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Human papillomavirus infection mediates response and outcome of vulvar squamous cell carcinomas treated with radiation therapy

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HIGHLIGHTS

- The first study of its kind to evaluate the impact of p16 on intact vulvar cancers.
- P16 expressing vulvar cancers have higher pathologic response rates.
- P16 expressing vulvar cancers have improved local control.

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ABSTRACT

Purpose. Human papillomavirus (HPV) is implicated as a causative factor in vulvar squamous cell carcinoma (VSCC). This study evaluates if p16-positivity, a surrogate for HPV, predicts for better response rates to chemoradiation therapy and survival.

Materials and methods. We conducted a retrospective chart review of women treated with neoadjuvant or definitive chemoradiation (CRT) therapy from 2000 to 2016 for VSCC. p16 stain-positivity was defined as diffuse strong "block" immunoreactivity within invasive tumor.

Results. Seventy-three women with median follow-up of 13.4 months were analyzed. Thirty-three (45.2%) had p16+ tumors. Median age was 73 years (range: 37–89); with p16+ tumors, the median age was 60 years vs 73 years for women with p16− tumors ($p < 0.001$). The distribution of tumor size and stage by p16−status were similar.

The complete clinical response (cCR) rate for p16+ tumors was 63.6% vs 35.0% for p16− tumors ($p = 0.014$). The pathologic complete response (pCR) rate for women treated neoadjuvantly was 53.8% vs 31.4% for p16+ vs p16−, respectively ($p = 0.067$). The combined complete response (cCR orpCR [CCR]) rate was 63.6% for p16+ and 30.0% for p16− ($p = 0.004$).

Two-year vulvar control (VC) for women with p16+ tumors was 75.5% vs. 49.5% for p16− ($p = 0.008$). In women with p16+ tumors who achieved CCR, 2-year VC was 92.3% vs 52.1% for CIR ($p = 0.009$). For p16− tumors, 2-year VC was 67.3% vs 41.1% for CCR and CIR ($p = 0.072$). No woman with a p16+ tumor developed distant metastases vs. 7 with p16− tumor ($p = 0.013$).

OS was not statistically different between p16+ cohorts, but was improved for p16− patients with CR vs CIR, 72.9% vs 18.8% ($p = 0.026$).

Conclusions. p16-positive tumors appear to have better clinical and pathologic response rates and clinical outcomes.

1. Introduction

Vulvar squamous cell carcinoma (VSCC) is a rare entity, with an estimated 6000 new diagnoses in 2017 and 1150 deaths. Over the past decade, the incidence has risen 0.6% per year and the death rate 1.2% per

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year [1]. The traditional precursor lesion is vulvar intraepithelial neoplasia (VIN), though women with a history of smoking, cervical cancer or cervical intraepithelial neoplasia (CIN), lichen sclerosus, or human papillomavirus (HPV) infection are at increased risk [2–6]. Similar to oropharyngeal carcinomas [7,8], it is now recognized that VSCC is driven by at least two separate pathways, one of which is HPV/p16-mediated [3,9], Though up to 25–40% of lesions are positive for HPV DNA or p16 [10,11], the data on the impact of HPV/p16 on prognosis has been conflicting [12–19].

Despite the now growing body of literature, response rates their connection to subsequent outcomes with HPV/p16-driven VSCC treated with chemoradiation therapy have not yet been reported. Herein, we sought to detail clinicopathologic response rates in women with intact vulvar carcinomas as related to the presence/absence of p16, which is a surrogate of HPV infection.

2. Materials and methods

2.1. Patient population

With institutional review board approval, a clinical database of all women diagnosed with vulvar squamous cell carcinoma (VSCC) and treated from 1991 to 2016 was established (n = 451). Women treated with radical vulvectomy alone or neoadjuvant/definitive radiation therapy were included in the database, and queries made to retrieve pathological specimen. Clinical data were collected on women with tissue available for pathological evaluation of p53 and p16. All cases were reclassified according to FIGO 2009 staging. Tumor, patient demographic, and treatment information was collected, as was information on prior therapeutic interventions in the case of salvage therapy after recurrence. Sites and dates of relapse were recorded and detailed as: vulvar, pelvic (including vulvar), and distant.

2.2. Pathological staining

Available tissue blocks were stained with p53 and p16(ink4a) antibodies on whole tissue sections and evaluated by two expert pathologists. Antibody and staining details are provided in Table 1. P53 nuclear expression and p16 nuclear and cytoplasmic expression were scored using a histochemical or H-score- like method where percentage cell staining was recorded for each intensity level (0: no staining, 1: weak staining intensity, 2: moderate staining intensity, 3: strong intensity staining). The percentage of cells staining for each intensity level was then multiplied by the intensity level. The resulting values for each intensity level were then added together to range from 0 (no staining) to 300 (diffuse strong staining). An H-score of 200 (indicative of strong staining in at least 2/3rd of the tumor) or higher was considered a positive result. For p16, most positive cases had H-scores closer to 300 (Fig. 1). Based on the coordinate expression of p53 and p16, four "tumor classes" were assigned: p53-driven (p53 positive, p16 negative), p16-driven (p16 positive, p53 negative), dual positive, and dual negative. Although in some gynecologic tumors (such as serous carcinomas), a complete absence of staining for p53 is considered indicative of p53 mutation, no definitive data (i.e. no large-scale study of such expression with correlation to mutation status) exist with respect to VSCC. Therefore, the p16 negative cases with p53 H-score of 0 were included in the "dual negative" category. Binary categorization of p16 and p53 status was also generated based on H-scoring. HPV DNA in-situ hybridization assay was not performed. Although strong diffuse p16 staining correlates with HPV positivity, discordance can be seen dependent upon the method of HPV detection and type of probes used [19,20].

2.3. Statistical methods

Analyses were performed using SPSS version 22 (IBM Corp, Armonk, NY). Correlations between staining patterns and clinical, pathological, and treatment characteristics were performed using chi-squared analysis and independent *t*-test. Estimates of vulvar recurrence-free survival (VRFS), distant metastases-free survival (DMFS), progression-free survival (PFS), and overall survival (OS) were calculated using the Kaplan-Meier method and cohort comparisons made with the log-rank test. Disease endpoints were calculated from the date of diagnosis until recurrence or most recent evaluation if without evidence of disease. Clinical response was determined at the completion of radiation therapy by either or both of the treating radiation oncologist or gynecologic oncologist. Women who were treated neoadjuvantly had a pathological assessment of tumor response by an expert gynecologic pathologist. Clinical response was assessed as complete (cCR) if no visible tumor or only ulceration remained; otherwise response was termed as incomplete (cPR). Pathologically, if no viable tumor remained after neoadjuvant treatment, a complete pathological response (pCR) was designated; else response was termed pathologically incomplete (pPR). Clinical and pathological response were combined into combined complete response (CCR) and combined incomplete response (CIR) in which women who achieved cCR after definitive non-operative therapy and women who achieved pCR following neoadjuvant therapy were considered to have a CCR. Women with residual clinical tumor (cPR) following non-operative therapy or viable tumor following neoadjuvant therapy (pPR) were considered to have a CIR.

3. Results

A total of 73 women were identified who had a pathological specimen available for immunohistochemical staining and had undergone neoadjuvant or definitive radiation therapy. Median follow-up was 13.4 months. 94.5% of women received concurrent sensitizing chemotherapy. Patient and treatment characteristics are listed in Table 2. A majority of women underwent neoadjuvant therapy followed by surgical resection (86.3%). Fifteen women had FIGO IB disease treated neoadjuvantly/definitively most commonly as a result of tumor location prohibiting adequate resection margins without compromising function of the anus, urethra, or clitoris. Women with p16+ tumors tended to be younger, but tumor size and stage distribution were not different. Women with p16+ tumors were, however, more likely to achieve a cCR to (chemo)radiation therapy (63.6% vs. 35.0% for p16−, p = 0.014), with a strong trend towards an improvement in pCR for those women who had surgery after neoadjuvant treatment (53.8% vs. 31.4% for p16−, p = 0.067). In women with p16+ tumors who achieved cCR, the likelihood of pCR was 85.7% vs. 61.5% for p16−. Similarly, in women with p53+ tumors who achieved cCR, the likelihood of pCR was 44.4% vs 88.9% if p53−. In women undergoing neoadjuvant treatment, median surgical margins did not differ (4.0 vs 3.5 mm for p16+ vs p16−, p = 0.609).

Women with p16+ tumors obtained a 63.6% CCR rate versus 30.0% for p16− tumors (p = 0.004). This translated into improved vulvar control, progression-free and overall survivals as can be seen in Table 3. p53 mutation expression resulted in a CCR of only 28.6% vs. 55.6% for p53−

Table 1
Antibody and staining details.

Antibody	Clone	Vendor	Dilution	Pre-treatment	Detection	Staining platform
P53	DO-7	Ventana Medical Systems, Inc., Tucson, AZ	Pre-dilute	CC1	i-view	Ventana Benchmark Ultra
P16 (ink4a)	E6H4	Ventana Medical Systems, Inc., Tucson, AZ	Pre-dilute	CC1	Opti-view	Ventana Benchmark Ultra

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