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Detection of human papillomavirus (HPV) in clinical samples: Evolving methods and strategies for the accurate determination of HPV status of head and neck carcinomas

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SUMMARY

Much recent attention has highlighted a subset of head and neck squamous cell carcinomas (HNSCCs) related to human papillomavirus (HPV) that has an epidemiologic, demographic, molecular and clinical profile which is distinct from non-HPV-related HNSCC. The clinical significance of detecting HPV in a HNSCC has resulted in a growing expectation for HPV testing of HNSCCs. Although the growing demand for routine testing is understandable and appropriate, it has impelled an undisciplined approach that has been largely unsystematic. The current state of the art has now arrived at a point where a better understanding of HPV-related tumorigenesis and a growing experience with HPV testing can now move wide scale, indiscriminant and non-standardized testing towards a more directed, clinically relevant and standardized approach. This review will address the current state of HPV detection; and will focus on why HPV testing is important, when HPV testing is appropriate, and how to test for the presence of HPV in various clinical samples. As no single test has been universally accepted as a best method, this review will consider the strengths and weaknesses of some of the more commonly used assays, and will emphasize some emerging techniques that may improve the efficiency of HPV testing of clinical samples including cytologic specimens.

Introduction

High risk human papillomavirus (HPV), particularly type 16, has been established as a causative agent for a significant proportion of head and neck squamous cell carcinomas (HNSCC) [1,2], and the incidence of these HPV-related carcinomas is on the rise [3–6]. Given the distinctiveness of HPV-related carcinoma as a biological and clinical variant of HNSCC, the need for routine HPV testing of oropharyngeal carcinomas is compelling and urgent. The increasing incidence of HPV-associated HNSCC, along with the growing importance of HPV status as a versatile biomarker, is spurring a growing expectation for HPV testing and inclusion of HPV status as a parameter of emerging molecular staging systems. Indeed, the College of American Pathologists has recently recommended routine HPV testing as part of the standard pathologic evaluation of resected oropharyngeal squamous cell carcinomas (http://www.cap.org/apps/docs/committees/cancer/cancer_protocols/2013/Pharynx_13protocol_3300.pdf), and Cancer Care Ontario has published evidence based guidelines for routine testing of HNSCCs (https://www.cancercare.on.ca/common/pages/UserFile.aspx?fileId=279836).

Despite an escalating expectation for the reliable determination of HPV status, there is not yet a standard strategy or method for HPV detection in head and neck cancers. Even fundamental questions regarding when and why to test for HPV still bewilder pathologists and treating clinicians alike. As a result, HPV testing is either never requested or it is indiscriminately demanded without any contextual regard for anatomic site, microscopic findings, clinical relevance and other factors that may influence the likelihood and significance of detecting HPV in a clinical specimen. Moreover, methods of HPV testing across laboratories vary considerably reflecting the biases and tendencies of individual pathologists, and the cost to benefit ratio of each technique [7]. Detection strategies vary not just in design, but in their detection targets. These targets have included HPV DNA, HPV RNA, viral oncoproteins,

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cellular proteins and HPV-specific serum antibodies. In the ongoing effort to establish a consensus approach, the challenge for the oncologic community is to implement standardized HPV testing using a method that is highly accurate, technically feasible, cost effective and readily transferrable to the diagnostic pathology laboratory in a way that is prognostically relevant and supports clinical care.

Relevance of HPV testing

Clinicians have not been able to rely on prognostic markers other than tumor stage in their care of patients with HNSCC. Numerous studies have addressed the prognostic relevance of cell proliferation (e.g. Ki67), p53 immunohistochemical staining, apoptosis, aneuploidy, Epidermal growth factor receptor overexpression and other markers of biologic activity, but none have proved consistently reliable across multiple studies [8–11]. Even histologic grade does not perform well as a prognosticator. Into this void, HPV detection has stepped in as a powerful biomarker indicating a more favorable clinical outcome for patients with HNSCC. Compared with patients with HPV-negative tumors, those with HPV-positive tumors have a lower risk of tumor progression and death, reflecting in part an enhanced sensitivity to ionizing radiation with or without chemotherapy [2,12–15].

When it comes to patients with HNSCC, the value of HPV testing is by no means restricted to mere prognostication. Detection of HPV is emerging as a valid biomarker for discerning the presence and progress of disease encompassing all aspects of patient care. HPV testing is increasingly used for more refined tumor staging: HPV positivity can be used as evidence of oropharyngeal origin in patients with large and bulky tumors that involve multiple contiguous anatomic sites, and in those patients who present with cervical lymph node metastases. In the near future, HPV status will help guide a more individualized therapeutic approach for patients with HNSCC. In particular, the less aggressive behavior associated with HPV positivity may justify less toxic doses of chemotherapy and/or radiation therapy (i.e. therapeutic de-intensification) for patients with HPV-positive HNSCCs [16]. Knowledge of HPV status is now compulsory for meaningful comparison of treatment responses for patients enrolled in clinical trials. Indeed, the direction of current clinical trials, where patient selection for specific therapies is predicated on HPV tumor status, dramatically heightens the stakes for accurate HPV detection. Finally, HPV assessment may play some present or future role in comprehensive cancer care including early cancer detection [17], post-treatment tumor surveillance [18,19], and more informed discussions with patients and their partners.

HPV testing by anatomic sub-site

HPV infection is strongly correlated with oropharyngeal location, particularly the palatine and lingual tonsils [20]. This preferential targeting likely reflects multifaceted biological interactions between HPV and the highly specialized lymphoepithelium lining the tonsillar crypts [11]. As one important example, the PD-1:PD-L1 interaction mediates complex immunomodulatory pathways that render the tonsillar epithelium an "immune-privileged" site for initial viral infection, and enhances adaptive immune resistance once a tumors is established [21]. Although HPV positivity is sometimes reported in HNSCCs arising outside of the oropharynx such as the sinonasal tract [22–24] and nasopharynx [25,26], expanding the scope of routine HPV testing is not warranted until studies establish a clear relationship between HPV infection at these non-oropharyngeal sites and a distinct natural history including treatment responses. Based on this localization of HPV-related HNSCC to the oropharynx, directives for routine HPV testing is generally restricted to those carcinomas arising from this specific anatomic sub-site https://www.cancercare.on.ca/common/pages/UserFile.aspx?fileId=279836). Current clinical practice appears to be out of step with clear directives for routine HPV detection restricted to oropharyngeal carcinomas. In one recent study, only 68% of North American head and neck practitioners routinely requested HPV testing of oropharyngeal carcinomas; and conversely, 32% routinely requested HPV testing of oral cavity carcinomas [27]. These findings underscore a need for further education to conform clinical practice with science-based guidelines.

In malignant transformation of the tonsillar epithelium, HPV does not act through a "hit and run" mechanism where its role is transient and limited to the initiation of tumorigenesis. Instead, the presence of HPV persists, and it is just as readily detected in metastatic implants as in the corresponding primary cancers [20,28]. Consequently, a lymph node metastasis is quite suitable as a substrate for HPV testing, obviating the need for additional tissue acquisition, particularly in those patients with small or even occult primary cancers. For those patients who present with neck metastases in the absence of an obvious primary tumor, HPV testing of lymph node metastases is an effective strategy for localizing the site of origin. In these patients, the detection of HPV in a lymph node metastasis is a reliable predictor of oropharyngeal origin [29,30]. Similarly, HPV status can be used to clarify tumor relationships in those patients with HNSCCs who go on to develop a squamous cell carcinoma at distant sites [28,31,32]. For example, the detection of HPV in a squamous cell carcinoma of the lung in a patient with a prior HNSCC helps identify the true nature of the lung cancer as a metastasis rather than a secondary primary [31,33].

Methods of HPV detection

There is currently no standard approach for HPV testing of clinical samples. Instead, methods of HPV testing across laboratories vary considerably reflecting the biases and tendencies of individual investigators, and the cost to benefit ratio of each technique [7,34,35]. Detection strategies vary not just in design, but in their detection targets. These targets have included HPV DNA, HPV RNA, viral oncoproteins, cellular proteins and HPV-specific serum antibodies. For widespread implementation in the clinical arena, detection methods must be accurate, cost effective and readily transferrable to the routine diagnostic laboratory.

The various strategies that are currently available are guided by an understanding of HPV-induced malignant transformation of oropharyngeal epithelium, particularly its interaction with key components of the retinoblastoma (Rb) tumor suppressor gene pathway [36]. The p16 tumor suppressor gene is a member of the INK4 class of cell-cycle inhibitors and represents a key component of the Rb pathway. The binding of the p16 tumor suppressor gene product with the cyclin-dependant kinases 4 and 6 block its interaction with the D-type cyclins, maintains the retinoblastoma (Rb) gene in a hypophosphorylated state that binds E2F transcription factor and, in turn, prevents cell cycle progression. HPV integration results in the deletion of the viral E2 gene promoter causing transcription of E6 and E7. Binding of the E7 oncoprotein to the Rb protein leads to Rb protein degradation and presumably to the compensatory overexpression of both cytoplasmic and nuclear p16 protein in HPV infected tumor cells [37]. Given this capacity to target and disrupt the Rb tumor suppressor gene pathway, HPV detection strategies may look to detect: (1) HPV DNA, (2) post-integration transcription of viral E6 and/or E7 mRNA, (3) the