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Perspective

The clinical significance of human papillomavirus and p16^{INK4a} in vulvar tumors

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Vulvar cancer is the fourth most common gynecological malignancy, with a global incidence of an estimated 45,240 new cases and 17,427 deaths in 2022.[1] The 5-year survival rate for vulvar cancer is about 70 % based on data from the SEER database.[2] More than 90 % of vulvar cancer was vulvar squamous cell carcinoma (SCC), which included keratinizing, non-keratinizing, basaloid, warty, and verrucous carcinoma.[3]

Based on the 2020 World Health Organization (WHO) Classification Criteria of Female Genital Tumors, vulvar SCC could be classified as human papillomavirus (HPV)-associated and HPV-independent SCC, which have different etiology and pathogenesis.[4] HPV-associated and HPV-independent vulvar SCC are usually preceded by the high-grade squamous intraepithelial lesion (HSIL) and differentiated-type vulvar intraepithelial neoplasia (dVIN), respectively.[5] HPV-associated vulvar SCC is caused by HPV infection, especially with high-risk HPV genotypes. Meanwhile, despite the etiology of HPV-independent vulvar SCC are not well studied, HPV-independent vulvar SCC is strongly associated with vulvar chronic inflammatory dermatosis, such as lichen sclerosis.[5]

1. The role of HPV and p16^{INK4a} positivity in vulvar neoplasms

As one of the well-established risk factors, HPV involvement in vulvar carcinogenesis was initially reported about four decades ago.[6] It is widely known that HPV plays a crucial carcinogenic role in the pathogenic mechanism of HPV-associated vulvar cancer, which appears to be similar to cervical cancer, penial cancer, and oropharyngeal cancer. Integration of HPV DNA into the host cell genome leads to the production of E6 and E7 oncoproteins. E6 causes the degradation of p53 and thus prevents cellular apoptosis. E7 functionally inactivates the retinoblastoma protein (pRb), which could further cause the increased expression of the tumor-suppressor protein p16^{INK4a}.[7] In the current version of WHO classification criteria of female genital tumors, the p16^{INK4a} block-type immunoreactivity and/or positive molecular testing for HPV is one of

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the essential diagnostic criteria for HPV-associated vulvar SCC.[4]

2. The prevalence of HPV and p16^{INK4a} positivity in vulvar neoplasms

To explore the prevalence of HPV (including high-risk HPV and low-risk HPV) and p16^{INK4a} positivity in vulvar cancer and VIN [including low-grade squamous intraepithelial lesion(LSIL), HSIL, and dVIN] worldwide, we conducted a meta-analysis, which included more than 160 relevant original studies.[8] The globally pooled prevalence of HPV in vulvar cancer and VIN was 39.1 % (95 % CI: 35.3–42.9) and 76.1 % (95 % CI: 70.7–81.1), respectively. In China, the prevalence of HPV in vulvar cancer and VIN was 44.2 % and 83.7 %, respectively.[9] The most two frequent HPV genotype in vulvar cancer was HPV16 (78.1 %, 95 % CI: 73.5–82.3) and HPV33 (7.5 %, 95 % CI: 4.9–10.7) worldwide.[8] However, the HPV16 and HPV18 were reported as the most predominant HPV genotypes in cervical cancer[10] and vaginal cancer.[11] The pooled prevalence of type-specific HPV among regions was also different, with the lowest prevalence in South America (54.3 %, 95 % CI: 30.2–77.4) and the highest in Oceania (89.0 %, 95 % CI: 67.6–99.5). Moreover, despite that HPV33 was the second most frequent type following HPV16 in vulvar cancer globally, the second most predominant type in South America and Asia was HPV18. Additionally, a relatively high prevalence of HPV52, HPV45, and HPV31 in Oceania and Africa was observed. These findings highlighted the vital significance of multi-valent HPV vaccination, which covers HPV16 and HPV33, in preventing vulvar neoplasm.[8]

Vulvar basaloid and warty carcinoma are generally considered to be associated with HPV infection. By contrast, keratinizing carcinoma is generally considered to be independent of HPV infection. The 2020 WHO Classification Criteria of Female Genital Tumors stated that there was considerable histo-morphological overlap between HPV-associated and HPV-independent vulvar SCC.[4] Our meta-analysis also observed a variation of the pooled prevalence between histological subtypes of vulvar cancer, with a relatively high prevalence of HPV in warty carcinoma (86.5 %, 95 % CI: 76.9–93.8) and lowest in keratinizing carcinoma (12.0 %, 95 % CI: 6.1–19.6).[8] The prevalence of HPV in warty carcinoma was 86.5 % instead of 100 %, which conveyed that not all vulvar warty carcinoma should be categorized into vulvar HPV-associated SCC. Meanwhile, the prevalence of HPV in keratinizing carcinoma was 12.0 % instead of 0.0 %, conveying that not all keratinizing carcinoma should be categorized into HPV-independent vulvar SCC. Hence, the classification between HPV-associated and HPV-independent vulvar SCC cannot be simply made by histo-morphological subtypes alone.

As a tumor-suppressor protein of the INK4 family, p16^{INK4a} binds to cyclin-dependent kinases 4 and 6, maintaining the hypophosphorylated status of pRb, which in turn binds to E2F transcription factor and prevents cell cycle progression. In HPV-related tumors, E7 oncoprotein could functionally inactivate pRb by disrupting the pRb from its binding to E2F transcription factor, and this inactivation could further lead to the accumulation of p16^{INK4a} by negative feedback, which can be detected by IHC.[7] p16^{INK4a} has emerged as a surrogate marker of persistent HPV infection, and been recommended as the biomarker for distinguishing HPV-associated and HPV-independent vulvar SCC, together with the detection of HPV.[4] In our meta-analysis, the definition of p16^{INK4a} positivity was as follows: strong and diffuse block staining, strong staining and/or staining of >70 % cells, or intense and/or moderate staining. The pooled prevalence of p16^{INK4a} positivity in patients with vulvar cancer and VIN was 34.1 % (95 % CI: 30.9–37.4) and 65.7 % (95 % CI: 52.5–77.7), respectively. Meanwhile, we did not detect a significant difference in the pooled prevalence of p16^{INK4a} positivity when considering the various criteria used to define it. Among patients with HPV-positive vulvar cancer, p16^{INK4a} positivity prevalence was 73.3 % (95 % CI: 64.7–81.2). In the HPV-negative vulvar cancer, we also observed a p16^{INK4a} positivity prevalence of 13.8 % (95 % CI: 10.0–18.1).[8] The discordance between the prevalence of p16^{INK4a} positivity and the prevalence of HPV DNA in the total vulvar cancer or each histological subtype should arouse the pathologists' attention to the accurate classification of truly HPV-associated and HPV-independent vulvar SCC.

3. The significance of HPV and p16^{INK4a} positivity for prognosis and treatment in vulvar neoplasms

The testing methods of HPV DNA and p16^{INK4a} IHC overexpression have their respective limitations in distinguishing HPV-associated and HPV-independent cancers. The testing of HPV DNA simply reflects the status of HPV infection, however, whether the positivity of HPV DNA represents a transient infection or a persistent infection as the carcinogenic role cannot be known.[12] Moreover, although regarded as a sensitive biomarker for HPV-induced transformation, p16^{INK4a} overexpression can also be caused by mechanisms independent of HPV.[7] It might be of great importance to integrate complementary results of these two testing methods to distinguish HPV-associated and HPV-independent vulvar SCC. Therefore, our meta-analysis investigated the pooled prevalence of double positivity for HPV and p16^{INK4a} positivity in vulvar cancer and VIN, which was 19.6 % (95 % CI: 16.3–23.0) and 44.2 % (95 % CI: 26.3–62.8) in vulvar cancer and VIN, respectively. We found that the pooled prevalence of double positivity for HPV and p16^{INK4a} is much lower than the prevalence of either HPV DNA or the p16^{INK4a} positivity alone.[8]

In recent years, the prognostic role of HPV status has been widely investigated in some HPV-related cancers, especially in oropharyngeal cancer. Specifically, HPV-positive patients showed a better survival outcome compared to HPV-negative in oropharyngeal cancer,[13] penile cancer[14] and anal cancer.[15] Previous studies on the association between HPV status and the prognosis of vulvar cancers often enrolled small study populations, which might lead to inconclusive findings. To evaluate the effect of HPV status on prognosis in vulvar cancer, Rasmussen and colleagues conducted the first relevant systematic review and meta-analysis in 2017 and reported a better overall survival (OS) as well as disease-free survival (DFS) in the patients with HPV-positive vulvar cancers than those with HPV-negative vulvar cancers.[16] The molecular mechanisms of the better prognosis in HPV-positive cancers were not well identified, and the increased radiosensitivity was proposed as one potentially important mechanism.[17]

In addition to HPV status, p16^{INK4a} overexpression has also been regarded as a potential prognostic marker.[18] Moreover, the better prognostic of double positivity for HPV DNA and p16^{INK4a} than that of either HPV or p16^{INK4a} positivity alone has aroused great interest of scholars.[19,20] In 2023, a multicentre, multinational study explored the prognostic implications of discordance between HPV and p16^{INK4a} status in oropharyngeal cancer. Among the total of 7654 patients with oropharyngeal cancer, patients with p16^{INK4a}+/HPV + oropharyngeal cancer had a significantly better prognosis than those with discordant oropharyngeal cancer (p16^{INK4a}–/HPV+ or p16^{INK4a}+/HPV–), meanwhile, patients with p16^{INK4a}–/HPV– oropharyngeal cancer had the worst prognosis.[20] In the field of vulvar cancer, a recent meta-analysis explored the prognostic role of p16^{INK4a} in vulvar SCC, which enrolled a total of 475 patients with vulvar SCC from 5 published studies, and showed that patients with p16^{INK4a} positive vulvar SCC had a significantly more favorable OS than those with p16^{INK4a} negative vulvar SCC (HR: 0.40, 95 % CI: 0.29–0.55).[21] However, the data on the prognostic value of combined HPV and p16^{INK4a} status in vulvar cancer was limited. Prospective randomized studies are needed to explore the association of different HPV and p16^{INK4a} positivity status with the survival outcomes in vulvar cancer patients, further guiding gynecologic oncologists to develop a more accurate prognostic stratification of vulvar cancer.

In summary, HPV DNA and p16^{INK4a} play an important role in the carcinogenesis, diagnosis, and prognostic evaluation of vulvar tumors. Active vaccination with multi-valent vaccines could significantly reduce the incidence of vulvar cancer. Additionally, a better understanding of the clinical significance of double positivity for HPV DNA and p16^{INK4a}

could help clinicians make a more accurate treatment plan and a better follow-up strategy for patients with vulvar cancer.

Authors contribution

PL and ZL: writing of the original manuscript; ZC and ZZ: investigation; KS: article supervision. BK and JM: conceptualization, review, and editing of the manuscript.

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Declaration of competing interest

The authors declare no conflict of interest.

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