p16 Expression in Laryngeal Squamous Cell Carcinoma

Subjects: Otorhinolaryngology

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Laryngeal squamous cell carcinoma (LSCC) is a common malignancy that, despite scientific advancements, has not seen an improvement in its prognosis. Few promising predictive markers have been found and none are relevant in clinical practice. p16^{ink4a}, an oncosuppressor protein involved in cell cycle arrest, with a prognostic impact on other cancers, has been widely used in the head and neck region as a surrogate marker of HPV infection. Published papers and meta-analyses seem to minimize the biological role of HPV in the context of LSCC's cancerogenesis, and to disprove the reliability of p16^{ink4a} as a surrogate prognostic marker in this context, while still highlighting its potential role as an independent predictor of survival.

Keywords: p16ink4a overexpression ; p16ink4a inactivation ; LSCC ; HPV ; prognostic marker

1. Introduction

The discovery and study of the role of high-risk Human Papillomaviruses (HPVs) in oropharyngeal squamous cell carcinomas (OPSCCs) during the last 20 years have completely changed our understanding of a subset of these tumors and have supported the identification of HPV-positive OPSCC as a distinct clinical entity [1][2][3][4][5][6]. The knowledge accumulated during this timeframe has led to the recent change in the American Joint Committee on Cancer (AJCC) TNM (tumor, node, metastasis) staging system and is supposed to ultimately pave the way to deintensification protocols in a selected cohort of patients [7]. An unintended byproduct of the rise in HPV-positive OPSCCs has been the shedding of light on the elected surrogate marker p16^{INK4a} [8], which is well known to oncologists and clinicians dealing with cervical cancers and less known to otolaryngologists and researchers in the field of head and neck cancers. In fact, in both the aforementioned eighth edition of the AJCC TNM staging system and current major guidelines, p16^{INK4a} immunohistochemistry (p16 IHC) is considered the reference technique for the detection of HPV in the oropharynx [9][10]. However, this technique has some relevant limitations that have been extensively discussed elsewhere [11][12].

2. p16^{ink4a} in the Context of LSCC

Laryngeal squamous cell carcinoma is a common head and neck cancer. It affects men more commonly than women. Its known risk factors are mainly tobacco and alcohol consumption, but dietary and environmental factors may also be involved, and the potential role of laryngopharyngeal reflux has been discussed [13]. LSCC is one of the rare cancers with a decreased 5-year survival rate during the last decades [14], and, for this reason, prognostic and predictive markers for LSCC are particularly sought-after. While no prognostic markers are routinely used or recommended in clinical practice for LSCC, a number of potential prognostic markers have been proposed over time, and a comprehensive review has recently been published by Cavaliere and colleagues [15]. The role of HPV infection in cancerogenesis and as a prognostic biomarker is much more controversial, and although a small subset of LSCC may be related to HPV, its clinical and prognostic relevance is probably limited [14][16]. However, most of the available data on p16^{INK4a} expression in LSCC derive from the use of p16IHC as a common technique of choice to demonstrate HPV involvement, often dismissing the possibility of overexpression related to different mechanisms.

By analyzing the literature, two distinct phases of the research effort covering the role of p16^{ink4a} in laryngeal cancer can be identified. The first one, between the 1990s and the turn of 2000, was more focused on the role of P16 inactivation as a step toward cancer progression, and the second phase, which started around the mid-2000s, was more focused on the role of p16^{ink4a} as a surrogate marker of HPV infection and on the role of its overexpression. In fact, almost 30 years ago, in the wake of works defining the inactivation of p16^{ink4a} as a potential step towards cancerogenesis in some head and neck cancers [17][18], the first evidence of the loss of heterozygosity (LOH) at the 9p arm in a subset of laryngeal cancers in a variable percentage of LSCCs emerged [19][20]. Later, along with allelic deletions, both point mutations and promoter hypermethylation were described as relatively frequent events involved in a subset of LSCCs, and LOH in particular seemed to be associated with more advanced and metastatic cases [21]. During this first wave of relevant papers, enough

evidence was building around the role of the inactivation of p16^{ink4a} in head and neck cancers to suggest gene therapy as a potential treatment to be considered [22][23]. Notably, p16^{ink4a} inactivation seemed to be present in more than 50% of patients [24]. The role of the LOH and mutations in head and neck cancers was later confirmed via next-generation sequencing analysis [25][26]. It has been suggested that the length of exposure to tobacco and alcohol (but not the intensity) is associated with the homozygous deletion of p16 [27], and that a lack of dietary folates is associated with p16 methylation [28]. An association between weak p16^{ink4a} expression and advanced disease was later confirmed by other authors [24][29], while p16 point mutations were found to be independently associated with the risk of relapse and death in advanced LSCCs, albeit only in a small subset of them [30]. In contemporary papers, p16 anomalies were also found to be potentially associated with the tumor grade [31][32] and invasiveness and regional lymph node metastasis [33][34][35][36]. However, not all research groups looking for a prognostic impact of p16 inactivation found the same results, sometimes confirming a higher frequency of genetic anomalies in more advanced cases [24][37][38][39]. In later years, characterized by papers focused on the potential role of HPV in non-oropharyngeal head and neck cancers, some papers started to point to the fact that while a subset of laryngeal cancers indeed overexpressed p16^{ink4a} (range: 4.7% [40]–39.02% [41], according to the papers cited), this did not seem to reflect relevant HPV involvement in the cancerogenic process, but it was still associated with a trend towards better survival [40][42][43][44] and progression-free survival [40][43]. Relevant exceptions exist at both extremes. In a series of 123 glottic LSCCs, p16-positive cases had a significantly better 2-year disease-free survival and fewer nodal relapses [41]. Allegra et al. found a positive impact on the 5-year overall survival (OS) and disease-specific survival in primarily operated cases, along with fewer nodal metastases [45]. A similar result was found by analyzing 95 consecutive LSCCs treated with different modalities. Researchers found a positive impact on the relapse-free survival (RFS) for the whole series and a positive impact on the OS in primarily operated cases [46]. In a study involving 812 patients, Zhu and colleagues found that p16^{ink4a}-positive patients had better OS, disease-specific survival, and RFS [47]. Other studies failed to find any trends or significant correlations between the p16^{ink4a} status and prognosis. Young and colleagues did not find any impact on the 2-year OS or RFS in a cohort of 307 patients, and other groups reported similar results for smaller cohorts [48][49]. Lastly, the paper by Larque and colleagues is a relevant outlier, as they found a better prognosis in patients with a negative p16^{ink4a} status. Notably, they also looked for p16^{INK4a} mRNA expression and gene mutations that did not correlate well with p16^{INK4a} protein expression [50]. Two recent papers, one a propensity-scored analysis of the National Cancer Database for survival outcomes by high-risk Human Papillomavirus status in non-oropharyngeal head and neck squamous cell carcinomas [14], and the other a systematic review and meta-analysis of the survival outcomes in Human Papillomavirus-associated non-oropharyngeal squamous cell carcinomas [16], offer us a broad view of the issue and some interesting insights. Tian and colleagues included in their analysis a total of 4804 LSCC patients, and an HPV+ status was associated with better survival at 1, 2, and 5 years of follow-up. A relevant issue with this paper is that it is focused on the HPV status, meaning that some papers, albeit a minority, did not use p16 to assess the HPV status and thus their analyses do not fairly reflect the impact of p16^{INK4a} on the prognosis. This limitation is overcome in the systematic review and meta-analysis by Sahovaler and colleagues, as they decided to group studies according to their detection techniques. Their paper included 24 studies and 9793 laryngeal cancer patients, and the subgroup analysis showed a significant survival improvement for p16^{INK4a}-positive patients but not for HPV-DNA-positive patients.

What has been discussed up to this point has directly and indirectly highlighted some relevant problems that prevent us from fully understanding the impact of p16^{INK4a} on laryngeal cancer, and especially on its prognosis. As seen, few papers have been published on the matter, and they are often not focused on p16^{INK4a} but prevalently or solely on the role of HPV in laryngeal cancer [51][52]. Moreover, different “non oropharyngeal tumors” are often grouped together, determining a loss of precious data. The discussed papers analyze the impact of p16^{INK4a} on tumors of different stages and treated with different treatment modalities. This could be relevant, as all the papers discussing a significant impact on the prognosis were mainly based on patients treated with surgery as their primary option [41][45][46][47], and the papers that stratified according to the treatment modality found no impact [47] or a negative one [46] on patients treated with nonsurgical modalities. As seen, there are two strikingly distinct phases of the research efforts, one focused on p16^{INK4a} inactivation as a step of LSCC cancerogenesis with a potential negative impact on prognosis, and the other focused on p16^{INK4a} overexpression both as a marker of HPV involvement in LSCC cancerogenesis and as a potential positive prognostic marker in and of itself. The two conditions might very well coexist, and this fact should be reflected properly in future research efforts, as both possibilities should be sought while looking for the prognostic impact of p16^{INK4a} expression. One problem that is tightly linked to the last one is the cutoff chosen to determine p16^{INK4a} expression. The most relevant papers discussed herein use a wide range of cutoffs, including the following: undefined [43][44]; expression (undefined level) in both the nucleus and cytoplasm [40]; >70% diffuse staining (nuclear and cytoplasmic) [41][47][53]; nuclear staining scored with the intensity reactivity score (IRS) with various cutoffs [45][46]; strong and diffuse (>25%) cytoplasmic and/or nuclear staining [48]; an intensity score of 2 (moderate) or 3 (strong) in ≥30% of tumor cells [49]; nuclear and cytoplasmic staining in >50% of cells [42]; nuclear staining in >50% of cells [54]; strong and diffuse cytoplasmic and nuclear staining in

all basal and suprabasal cells [50]. This variability is detrimental to any attempt at a coherent analysis and interpretation of the data. It is not possible to tell by the state of the art how these different cutoffs affected the findings of the papers, or whether any of them correlate better with the clinicopathological characteristics or survival trends. Moreover, one thing to consider is the selection of a cutoff that reflects HPV positivity (a 70% cutoff with nuclear and cytoplasmic expression with at least moderate-to-strong intensity is recommended by the NCCN citing the guideline from the College of American Pathologists [55]), and another is a cutoff that reflects $p16^{INK4a}$ overexpression or inactivation when considering it as an independent marker. The matter is further complicated by early evidence that the staining pattern of $p16^{INK4a}$ might, in and of itself, be predictive of certain clinicopathological characteristics of the tumor [56][57]. Lazăr and colleagues [57] analyzed 88 cases of LSCCs looking for different patterns of the distribution/intensity of the staining and their respective correlations with the clinicopathological characteristics. They found that different patterns were associated with different levels of nodal involvement. A similar observation was made by Zhao and colleagues [56]. Future research focusing on $p16^{INK4a}$ as a prognostic marker will need to properly assess the ideal cutoffs and analyze different staining patterns and their respective associations with the clinicopathological characteristics and survival outcomes.

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