and these were not included in the study. Five tumors did not have sufficient material for immunohistochemical analysis, and in four tumors, we were not able to classify the adjacent vulvar intraepithelial neoplasia lesion because of a massive inflammatory response or ulceration. A total number of 130 patients were available for the study. Clinicohistopathologic characteristics and follow-up data were obtained from medical charts (last date of follow-up: August 1, 2008).

Histology of the Adjacent Vulvar Intraepithelial Neoplasia Lesion. Based on the specific criteria established for the histopathologic characteristics, premalignant lesions flanking vulvar squamous cell carcinoma were classified as differentiated vulvar intraepithelial neoplasia, with or without the presence of lichen sclerosis, and usual vulvar intraepithelial neoplasia (26, 27). All slides were re-evaluated by an expert gynecopathologist (J. Bulten).

p16^{INK4A} Expression. Tissue sections (4 μ m) of the archival paraffin-embedded tissue samples adjacent to that used for H&E slides to assess the adjacent vulvar intraepithelial neoplasia lesion were mounted onto SuperFrost glass slides (Menzel-Gläser) and dried overnight at 37°C. The sections were dewaxed in xylene and rehydrated through graded alcohols. Endogenous peroxidase was blocked with 3% hydrogen peroxide in PBS (pH 7.4) for 30 min. Slides were rinsed three times in PBS for 5 min, and antigen retrieval done with boiling citrate buffer (0.01 mol/L; pH 6.0) for 10 min. After cooling down to room temperature, slides were briefly washed in PBS for 10 min. Subsequently, the slides were preincu-

bated with 20% normal horse serum and then incubated with the primary antibody p16^{INK4A} (clone JC-8, Immunologic) diluted 1:200 in PBS with 1% bovine serum albumin (overnight). Slides were rinsed in PBS (10 min), and postantibody blocking was done for 30 min (horse anti-mouse immunoglobulin G). Staining was developed with diaminobenzidine/hydrogen peroxide for 5 min, intensified with copper sulfate and counterstained with Mayer's hematoxylin. The slides were dehydrated through graded alcohols and xylene and finally mounted with Permount mounting medium (Fisher Chemicals). Negative controls (buffer only) and HPV-positive Cervical Intraepithelial Neoplasia CIN III lesions served as p16^{INK4A}-positive controls in each run.

Interpretation of p16^{INK4A}. Nuclear and cytoplasmic p16^{INK4A} staining were considered as a positive reaction. The results were reported in a semi quantitative fashion: negative (–) if <5% of the cells had nuclear or cytoplasmic staining, slightly positive (1+) if 5% to 25% of the cells were stained, moderately positive (2+) if staining was present in 25% to 75% of the cells, and markedly positive (3+) if >75% of the cells showed nuclear or cytoplasmic staining (11, 13).

HPV Presence and Genotyping. DNA was isolated from formalin fixed paraffin-embedded tissue sections (6 μ m) with the EZ1 robot (with the DNA tissue kit of Qiagen), according to standard procedures (28), and used for PCR analysis. A negative water control was included with each batch of 10 samples. Broad-spectrum HPV DNA amplification was done using a short PCR fragment assay. The short PCR fragment system amplifies a 65-bp fragment of the L1 open reading frame, allowing the detection of at least 43 HPV types. Subsequent HPV genotyping was done via a reverse hybridization line probe assay, allowing simultaneous typing of the 25 HPV genotypes. The combined short PCR fragment–line probe assay system for detection and genotyping of HPV has been described in detail elsewhere (28).

Physical Status of HPV by *In situ* Hybridization. The *in situ* hybridization procedure for HPV16/18 and HPV31/33 on 3- μ m-thick paraffin sections was done as described previously (29, 30). In brief, tissue sections were pretreated with proteolytic reagents and hybridized, and the biotinylated DNA–DNA hybrids were immunohistochemically detected by the peroxidase-labeled avidin-biotin complex. For detection of HPV16/18/31/33 DNA sequences, *in situ* hybridization was followed by an immunohistochemical detection method using catalyzed reporter deposition signal amplification, which enables the detection of low copy numbers. Only a punctate signal in the nuclei was considered to represent viral integration (31, 32).

Statistical Methods. Clinical data were entered in a computerized database and analyzed using SPSS software (version 16.0.1 for Windows; SPSS). Disease-specific survival was defined as the time from the date of primary treatment to the date of death due to vulvar squamous cell carcinoma or last date of follow up. Differences in disease-specific survival between the tumors were investigated using Kaplan-Meier statistical method. χ^2 And Student's *t* tests were used to calculate differences in clinicohistopathologic characteristics. For all tests, a significance level of *P* < 0.05 was chosen.

Results

Histopathologic review of vulvar squamous cell carcinoma slides showed 25 (19.2%) cases of usual vulvar intraepithelial neoplasia adjacent to vulvar squamous cell carcinoma and 105 cases (80.8%) of differentiated vulvar intraepithelial neoplasia (in combination with lichen sclerosis in 59 cases; Fig. 1A and D for representative H&E slides of usual vulvar intraepithelial neoplasia and differentiated vulvar intraepithelial neoplasia). Lichen sclerosis as a solitary adjacent lesion was present in three cases and was considered to belong to the differentiated vulvar intraepithelial neoplasia-associated type (33-36).

To investigate whether the tumor tissues displayed HPV activity, we did p16^{INK4A} staining (Fig. 1B, C, E, and F). Ninety-one tumors (70%) showed a p16^{INK4A} expression pattern in which <5% of the tumor cells displayed cytoplasmic and/or nuclear p16^{INK4A} positivity. In four tumors, 5% to 25% positive cells were observed. Twenty-five percent to 75% and >75% positive cells were observed in nine and 26 tumors, respectively, and were considered a true positive result. All 25 usual vulvar intraepithelial neoplasia-associated tumors displayed high p16^{INK4A} expression (>25% positive tumor cells) compared with only 10 (9.5%) of 105 differentiated vulvar intraepithelial neoplasia-associated tumors (Fig. 2).

All vulvar squamous cell carcinomas were analyzed for presence of HPV DNA. Forty-five of the 130 tumors (34.6%) tested positive for HPV, of which 33 (25.4%) for high-risk HPV types. Of the high-risk HPV types, HPV 16 was most common and present in 18 (54.5%) of the 33 tumors as the only HPV type, and in two tumors (6%), a combination with HPV 16 and another HPV type was found. HPV 33 was the second most common type and detected in seven of the 33 HPV-positive tumors (21.2%). HPV 18 was present in two tumors as the solitary subtype and in combination with HPV 16 and 54 in one

additional tumor (Table 1). In 24 (96%) of 25 usual vulvar intraepithelial neoplasia-associated tumors, high-risk HPV DNA was detected, whereas 11 (10.5%) differentiated vulvar intraepithelial neoplasia-associated tumors were found positive for high-risk HPV DNA (Fig. 2). However, of those 11 differentiated vulvar intraepithelial neoplasia-associated high-risk HPV-positive tumors, only one single tumor displayed high p16^{INK4A} expression. The remaining 10 differentiated vulvar intraepithelial neoplasia high-risk HPV-positive samples were all p16^{INK4A} negative, suggesting that the HPV found in these differentiated vulvar intraepithelial neoplasia-associated tumors was not active. Because p16^{INK4A} expression is linked to HPV activity, the physical status of high-risk HPV DNA by *in situ* hybridization for HPV types 16, 18, and 33 was shown. Twenty-three usual vulvar intraepithelial neoplasia-associated squamous cell carcinomas were positive for HPV 16, 18, or 33. One tumor was positive for HPV 58. All 23 HPV 16-, 18-, and 33-positive usual vulvar intraepithelial neoplasia-associated squamous cell carcinomas were analyzed, together with all differentiated vulvar intraepithelial neoplasia-associated squamous cell carcinomas that tested positive for HPV DNA and/or displayed high p16^{INK4A} expression. Integration was observed in all 23 HPV 16-, 18-, or 33-positive usual vulvar intraepithelial neoplasia-associated squamous cell carcinomas (see Fig. 3A for a representative slide of a tumor positive for HPV 31/33 by *in situ* hybridization). Of these, 17 were positive for HPV 16 and/or 18. However, none of the p16^{INK4A} expressing and/or HPV 16, 18, or 33 DNA containing differentiated vulvar intraepithelial neoplasia-associated vulvar squamous cell carcinomas displayed HPV integration (Figs. 2 and 3B for a representative slide of a tumor negative for HPV 16/18 by *in situ* hybridization), suggesting a nonsignificant clinical presence of high-risk HPV in differentiated vulvar intraepithelial neoplasia-associated squamous cell carcinomas.

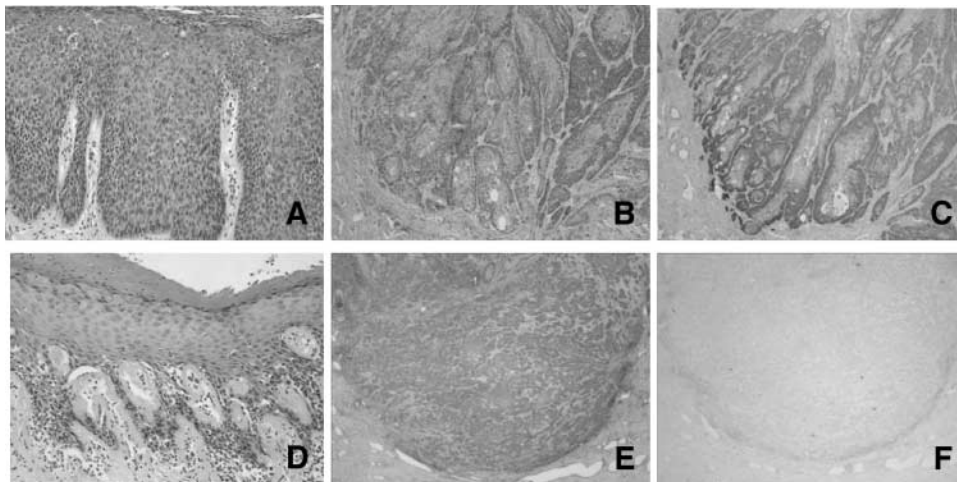


Figure 1. Examples of vulvar intraepithelial neoplasia lesions adjacent to vulvar squamous cell carcinomas, squamous cell carcinomas associated with both types of vulvar intraepithelial neoplasia, and p16^{INK4A} expression. Subdivision of vulvar squamous cell carcinomas was among other things based on the histology of the adjacent vulvar intraepithelial neoplasia lesion and p16^{INK4A} staining. Usual vulvar intraepithelial neoplasia (A)-associated tumors (B) showed >25% p16^{INK4A} expression in all cases (C). Differentiated vulvar intraepithelial neoplasia (D)-associated tumors (E) displayed ≤25% p16^{INK4A} expression (F) in 90.5% of the squamous cell carcinomas.

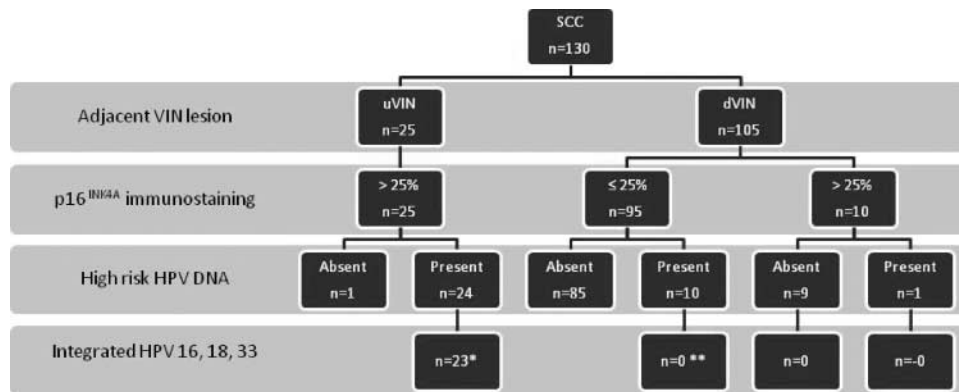


Figure 2. Schematic overview of histology of the adjacent vulvar intraepithelial neoplasia lesion, p16^{INK4A} expression, high-risk HPV presence, and integration. A causal relation between high-risk HPV and vulvar squamous cell carcinoma is only plausible for usual vulvar intraepithelial neoplasia-associated squamous cell carcinomas. In differentiated vulvar intraepithelial neoplasia-associated vulvar squamous cell carcinomas positive for high-risk HPV or with high p16^{INK4A} expression, this was not accompanied by HPV integration. *Asterisk*, of the 24 usual vulvar intraepithelial neoplasia-associated squamous cell carcinomas, 23 were positive for 16, 18, or 33 and available for integration analysis. *Double asterisk*, of the 10 HPV-positive differentiated vulvar intraepithelial neoplasia-associated squamous cell carcinomas with low p16^{INK4A} expression, six were positive for HPV 16, 18, or 33 and available for integration analysis.

With the aim to find out whether there are differences in disease-specific survival between the two types of vulvar squamous cell carcinomas, the disease-specific survival of all vulvar squamous cell carcinoma patients and subdivided between usual vulvar intraepithelial neoplasia and differentiated vulvar intraepithelial neoplasia-associated vulvar squamous cell carcinomas was determined. In addition, disease-specific survival for vulvar squamous cell carcinomas subdivided between high-risk HPV positive and negative was determined. For all vulvar squamous cell carcinoma patients, 5-year disease-specific survival rate was 77% (Fig. 4A). A subdivision between usual and differentiated vulvar intraepithelial neoplasia showed a 5-year disease-specific survival rate of 70% for differentiated vulvar intraepithelial neoplasia-associated squamous cell carcinoma patients. Survival for usual vulvar intraepithelial neoplasia-associated squamous cell carcinoma patients was significantly better, with a 5-year disease-specific survival rate of 100% (log rank $P = 0.012$; Fig. 4B). A subdivision between high-risk HPV positive or negative did

not result in a different disease-specific survival (log rank $P = 0.646$; Fig. 4C). Stratification for usual vulvar intraepithelial neoplasia, HPV negative differentiated vulvar intraepithelial neoplasia, and HPV positive differentiated vulvar intraepithelial neoplasia associated vulvar squamous cell carcinomas revealed an unexpected worse survival of the latter (Fig. 4D). Similarly, stratification for HPV positive/p16^{INK4A} positive (24 usual vulvar intraepithelial neoplasia associated vulvar squamous cell carcinomas and one differentiated vulvar intraepithelial neoplasia associated vulvar squamous cell carcinomas), HPV positive/p16^{INK4A} negative (10 differentiated vulvar intraepithelial neoplasia associated vulvar squamous cell carcinomas), and HPV (94 differentiated vulvar intraepithelial neoplasia associated vulvar squamous cell carcinomas and one usual vulvar intraepithelial neoplasia associated vulvar squamous cell carcinomas) vulvar squamous cell carcinomas showed reduced disease-specific survival for the HPV positive/p16^{INK4A} negative subset (Fig. 4D, *inset*). As such, the lack of a difference in disease-specific survival between HPV-positive and HPV-negative tumors (Fig. 4C) can be explained, but why these HPV positive/p16^{INK4A} negative differentiated vulvar intraepithelial neoplasia-associated squamous cell carcinoma carry a poor disease-specific survival remains elusive.

Table 1. Overview of HPV types in 130 vulvar squamous cell carcinomas

	n	Percentage
HPV negative	85	65.4
Low-risk HPV types	12	9.2
54	1	91.7
X	11	8.3
High-risk HPV types	33	25.4
16	18	54.4
16, 18, 54	1	3
16, 58	1	3
18	2	6.1
33	7	21.2
52	1	3
52, 66	1	3
53	1	3
58	1	3

Discussion

HPV detection by PCR alone results in an overestimation of the percentage vulvar squamous cell carcinomas that are causally associated with HPV. We showed the presence of high-risk HPV in 26.9% of the vulvar squamous cell carcinomas, but our more detailed analysis showed a plausible causal relationship between high-risk HPV and vulvar squamous cell carcinoma carcinogenesis only in usual vulvar intraepithelial neoplasia-associated vulvar squamous cell carcinomas (19.2%). In differentiated vulvar intraepithelial neoplasia-associated squamous cell carcinomas the detected

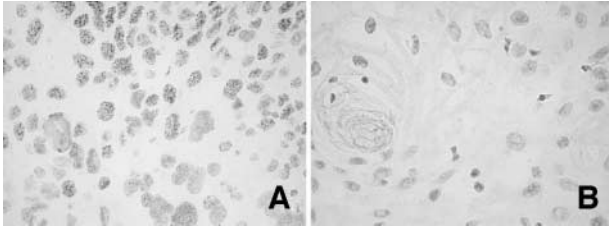


Figure 3. Examples of tumor cells positive or negative for *in situ* hybridization for HPV types 16/18 and 31/33. Integration analysis was done to fine tune the etiologic role of HPV in vulvar squamous cell carcinomas. All usual vulvar intraepithelial neoplasia-associated squamous cell carcinomas showed HPV in an integrated form (A; positive for HPV 31/33), whereas none of the HPV-positive or high-p16^{INK4A} expressing differentiated vulvar intraepithelial neoplasia-associated squamous cell carcinomas had HPV integrated (B).

HPV was not found integrated or activated and thus was not considered to be causally involved. Thirteen percent of the vulvar squamous cell carcinomas were considered to be caused by HPV 16 and/or 18. In addition, the usual vulvar intraepithelial neoplasia-associated vulvar squamous cell carcinomas had a better disease-specific survival than differentiated vulvar intraepithelial neoplasia-associated vulvar squamous cell carcinomas.

To assess the extent of the etiologic role of HPV in the carcinogenic process, we established histology of adjacent vulvar intraepithelial neoplasia lesion, p16^{INK4A} overexpression, and determination of HPV integration status. Because differentiated vulvar intraepithelial neoplasia is not associated with HPV (24), it is unlikely that the tumor directly adjacent to this differentiated vulvar intraepithelial neoplasia lesion is caused by HPV. In our study, a subdivision based on adjacent vulvar intraepithelial neoplasia lesion resulted in two clearly perceptible groups of vulvar squamous cell carcinomas

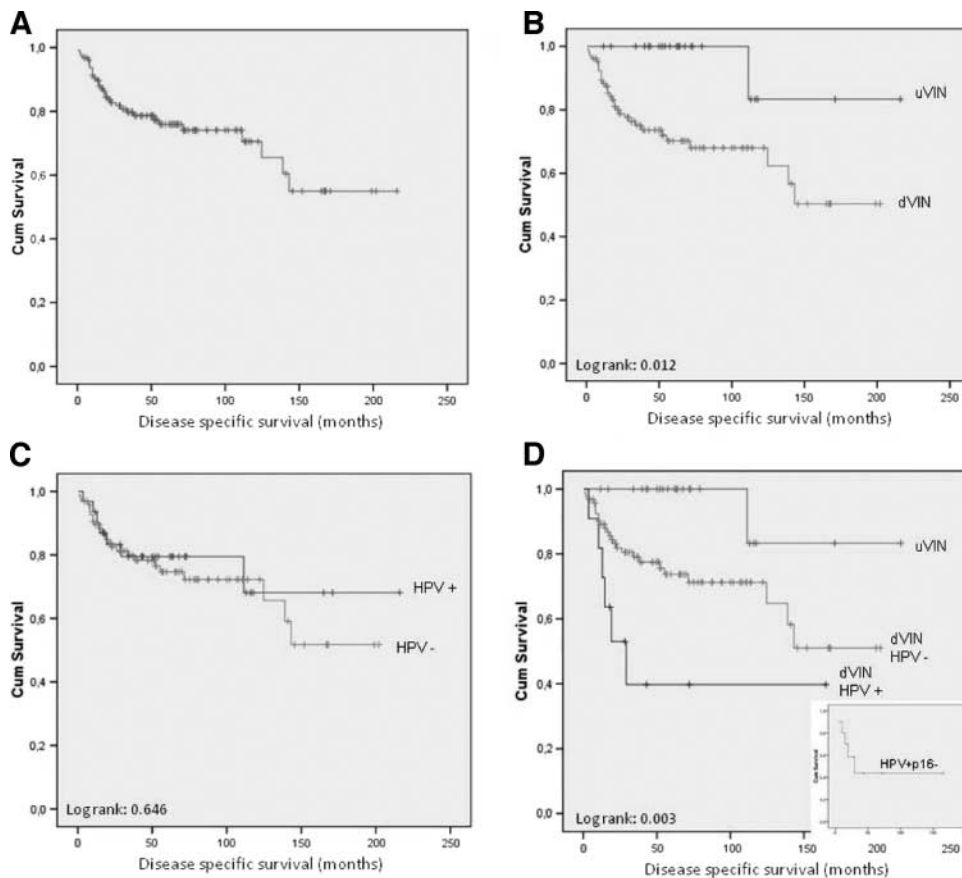


Figure 4. Disease-specific survival curves. Five-year disease-specific survival for all vulvar squamous cell carcinoma patients is 77% (A). Subdivision between the vulvar squamous cell carcinomas based on histology of adjacent vulvar intraepithelial neoplasia lesion showed a better disease-specific survival for usual vulvar intraepithelial neoplasia-associated tumors than differentiated vulvar intraepithelial neoplasia-associated tumors ($P = 0.012$; B). Five-year disease-specific survival for usual vulvar intraepithelial neoplasia-associated tumors is 100%, whereas disease-specific survival for differentiated vulvar intraepithelial neoplasia-associated tumors is 70%. Based on high-risk HPV positivity, no significant differences could be shown ($P = 0.646$; C). This was mainly due to the attribution of high-risk HPV-positive differentiated vulvar intraepithelial neoplasia-associated squamous cell carcinomas (D) or HPV positive and p16^{INK4A} negative (*inset*) with a worse disease-specific survival. When all usual vulvar intraepithelial neoplasia-associated tumors may be prevented by vaccines in the future, the disease-specific survival of vulvar squamous cell carcinoma patients will slightly decrease from 77% to 70% (A and B).

(Fig. 2). High p16^{INK4A} expression was observed in 9.5% of the differentiated vulvar intraepithelial neoplasia-associated squamous cell carcinomas and suggested active HPV involvement, but only in one tumor the high p16^{INK4A} expression was accompanied by high-risk HPV presence. Although p16^{INK4A} overexpression is strongly related to active HPV infection as a result of functional inactivation of pRb, it may also occur as an endogenous response toward abnormal cell proliferation due to an eroded checkpoint function (11, 13). p16^{INK4A} thus is an indicator that a carcinogenic cascade has commenced but may also be expressed in HPV-independent tumors, wherein its overexpression may counteract signals that abnormally drive cell proliferation, a checkpoint function that is eroded in premalignant and tumor cells (37). Furthermore, the observation that integration of HPV was not observed in HPV-positive differentiated vulvar intraepithelial neoplasia-associated tumors supports our suggestion that HPV did not contribute to the development of this tumor type. However, we cannot rule out that episomal HPV does not have an oncogenic potential. Viral integration is not a prerequisite for oncogenic activity because a proportion of HPV 16-positive cervical tumors contains episomal HPV DNA either alone or in coexistence with integrated HPV sequences (38, 39). In contrast with differentiated vulvar intraepithelial neoplasia-associated tumors, usual vulvar intraepithelial neoplasia-associated tumors were causally associated with high-risk HPV; all expressed high p16^{INK4A} expression, and the HPV types found were integrated in the genome.

The analysis of the presence and integration of high-risk HPV, expression of p16^{INK4A}, and histology of the adjacent lesion indicated that it is very unlikely that high-risk HPV is causally related to the development of differentiated vulvar intraepithelial neoplasia-associated vulvar squamous cell carcinomas. When performing a survival analysis for differentiated vulvar intraepithelial neoplasia and usual vulvar intraepithelial neoplasia-associated squamous cell carcinoma, we found a significantly worse disease-specific survival for differentiated vulvar intraepithelial neoplasia associated vulvar squamous cell carcinoma (Fig. 4B). Unexpectedly, we found reduced disease-specific survival for HPV positive differentiated vulvar intraepithelial neoplasia associated vulvar squamous cell carcinoma compared with HPV negative differentiated vulvar intraepithelial neoplasia-associated vulvar squamous cell carcinomas (Fig. 4D). We cannot provide an explanation why presence of HPV, which most likely did not induce the differentiated vulvar intraepithelial neoplasia-associated vulvar squamous cell carcinoma, is associated with poor disease-specific survival. The low sample number of HPV positive differentiated vulvar intraepithelial neoplasia ($n = 11$) compared with HPV negative differentiated vulvar intraepithelial neoplasia ($n = 94$) may have influenced this finding. Further retrospective studies using samples from other centers are needed to determine whether the poor disease-specific survival of HPV positive differentiated vulvar intraepithelial neoplasia associated vulvar squamous cell carcinoma is actually a real finding or a chance occurrence. Stratification based on p16^{INK4A} expression did not result in a different allocation of samples in the three subsets because the sample set contains only one HPV positive/p16^{INK4A} positive differentiated vulvar intraepithelial neoplasia

and one HPV- usual vulvar intraepithelial neoplasia-associated vulvar squamous cell carcinoma, and therefore disease-specific survival curves were highly comparable with usual vulvar intraepithelial neoplasia, HPV positive differentiated vulvar intraepithelial neoplasia, and HPV negative differentiated vulvar intraepithelial neoplasia associated vulvar squamous cell carcinoma.

In this study we show that, with HPV DNA detection alone, the number of vulvar squamous cell carcinomas with a clinically relevant infection are overestimated. This has implications for the protective role of HPV vaccines for vulvar squamous cell carcinomas. There is increasing evidence that the recently introduced vaccines against HPV 16 and 18 will significantly reduce the incidence of cervical carcinoma and its premalignancies (40, 41). This has led to the implication of HPV vaccination in nationwide vaccine programs in most European countries. Although most studies have focused on the impact these vaccines will have on cervical cancer, limited research has been done on how the vaccines may affect vulvar squamous cell carcinoma incidence. Still, in September 2008, the Food and Drug Administration approved one vaccine (Gardasil) to be used to prevent some cancers of the vulva and vagina (42). In our detailed analysis, it seems that only usual vulvar intraepithelial neoplasia-associated (25 of 130) vulvar squamous cell carcinomas are causally associated with high-risk HPV. Of those usual vulvar intraepithelial neoplasia-associated vulvar squamous cell carcinomas, 17 (13% of all vulvar squamous cell carcinomas) were HPV 16/18 positive and will possibly be prevented by the current HPV vaccines. We show that the usual vulvar intraepithelial neoplasia-associated vulvar squamous cell carcinoma have a more favorable prognosis compared with differentiated vulvar intraepithelial neoplasia-associated vulvar squamous cell carcinomas. Vaccination may therefore prevent a small subset of vulvar squamous cell carcinoma with relatively good prognosis, without broadly affecting vulvar squamous cell carcinoma incidence and survival rates. Currently, HPV vaccines covering more high-risk HPV types are under development, and most, if not all, usual vulvar intraepithelial neoplasia-associated carcinomas may be prevented by these vaccines in the future. Because this strategy eliminates the tumors with a relatively good prognosis, 5-year survival for vulvar squamous cell carcinoma patients could slightly decrease from 77% in the prevaccination era to 70% after the possible prevention of all usual vulvar intraepithelial neoplasia-associated vulvar squamous cell carcinomas (Fig. 4A–B).

In conclusion, high-risk HPV is causally associated with the development of usual vulvar intraepithelial neoplasia-associated vulvar squamous cell carcinomas. Although high-risk HPV was found in 10.5% of differentiated vulvar intraepithelial neoplasia-associated vulvar squamous cell carcinomas, it does not seem to be causally associated with tumor development. Integration of HPV 16 or 18 was observed in 17 usual vulvar intraepithelial neoplasia-associated vulvar squamous cell carcinomas (13%), which may be prevented by HPV vaccines in the future. This is lower than previously supposed and leaves most vulvar squamous cell carcinomas with worst disease-specific survival unsolved.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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