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## Lessons from Australia: HPV is not a major risk factor for esophageal squamous cell carcinoma

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### Keywords

HPV; esophageal squamous cell carcinoma; epidemiology; contamination

The incidence of esophageal cancer exhibits great geographical diversity, with up to 20-fold differences in rates between high- and low-risk areas (1). Despite stable or even declining worldwide esophageal cancer rates over the last few decades (2,3), this disease remains a substantial public health problem with a continued need for effective cancer prevention strategies (3). Human papillomavirus (HPV) has been hypothesized to cause ESCC, but as described in this issue by Antonsson *et al.*, it does not seem to be a major risk factor (4). Determining the extent to which HPV may contribute to ESCC etiology is important given the recent clinical discoveries related to HPV-associated tumors.

The prophylactic HPV vaccine has high vaccine efficacy against HPV16- and 18-related cervical disease (5,6), vulvar and vaginal precancers (7), and HPV6- and 11-related condyloma acuminata (i.e., genital warts) in men and women (8,9). For HPV-associated oropharyngeal cancer, a direct evaluation of the vaccine efficacy has not been conducted, but researchers are optimistic that the vaccine will have similar efficacy against HPV16 infection in the head and neck region as in anogenital mucosal sites. Thus, the effectiveness of the HPV vaccine may extend beyond the cervix to other anatomic sites where HPV-induced carcinogenesis occurs.

In addition to the potential for cancer prevention through vaccination, HPV has been associated with improved survival in patients with oropharyngeal cancer (10–14), possibly due to better response to chemotherapy or chemoradiation in HPV-positive tumors (15). An on-going clinical trial is evaluating radiation dose de-intensification among HPV-positive oropharyngeal cancer patients (16). Although the current standard of care for ESCC treatment is surgical resection (17), theoretically there could be improvements in treatment for ESCC if HPV caused a fraction of these cancers.

Antonsson *et al.* conducted a study of HPV in ESCC in tissue from cases in Australia (4), where esophageal cancer incidence follows the pattern of low-risk Western countries, with age standardized incidence rates of 1.4 and 1.0 per 100,000 for men and women, respectively (18). Using PCR for HPV detection, the authors found DNA from HPV types 16 and 35 in 8 of 222 cases (3.6%), and they found p16<sup>INK4a</sup> overexpression, consistent with

HPV *E7* oncogene expression (19–21), in only 4 of the HPV DNA-positive cases (1.8%). These results are similar to those found in other large studies that have taken care to avoid contamination in specimen collection, processing, and/or laboratory testing and have found low or no prevalence of HPV in ESCC tumor tissue (22–25).

The study by Antonsson *et al.* highlights the need to investigate HPV DNA-positive cases to determine if the detected HPV was actively expressed in a way that might have contributed to tumor development or was simply an incidental infection or contaminant. In this study, there were several indications that HPV was not etiologically related to cancer development in some of the cases where it was detected. First, HPV DNA-positive cases were often equivocally or weakly positive instead of having a strong PCR signal. Second, HPV DNA was not always detected concordantly in duplicate tumor specimens from the cases with an additional specimen available. Third, half of the HPV DNA positive cases were negative for p16<sup>INK4a</sup> overexpression, suggesting that the detected DNA did not contribute to the development of cancer in these cases.

Based on the results from Antonsson *et al.* and other studies, HPV infection contributes to very few, if any, cases of ESCC. As such, any potential involvement of HPV in ESCC will affect neither HPV vaccination policy nor treatment for ESCC. Given the lack of public health benefit and the small number of cases to which HPV may contribute, as indicated by studies from areas with high (23) and low rates of ESCC (4,22,24,25), it may not be an efficient use of resources to continue to investigate HPV as an etiologic agent in ESCC.

The study by Antonsson *et al.* raises important general considerations for investigating HPV in extra-cervical tumor tissues. PCR is the gold standard for HPV DNA detection, but it is highly prone to contamination (26–31). Thus, investigators should take precautions against contamination in sample collection, processing, and testing. If HPV DNA is detected, follow-up tests should be performed to help clarify the role of the detected HPV. The additional testing for p16<sup>INK4a</sup> overexpression by Antonsson *et al.* is a strength of this study, and it suggests that at least some of the HPV initially detected by PCR was unlikely to reflect active infection that contributed to the development of the tumor. p16<sup>INK4a</sup> staining, however, also has limitations. For example, p16<sup>INK4a</sup> is sometimes underexpressed in ESCC due to hypermethylation (32,33) or mutation (34). Conversely, p16<sup>INK4a</sup> may be overexpressed due to non-HPV related changes in the pRB pathway; a recent study of ovarian cancers found focal homogeneous or complete immunostaining with p16<sup>INK4a</sup> in 74% of cases but no evidence of HPV DNA (35). Thus, p16<sup>INK4a</sup> overexpression may provide additional information about HPV oncogene expression among HPV DNA-positive cases, but to better interpret the meaning of p16<sup>INK4a</sup> overexpression in HPV DNA-positive cases, it may be important to stain some of the HPV DNA-negative cases as well to establish the background level of p16<sup>INK4a</sup> overexpression in ESCC or other tumor tissues.

Additional follow-up tests may also be useful. For example, real-time (RT)-PCR can be used to measure viral load, which can help distinguish between contamination with incidental infection (usually a low viral load) and active infection (usually a high viral load, with at least one copy of the virus present in every tumor cell (36)). Also, HPV *E6* and *E7* mRNA expression can be measured directly (37–39) to assess the expression of *E6* and *E7* oncoproteins, which is required for HPV-related malignancy (40–43). The presence of HPV can also be confirmed by *in situ* hybridization (ISH), which is less prone to detecting HPV DNA introduced through contamination because it does not amplify the DNA. ISH also permits visualization of HPV DNA localized to the tumor cell nucleus, which can identify integrated DNA as a distinct dot rather than dispersed throughout the nucleus as episomes (44). Although some cervical tumors do not contain integrated HPV (45), integration is a major mechanism in HPV-associated carcinogenesis (46) and usually occurs in HPV-

associated oral cancers (47,48). Another evidence of HPV activity is E6/E7 seropositivity, which indicates the presence of an HPV-associated tumor somewhere in the body (49,50), but it is only about 50% sensitive for invasive cervical cancer (36) and does not always occur in oral cancer patients either (51). Future tissue-based studies would be well-advised to include positive controls, such as cervical cancer tissue, and negative controls, for example stomach or brain cancer tissue (where HPV is not known to cause cancer), collected in the same way as the tissue of interest. Given the current lack of a single, definitive test for determining if HPV caused an individual tumor, the use of several tests can provide a more complete picture of the functionality of HPV in HPV DNA-positive cases without relying too heavily on any one test. The results of these tests must be taken together, with interpretation based on the balance of evidence.

In conclusion, several recent studies of HPV in ESCC that took great care to avoid contamination suggest that HPV does not contribute to the majority of cases, either in areas with high or low rates of ESCC. Although it is true that HPV is increasingly recognized as a cause of some extra-cervical cancers, including oropharyngeal, vulvar, vaginal, penile, and anal cancers (52), the attributable fraction of preventable cancers worldwide is still driven by cervical cancer (53,54). While recent vaccine efficacy data further expand the use of the HPV vaccine to boys for the prevention of genital warts and may expand it further to rare cancers in the anus and oropharynx, current cost-benefit analyses that have considered the totality of HPV-associated diseases still indicate that vaccine administration should be focused on obtaining high coverage among young girls; this strategy has the potential to prevent more cancers than vaccinating a similar number of individuals, half of whom are boys (55). We still have much to learn about HPV-related carcinogenesis, even in the cervix, and it is possible that HPV may cause cancer at additional anatomical sites. In the esophagus, however, HPV causes only a small proportion of ESCC cases, if any. The impetus for HPV vaccination remains driven by the burden of cervical cancer.

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