In this issue of Cancer Research, 2 studies by Holzinger and colleagues (1) and Liang and colleagues (2) investigate which biomarker(s) of human papillomavirus (HPV) infection are more prognostic in oropharyngeal and head and neck squamous cell carcinomas (HNSCC). In the former study, authors conclude that viral load and RNA pattern analysis are better prognostic markers than p16INK4A in identifying oropharyngeal squamous cell carcinomas (OPSCC) driven by HPV-16 infection. In the latter, authors conclude that adding HPV E6/E7 serology to definition HPV-associated HNSCC yields a stronger association of HPV presence with prognosis.

For over a decade now, there has been a plethora of studies documenting the role of high-risk HPV infection (i.e., HPV-16 and -18) in HNSCC. The issue is of significant importance because HPV infection is related to sexual practices (3, 4) and understanding that HPV is a risk factor for OPSCC (3); that those with HPV(+) OPSCC, particularly in men, is on the rise (5). We now understand that HPV is a risk factor for OPSCC (3); that those with HPV(+) OPSCC tend to be younger, smoke less, and have smaller tumors than those with HPV(−)OPSCC (6, 7); that somatic mutations (including those in the TP53 gene) are less frequent in HPV(+)-OPSCC (7); and finally, that HPV(+) OPSCCs respond better to treatment and have better outcomes (8, 9). It is also clear that the higher prevalence of high-risk HPV infection is confined to the oropharynx (10, 11) and that the survival advantage associated with HPV infection in patients with OPSCC has not been well-documented for tumors outside the oropharynx—likely reflecting the interplay between the virus and lymphoid tissue in accounting for differences in the biology of the disease and treatment response.

Recognizing then that HPV(+) OPSCC represents a distinct entity with different treatment response than nonoropharyngeal carcinomas, HPV infection—or surrogate markers thereof—has now become the first clinically relevant prognostic indicator for OPSCC. Questions have naturally followed with regards to what is/are the best prognostic biomarker(s) for OPSCC. In addition, as HPV(+) OPSCC is associated with better outcomes, there is now a window to consider treatment de-escalification. If the current trials aimed to answer this question prove that de-escalation schemes are safe and effective, then we will soon use HPV’s association with OPSCC not only to prognosticate but to guide treatment.

Establishing the presence of HPV DNA, however, does not seem sufficient. Studies have shown that an important distinction must be made between the presence of HPV in OPSCC and where HPV infection is causal (12–14). It is generally believed that the detection of active HPV infection can be used to infer whether OPSCC is driven by HPV. However, this can vary according to the methods used to detect HPV infection, which can include PCR-based detection of E6/7 mRNA, L1 mRNA or HPV-16 DNA (with or without viral load analysis), HPV-16 DNA by in situ hybridization, E6/7/L1 serum antibodies (15), and the newer MassARRAY system that couples standard (PCR) techniques with mass spectrometry to determine viral load and gene expression (16, 17). Advances in our understanding of oncogenic HPV biology have shown that as HPV E6/E7 proteins sequester the tumor suppressor p53 and the retinoblastoma (Rb) gene product pRb that this, in turn, causes overexpression of p16INK4A protein via a negative feedback mechanism. The ease of adapting immunohistochemistry (IHC) in diagnostic laboratories, coupled with the common practice of working with fixed tumor blocks from patients’ biopsies, has popularized the use of p16INK4A-IHC as the surrogate oncogenic HPV marker of choice. In addition, p16INK4A expression has been shown to be associated with overall and progression-free survival in OPSCC and this association was similarly as significant as for HPV status (9). So given this, is p16INK4A-IHC enough?

The 2 studies in this current issue provide further insight on this issue (1, 2). In the study by Holzinger and colleagues, authors investigate the prognostic accuracy of viral RNA patterns, high viral loads, and p16INK4A immunostaining in OPSCC in Western European patients. The results of this study support the notion that p16INK4A immunostaining alone may not be an appropriate surrogate marker for HPV active infection in this patient cohort and may highlight differences in risk factors such as smoking between populations. Interestingly, in addition to p16INK4A immunostaining, the authors used an algorithm that borrowed from the cervical cancer literature to ascertain active HPV infection [i.e., viral mRNA expression patterns seen in cervical cancer, CxCaRNA(+) as well as viral load as determined by PCR. The strongest association with better prognosis was with either CxCaRNA(+) tumors or with high viral load tumors. In contrast, a weaker association was found for OPSCC that were HPV(+) and p16INK4A(+) and only for overall survival; no significant association was found for progression-free survival in this group. In contrast to the above studies with North American patients, neither HPV DNA nor p16INK4A alone showed significant associations with survival—although similar trends were noted—and there was no
difference in OPSCC p16\textsuperscript{INK4A} expression based on HPV DNA status. Of note, detection of HPV-16 infection in OPSCC cases, as measured by both DNA (49%) and viral mRNA expression (20%), was lower than for other North American and international multicenter studies (6, 9). Together, these differences should caution readers on the generalizability of these findings to the other populations.

The study by Liang and colleagues investigated the association between HPV infection and overall survival in a cohort of patients with HNSCC. They concluded that the detection of HPV positivity via DNA markers or p16\textsuperscript{INK4A} alone was not accurate in identifying patients with OPSCC with better prognosis and that adding measurements for E6/E7 antibodies was a superior strategy. Moreover, in support of the idea that p16\textsuperscript{INK4A} overexpression may be modulated by other unfavorable factors aside from HPV, patients with p16\textsuperscript{INK4A} (+) tumors who were seronegative for E6/E7 antibodies had significantly increased risk of death. There were several issues that are worth mentioning. For one, the correlation between these markers was lower than previously reported (6, 15), and in particular, so was the case for detection of HPV-16 DNA by 2 separate methods (i.e., \( \kappa = 0.18 \) between MPO and SFCPR methodologies). In addition, a combination that would be clinically useful, HPV-16 DNA and p16\textsuperscript{INK4A} immunostaining was not examined. Given these issues, the authors' conclusions that “the combination of HPV 16 DNA or p16\textsuperscript{INK4A} immunostaining with HPV E6/E7 antibodies represents the most clinically valuable surrogate markers for the identification of patients with HNSCC who have a better prognosis” should be substantiated further.

In general, studies have shown good correlation between p16\textsuperscript{INK4A} overexpression and HPV infection. A study by Smeets and colleagues showed a sensitivity and specificity of 100% and 79% (15). Similar findings were seen by others (6, 9, 18). However, the biology involved in p16\textsuperscript{INK4A} expression is complex and can be modulated by other factors, which may negatively impact prognosis and that are independent of HPV, such as Rb gene methylation and/or p16\textsuperscript{INK4A} loss via genetic or epigenetic changes. Inspired by this, a recent study in the \textit{British Journal of Cancer} looked at this issue, further considering the different biologic scenarios that could alter p16\textsuperscript{INK4A} expression. Interestingly, the authors found that p16\textsuperscript{INK4A} nuclear localization may inform differences in survival outcomes in HNSCC regardless of site or HPV status (19). Complicating matters further, even in those OPSCCs where HPV infection is determined, the prognosis in these patients seems to also depend on other factors such as HPV copy number (20), smoking (9), p53, and EGFR (21) among others.

What can be stated from these 2 studies and others is that p16\textsuperscript{INK4A} status, particularly if dichotomized, should be interpreted with caution. Further testing to confirm HPV active infection may be warranted, particularly in consideration of de-escalation regimens. In addition, other prognostic clinical parameters should be considered. For example, a study investigating p16\textsuperscript{INK4A} as a prognostic biomarker of treatment response in a cohort of patients treated with radiotherapy alone in the Danish Association of Head and Neck Cancer Group 5 trial found that, in addition to p16\textsuperscript{INK4A} expression, low tumor classification (T1/2) and negative lymph nodes were independent predictors of good prognosis (22). On the other hand, a study by Ang and colleagues showed that the nodal stage (N0-N2a vs. N2b-3) and tobacco smoking (<10 vs. >10 pack-years) were major determinants of overall survival in patients with HPV (+) OPSCC (9). As long as p16\textsuperscript{INK4A} immunostaining is more routinely conducted, it is perhaps around these risk factors that debate should focus on which p16\textsuperscript{INK4A} (+) OPSCCs does HPV status needs to be clearly ascertained, which have better prognosis, and which are better candidates for de-escalation regimens.

References


Biomarkers of HPV Infection in Oropharyngeal Carcinomas: Can We Find Simplicity in the Puzzle of Complexity?

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