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Laboratory-Clinic Interface

From HPV-positive towards HPV-driven oropharyngeal squamous cell carcinomas

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ABSTRACT

The incidence of HPV-positive oropharyngeal squamous cell carcinoma (OPSCC), which is both biologically and clinically distinct from tobacco- and alcohol-related OPSCC, is dramatically increasing. The finding that individuals with HPV-positive local/regionally advanced OPSCC have a significantly better prognosis than their negative counterparts have led to efforts to de-escalate treatment in those patients to avoid serious side effects and to improve their long-term quality of life, while maintaining treatment efficacy. Identifying diagnostic tests that are able to distinguish cancers etiologically associated with HPV is thus becoming a pressing challenge for researchers. The purpose of this review is to provide an overview of the diagnostic tools presently available to evaluate HPV status in patients with OPSCC and, in particular, to discuss their strengths and weaknesses in identifying those infections that are the real driving force in the oropharyngeal carcinogenesis process.

Introduction

According to recent estimates, cancers of the upper aero-digestive tract (UADT) are expected to affect approximately 742,000 and 144,000 new patients, respectively, worldwide and in Europe [1]. About 90% of those are squamous cell carcinomas (SCC), which develop in the epithelial linings of the oral cavity, pharynx, and larynx [reviewed in [2].

UADT-SCC is strongly associated with tobacco use, heavy alcohol consumption, betel quid chewing, and poor oral hygiene [3–5], and although these risk factors still globally account for the majority of these cancers, high risk oncogenic human papillomavirus (HPV) types, frequently HPV type 16 (HPV16), are known to be causally associated with a subset of oropharyngeal squamous cell carcinomas (OPSCC) arising from the crypt epithelium of the palatine and lingual tonsils [6–10]. HPV-driven OPSCC and tobacco- and alcohol-related OPSCC are biologically distinct entities [8,11–15], and the alterations of the p53- and pRb-pathways, the mechanisms by which perturbations are achieved, are radically different. In the former, p53 and pRb are both inactivated at the protein level by, respectively, E6 and E7 viral oncoproteins. The majority of HPV-negative tumors, instead, harbor mutations in the TP53 gene, which inactivate the functions of cellular tumor suppressor p53, show loss of $p16^{INK4a}$ tumor suppressor, and frequently have cyclin D1 amplification with a consequent decrease in the growth-suppressive hypo-phosphorylated form of pRb. HPV-driven tumors have, moreover, fewer gross chromosomal aberrations and approximately one half of the mutation rate of HPV-negative ones [16].

While the overall incidence of OPSCC has been increasing, survival rates have also been rising over the last three decades and this may be explained by the positive prognostic impact of HPV transforming infection [17–21]. Retrospective analyses of archival tumor specimens from patients enrolled in phase III trials and a recent meta-analysis concur in confirming that subjects with HPV-positive local/regionally advanced OPSCC have indeed a significantly better prognosis than their HPV-negative counterparts, and, in fact, HPV-status is currently considered the best independent predictor of survival in patients with advanced OPSCC [22–25].

But beyond its prognostic significance, HPV evaluation in OPSCC has also proven to be valuable to: (a) detect occult OPSCC [26], (b) stratify patients for treatment de-escalation trials [25], (c) personalize post-operative surveillance strategies [27] and (d) facilitate early detection of primary SCC and recurrence [28]. In the future, HPV-status may serve as a guide for treatment strategy/selection and, further along in time, a treatment target itself [29]. In view of these considerations, routine HPV testing has recently been rec-

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ommended by both the College of American Pathologists and the American Joint Committee on Cancer as part of the standard pathologic assessment of OPSCC specimens [30]. For the moment, however, the methods and strategies for HPV detection have not yet been entirely defined [reviewed in [31].

This review aims to provide an overview of the tests now available to evaluate HPV status in patients with OPSCC and, in particular, their strengths and weaknesses in identifying those infections that are the real driving force in oropharyngeal carcinogenesis. The term HPV-*positive* OPSCC will be used here to refer to carcinomas of the oropharynx *presumed to be associated with HPV* mostly on the basis of positivity to HPV-DNA or $p16^{INK4a}$ immunohistochemistry (IHC). The term HPV-*driven* OPSCC will, instead, be used to refer to carcinomas in which sensitive biomarkers and/or surrogate markers have identified the HPV infection which we consider *the driving force* maintaining the transformed phenotype.

Standard HPV-status determination in tissue samples from OPSCC

HPV status in OPSCC patients is determined in the clinical setting on the basis of samples testing positive to HPV-DNA or IHC for $p16^{INK4a}$, considered a surrogate marker for active HPV involvement in OPSCC tumorigenesis [32,33].

Testing for viral DNA by PCR amplification of HPV-DNA and in situ hybridization (ISH) is the most widely used method to diagnose HPV infection in clinical samples. HPV-DNA alone is not, however, sufficient to identify HPV-driven cancers of the head and neck region [12,34,35]. While PCR-based assays are, in fact, highly sensitive, DNA-positivity in tumor tissues can result not only because HPV is the real driving force in carcinogenesis but also because a non-transforming infection may have been identified in the tumor or neighboring tissue (a past infection that has not progressed to cancer or recent oral HPV exposure); cross-contamination between tissue samples or laboratory contamination are also possible. ISH using HPV-type specific probes allows direct visualization of HPV in tissue samples and may, theoretically, discriminate between oncologically relevant and non-relevant infections. ISH testing has, however, been found to lack satisfactory sensitivity; one study, in fact, reported that 79% of HPV ISH negative OPSCC patients were positive to both HPV PCR and $p16^{INK4a}$ [33].

Some consider $p16^{INK4a}$, whose expression is thought to be up-regulated due to pRb degradation induced by high-risk HPV E7 protein, a surrogate marker that is able to identify tumors with relevant viral oncogene expression [36]. $p16^{INK4a}$ immunostaining has, nevertheless, shown both insufficient sensitivity [7,35,37–39] and specificity [33–35,37–39] when used as a stand-alone test. Interestingly, 22% of OPSCC with transcriptionally active HPV16 infection were found to be $p16^{INK4a}$ negative, whereas an up-regulation of $p16^{INK4a}$ was identified in 14–21% of tumors with no evidence of HPV16 transforming infection [35]. Recently, $p16^{INK4a}$ positive-HPV-DNA negative tumors were classified as non HPV-driven on the basis of genetic profiling [40].

HPV-driven OPSCC

While increasing evidence has emerged indicating that HPV DNA-positive OPSCC is a heterogeneous entity as far as biological and clinical behavior are concerned [8,9,11–15,22,41–45], according to some data a small percentage of HPV DNA-positive OPSCC patients have an unfavorable prognosis. This inconsistency could be explained by the quantity of cigarettes smoked a year, comorbidities, the impact of the T and N category, and the pattern of nodal metastasis; all of these can modify the prognosis of HPV-positive OPSCC [22,25,44,45]. At the same time, it is also possible that the tumor was classified as HPV-positive simply on the basis of a single marker with poor specificity [11–13,22,35,39,46]. In a large series of OPSCCs ($n = 199$), HPV-DNA status and $p16^{INK4a}$ as stand-alone tests were not found to be sensitive discriminators, respectively, of overall and progression free survival on univariate and multivariate analysis [35]. Similarly, a recent large study carried out in Holland reported that the survival curve of 723 patients with a positive $p16^{INK4a}$ but a negative HPV DNA test result was found to be virtually identical to that in patients whose results for both tests were negative [25]. The wide variation in HPV prevalence across different studies could also be partially ascribed to the different methods used to detect HPV [47].

Beyond a matter of biological curiosity, accurate detection of HPV-driven tumors is important for treatment decisions, to predict individual patients' outcomes, and to calculate the burden of this type of carcinoma in different populations [21,35,39]. Finally, when treatment de-escalation trials in HPV-positive SCC are being designed, every effort must be made to avoid the risk of recruiting patients with transient HPV infections whose outcome could be negatively influenced by suboptimal therapy [25]. The primary aim underlying HPV testing of clinical samples from OPSCC is thus not only that of classifying the tumor as a HPV-positive or -negative cancer but also of discriminating between a transient, transcriptionally silent infection and a biologically relevant one *driving* the cancerogenesis.

A comprehensive cancer model for HPV-induced cell transformations has been developed over the last two decades. The following elements support the causal role of mucosal HPV in the pathogenesis of OPSCC (a) the presence of at least one viral genome copy per tumor cell, (b) active transcription of the viral E6 and E7 oncogenes, and (c) interaction of the viral oncoproteins with cell-cycle regulatory proteins and apoptosis-inducing proteins (Table 1) [48,49].

Table 1
HPV as the driving force in mucosal cancer (Refs. [48,49]).

Necessary features		Consequences		Markers
⩾1 Viral genome in each tumor cell	→	Presence of HPV-DNA	→	HPV-DNA detection by PCR or ISH
Active transcription of the E6 and E7 oncogenes	→	Presence of E6 mRNA and E7 mRNA	→	HPV E6 and E7 mRNA detection by PCR
Interaction of the viral oncoproteins with cell-cycle regulatory proteins	→	Degradation of pRb oncosuppressor	→	Low pRb expression levels by IHC
		Degradation of p53 oncosuppressor	→	Low p53 expression levels by IHC with wild-type TP53
		Up-regulation of the CDNK2a gene	→	High $p16^{INK4a}$ expression levels by IHC
		Down-regulation of the CCND1 gene	→	Low cyclin D1 expression levels by IHC

HPV: human papillomavirus; PCR: polimerase chain reaction; ISH: in situ hybridization; IHC: immunohistochemistry.