Oral Oncology xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Oral Oncology

journal homepage: www.elsevier.com/locate/oraloncology

Review

Do high-risk human papillomaviruses cause oral cavity squamous cell carcinoma?

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ARTICLE INFO

Article history:
Received 7 October 2014
Received in revised form 16 November 2014
Accepted 19 November 2014
Available online xxxx

Keywords:
Oral/oropharyngeal/oral cavity/head and neck cancer
HPV
Young patients
Non-drinkers/non-smokers

SUMMARY

High-risk human papillomaviruses (HR-HPV) are an established etiologic factor for a growing number of oropharyngeal cancers. However, their potential role in other upper aerodigestive tract locations is still a matter of debate, particularly in the oral cavity. This is of paramount importance as in the future diagnosis, treatment and follow up in head and neck squamous cell carcinoma may vary according to HPV status. This article reviews the recent published data and highlights some of the pitfalls that have hampered the accurate assessment of HR-HPV oncological role outside the oropharynx. We demonstrate that, in contrast to the oropharynx, only a small fraction of cancers located in the oral cavity seem to be HPV-related even in young non-smoking non-drinking patients. We emphasize several relevant factors to consider in assumed HPV-induced oral cavity cancers and discuss the current theories that explain why HPV-induced cancers arise preferentially in the oropharynx.

Introduction

Oral squamous cell carcinomas are a major global health issue. These tumors represent a heterogeneous group affecting the oral cavity and oropharynx. Tobacco, alcohol and betel quid abuse are the traditional risk factors. Their annual estimated incidence is around 275,000 for oral cavity and 85,000 for oropharyngeal cancers [1,2]. There is wide geographical variation in the incidence of these cancers and the highest rates of oral cavity squamous cell carcinoma (OCSCC) are found in the Indian subcontinent (i.e. India, Pakistan, Sri Lanka, Bangladesh, etc.). In these countries, OCSCC is the most common cancer in men and may contribute up to 25% of all new cancer cases [3]. In Western countries, as tobacco consumption drops, the incidence of OCSCCs and more generally of head and neck squamous cell carcinomas (HNSCC) is stabilizing or falling [4]. However, amongst these tumors those arising in the oropharynx are on the increase. This epidemiologic change has been attributed to high-risk human papillomavirus (HPV) and particularly to type 16, which is now recognized as a causative agent in a growing subset of oropharyngeal squamous cell carcinomas (OPSCCs) [5]. Indeed, numerous studies have demonstrated a two to three fold increase in the prevalence of HPV-driven OPSCC over the last three decades, especially in North America and Northern Europe [6–8]. The underlying reasons are still poorly understood and several hypotheses have been proposed: changes in sexual behavior [9], decreased rates of tonsillectomy performed in the pediatric population since the 70s [10] and progress in the diagnostic work up and HPV testing assays [11]. Considering current trends, it is estimated that high-risk HPV (HR-HPV) will become the dominant etiologic factor for OPSCC, in the coming decades, in most Western countries [6]. These tumors have distinct epidemiologic features and oncogenic mechanisms that differ from their HPV-negative counterparts [12,13]. Additionally, their prognosis is much more favorable which has led the medical community to consider new treatment strategies. Indeed, it is possible that less intensive treatment regimens could achieve similar efficacy with less toxicity and improved quality of life [14]. In spite of considerable advances in the understanding of these tumors, numerous issues are still unresolved, particularly the potential role of HR-HPV in oral cavity carcinogenesis [15]. Many studies, using a variety of techniques, have demonstrated the presence of the HPV genome in OCSCCs. However, compared to the evidence of a link between HR-HPV and oropharyngeal cancers, the role of HR-HPV

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http://dx.doi.org/10.1016/j.oraloncology.2014.11.011
1368-8375/

in oral cavity cancers remains uncertain. This issue is of paramount importance, as in the coming years, diagnosis, treatment and follow up in HNSCC may vary according to HPV status. This article reviews the published data on the potential role of HR-HPV in OCSCCs.

Oral cavity squamous cell carcinoma and HR-HPV infection

Many studies [16–29] have identified a high proportion of OCSCC with detectable HPV DNA (Table 1). In a recent systematic review, summarizing 60 publications on 4195 patients with OCSCC, Isayeva et al. [30] found that 705 (16.8%) of these tumors contained HPV DNA especially the HPV16 genotype. The rates of HPV-positive cancers ranged from 0 to 94.7% in the studies that were analyzed and the weighted prevalence was 20.2% (95% CI 16–25.2%). These findings are mirrored by Kreimer et al. [31] and Termine et al. [32] who performed similar work based respectively on 35 studies enrolling 2642 patients (cumulative pooled prevalence of HPV DNA detection: 23.5%, (95% CI 21.9, 25.1)) and 47 studies on 4852 patients (cumulative pooled prevalence of HPV DNA detection: 39.9%, (95% CI 30.2, 49.8)). All the studies that were analyzed in these three systematic reviews used PCR-based tests to identify HPV DNA (end point PCR or quantitative PCR).

However, most of the studies, that have assessed the prevalence of HPV DNA in OCSCC, have limitations that must be addressed. Firstly, it is important to highlight that despite widespread use of successive versions of the World Health Organization's International Classification of Diseases (ICD) and the Classification of Diseases for oncology (ICD-O), the literature in this field has either suffered from significant site misclassification or has lacked adequate site separation to allow accurate conclusions to be drawn. For instance, some studies have grouped base of tongue cancers with mobile tongue cancers as OCSCC even though the base of tongue belongs to the oropharynx subsite [33]. Such confusion has probably falsely increased the rate of HPV-DNA identification in OCSCC. Most recent publications have resolved this problem, but these issues should be considered when reviewing and interpreting existing data. Secondarily, the use of PCR-based techniques to identify HPV DNA leads to several problems. PCR is a very sensitive tool, but it can provide false positive results as it lacks specificity. Despite the use of stringent procedures and proper controls, previously amplified material can potentially contaminate negative specimens. Moreover, there is no tissue context. The result is either positive or negative and it is not possible to determine if viral DNA arises from the population of cancer cells, or the surrounding non-neoplastic tissue. Indeed, analyses of healthy oral and oropharyngeal mucosa have shown HPV infection in at least 5–15% of cases, but rates exceeding 50% have also been reported [34]. Finally, even if the viral DNA that was amplified and detected comes from the tumor itself, this does not necessarily support a causal role in tumorigenesis, which is the key question. HPV DNA detection by PCR does not distinguish whether HPV is actively involved in carcinogenesis (driver infection) or not (passenger/bystander infection). Consequently, molecular markers of HPV oncogenic activity are necessary to consider causality. Expression of E6 and E7 viral oncogenes is considered the gold standard for clinically relevant HPV infection [21,35]. The main factors supporting this concept are:

- E6 and E7 viral oncogenes, by inhibiting TP53 and pRb respectively, play a key role in the abrogation of cell cycle control, apoptosis and promotion of genetic instability that contributes to the development of cancer (Fig. 1) [36].
- In vitro studies have demonstrated that their expression induces keratinocytes immortalisation and that their inhibition, in HPV-induced cancer cell lines, results in the loss of the transformed phenotype [37,38].
- Finally, several authors have demonstrated that only tumors containing transcriptionally active HPV (E6/E7 mRNA) represent a specific subgroup. These tumors are characterized by the absence of TP53 mutation [39], a significantly decreased number of chromosomal abnormalities [12], and a specific gene expression profile when compared to HPV-negative and transcriptionally inactive HPV-positive OPSCC [40] (those tumors that contain HPV DNA but do not express viral oncogenes). Additionally, survival analyses have shown that among HPV-positive OPSCC, only transcriptionally active tumors have significantly better survival. Transcriptionally inactive and HPV-negative tumors have poorer survival [40].

Therefore, studies based upon HPV DNA detection by PCR, solely, overestimate the fraction of OCSCC that are potentially HPV-induced. This point was previously well described in OPSCC in which up to 50% of HPV DNA-positive cancers are negative for E6/7 mRNA expression [12,41].

Table 1
HPV DNA detection frequencies in oral cavity squamous cell carcinomas.

Author	Year, country	Sample	Method (primer sets)	n° OCC	n° HPV + OCC
Gillison et al. [16]	2000, USA	Frozen	PCR (MY09/MY11, HMB01 HPV16/18 E7 specific primers)	84	10 (11.9%)
Ragin et al. [17]	2006, USA	FFPE	PCR (PGMY09/MY11, GP5+/6+)	91	18 (19.7%)
Smith et al. [18]	2012, USA	FFPE	PCR (MY09/MY11, GP5+/GP6+)	145	19 (13.1%)
Sharma et al. [19]	2012, USA	Fresh	PCR (PGMY09/11, E6 specific primers)	166	22 (13%)
Klussmann et al. [20]	2001, Germany	Frozen	PCR (consensus primers, HPV16 specific primers)	22	4 (18.2%)
Smeets et al. [21]	2007, Netherlands	Frozen	PCR (GP5+/GP6+)	30	9 (30%)
St Guily et al. [22]	2011, France	FFPE	PCR (SPF10)	209	22 (10.5%)
Rautava et al. [23]	2012, Finland	Frozen	PCR, (MY09/MY11, GP5+/GP6+)	37	28 (75%)
Laco et al. [24]	2012, Czech republic	FFPE	PCR (GP5+/GP6+, PGMY09/11)	48	7 (14.5%)
Ribeiro et al. [25]	2011, multiple countries	Frozen	PCR (PGMY09/11)	132	0 (0%)
Deng et al. [26]	2012, Japan	Frozen	PCR (GP5+/GP6+, MY09/11)	31	10 (30%)
Barwad et al. [27]	2011, India		PCR (MY09/MY11)	34	16 (47.1%)
Kaminagakura et al. [28]	2011, Brazil	FFPE	PCR (GP5+/GP6+)	114	22(19.3%)
Chang et al. [29]*	2013, USA	FFPE	Wide spectrum ISH	126	3 (3.2%)

PCR: polymerase chain reaction, and ISH: in situ hybridization.
PGMY09/11 (a modified version of MY09/11), GP5+/GP6+, and SPF10 LiPA are consensus primer sets that are designed to bind to highly conserved regions, of varying length within the HPV L1 gene, allowing simultaneous identification of a large range of HPV types. Samples genotyping is performed after DNA amplification by several methods including hybridization with dedicated probes and pyrosequencing.
* In the study of Chang et al. [29], HPV status was assessed only in 93 patients (these patients were all affected by pT1–2 oral tongue squamous cell carcinoma, thus minimizing the issue of site misclassification). ISH was performed using probes targeting 37 distinct HPV subtypes, including 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, and 52.

Please cite this article in press as: Mirghani H et al. Do high-risk human papillomaviruses cause oral cavity squamous cell carcinoma? Oral Oncol (2014), http://dx.doi.org/10.1016/j.oraloncology.2014.11.011