HPV and cancer of the oral cavity

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Abbreviations: HPV, human papillomavirus; HNSCC, head and neck squamous cell carcinoma; OPSCC, oropharyngeal squamous cell carcinoma; OSCC, oral squamous cell carcinoma.

Increased awareness of human papillomavirus (HPV) as an etiological cause of head and neck squamous cell carcinoma has increased the interest in analysis of distinct oral sub-sites. It is currently under debate, whether HPV plays a role in the development of squamous cell carcinoma of the oral cavity (OSCC). The weakness in most published studies is the lack of performing different HPV detection tests combined with analysis for biological activity of the virus. In addition, different sub-sites of the oral cavity had been combined to a single entity, which retrospectively leads to a highly heterogeneous basis of data. In this review we mainly discuss the unclear role of HPV in OSCC development.

HPV Positivity in Head and Neck Cancers

Squamous cell carcinoma of the head and neck (HNSCC) is an anatomically heterogeneous group of neoplasms arising from the mucosal surface of the oral cavity, oropharynx, hypopharynx, larynx and nasopharynx. Each year approximately 263,000 cases of oral cavity cancer and 135,000 cases of pharyngeal cancer are diagnosed worldwide. Recent epidemiological work suggests considerable differences between HNSCC related to tumor sub-site. Unfortunately, a shortcoming in many publications is the fact that a simplified grouping of cancers of different head and neck regions as a single entity is retrospectively leading to a highly heterogeneous basis of data. Therefore, important distinctions between anatomic sub-sites and their natural histories have not been attributed in detail. Over the last decade it has become clear that human papillomaviruses (HPV) not only cause genital oncoproteins E6 and E7. These proteins are the key drivers of tumourigenesis by inactivating 2 of the most important tumor suppressors, pRb and p53. In premalignant and malignant lesions, E2 function gets abrogated, which subsequently leads to higher expression levels of E6 and E7. The inhibition of the tumor suppressor proteins p53 and pRb alters cell cycle pathways regulating cellular proliferation, apoptosis, as well as genetic stability, which can lead to the formation of epithelial lesions. The binding of high-risk HPV E7 protein with pRb results in the release of the transcription factor E2F from the pRb-E2F protein complex and the promotion of cell cycle progression and also leads to the release of the p16INK4A gene from its transcriptional inhibition. As a consequence, p16INK4A protein is expressed at a high level and is thus considered as a reliable surrogate marker for high-risk HPV infection.

Oncogenic Function of HPV

HPV is a small DNA virus with a specific tropism for squamous epithelia. To date, 202 different HPV types have been isolated (International HPV Reference Center; http://ki.se/en/labor med/international-hpv-reference-center) and HPV types infecting the mucosa are further classified into high- and low-risk groups based on the relative malignant potential of the lesions they cause. Whereas low-risk HPVs, such as HPV6 and HPV11, cause benign warts, high-risk HPV, such as HPV16 and HPV18, cause premalignant squamous intraepithelial neoplasias that can progress to cancer. In a persistent infection the viral E2 protein is tightly controlling the expression of the main viral oncoproteins E6 and E7. These proteins are the key drivers of tumourigenesis by inactivating 2 of the most important tumor suppressors, pRb and p53. In premalignant and malignant lesions, E2 function gets abrogated, which subsequently leads to higher expression levels of E6 and E7. The inhibition of the tumor suppressor proteins p53 and pRb alters cell cycle pathways regulating cellular proliferation, apoptosis, as well as genetic stability, which can lead to the formation of epithelial lesions.

HPV Infection of the Oral Cavity

While HPV is an important cause of OPSCC, it is currently unclear whether HPV may also have a role in other HNSCC sub-sites. It has been hypothesized in 1983 and since then is still debated. The generally accepted risk factors for cancers of the oral cavity (including tumors of the tongue, floor of the mouth, gingiva, gum, palate, lip mucosa and other sites of the mouth) are tobacco habits. Recent publications showed an increased incidence of HPV infections in HNSCC of approximately 50% with HPV16 being the most prevalent type in at least 90% of this cancer. Whereas the majority of HPV-driven cancers of the head and neck are oropharyngeal squamous cell carcinoma (OPSCC) comprising the tonsils and base of the tongue, it is currently in debate whether HPV may also have a role in other HNSCC sub-sites.
| Reference            | Article type | No. of OSCC | OSCC sub-sites and site-specific HPV-positivity | HPV detection methods | Overall HPV DNA positivity | Concordance between HPV DNA positivity and other methods | Cancers of other sites tested |
|---------------------|--------------|-------------|-----------------------------------------------|-----------------------|--------------------------|------------------------------------------------------|-----------------------------|
| Syrjänen 2011       | Review       | 1885        | Lip mucosa (66.7%)                             | GP5+ / GP6+ PCR, type specific E6/E7 PCR, P16INK4A / p53 / EGFR IHC, ISH | OR = 3.98; 95% CI: 2.62 - 6.02 WP = 20.2%; 95% CI 16.0% - 25.2% | No                      | No larynx, sinonasal tract, nasopharynx              |
| Isayeva et al., 2012 | Review       | 4195        | WP D                                            | GP5+ / GP6+ PCR, type specific E6/E7 PCR, P16INK4A / p53 / EGFR IHC, ISH | 44% | No                      | No                                          |
| Durya et al., 2012  | Research article | 162         | Lip mucosa (66.7%)                             | GP5+ / GP6+ PCR, type specific E6/E7 PCR, P16INK4A / p53 / EGFR IHC, ISH | 44% | No                      | No                                          |
| Duncan et al., 2013 | Research article | 81          | Lip mucosa (25.0%)                             | PCR, p16INK4A IHC     | 8.6% | Yes                     | No                                          |
| Lingen et al., 2013 | Research article | 409         | Lip mucosa (25.0%)                             | SPF10 PCR, TaqMan qRT-PCR, ISH p16INK4A IHC | 5.9%; 95% CI 3.6 - 8.2 | No                      | No                                          |
| Reuschenbach et al., 2013 | Research article | 275         | Lip mucosa (25.0%)                             | PCR-EIA, ISH p16INK4A IHC | 25.1% | No                      | No                                          |
| Walline et al., 2013 | Research article | 104         | Lip mucosa (25.0%)                             | PCR Mass-array, ISH, p16INK4A IHC, p16INK4A IHC, ISH | 9.6% | No                      | Oropharynx, Nasopharynx                        |
| Chung et al., 2014  | Research article | 89          | Lip mucosa (25.0%)                             | p16INK4A IHC, ISH     | 14.6% | No                      | Oropharynx, Nasopharynx                        |
| Upile et al., 2014  | Research article | 102         | Lip mucosa (25.0%)                             | HPV16-E6 qPCR         | 4%   | No                      | Oropharynx, Nasopharynx                        |

WP = weighted prevalence, OR = odds ratio; CI = confidence interval
ISH = in situ hybridization, IHC = Immunohistochemistry, EIA= enzyme linked immuno assay
EGFR: epidermal growth factor receptor
n.d. = not described
smoking, and betel quid chewing and alcoholic beverage drinking. The healthy adult population shows a prevalence for any HPV type of 2–8% in the oral cavity, with HPV16 being the most commonly identified type. Notably, the prevalence of HPV seems to be significantly higher in men than in women. Furthermore, high-risk sexual behavior including oral-genital sex has been found to be associated with transmission of HPV infections between oral and genital sites. Immunodeficiency (e.g., HIV infection) and smoking seem to increase the risk for oral HPV infection, i.e., make infections more likely to persist. Initial studies suggest that most oral HPV infections are likely to be cleared within a year. Thus, persistence of the virus might be the critical factor for the development of HPV-related diseases.

Subclinical and Premalignant oral HPV Infection

In other parts of the body, such as the genital tract, HPV infects exclusively the basal cells of the epithelium, where the virus can remain latent. There is evidence that HPV also infects gingival tissue. The periodontal pocket is the only location of the gingival mucosa where basal cells are exposed to the environment. The periodontal pocket enlarges during progression of periodontitis as a result of chronic inflammatory processes. In the presence of chronic inflammation, increased basal cell proliferation leads to higher viral load in saliva as well as higher risk of HPV transmission. In a recent hospital-based case-control study with histologically confirmed HNSCC cases, periodontitis was associated with more than 4-fold increased odds of HNSCC. The strength of association was greatest in the oral cavity, followed by oropharynx and larynx. This led to the hypothesis that chronic inflammation and continuous epithelial proliferation in the junctional gingiva could favor the replication of HPV and might be an important reservoir for HPV in the oral mucosa.

In (potentially) premalignant oral lesions an increased HPV DNA positivity of 20–64% was described. A recent report by McCord et al. (2013) combining in situ hybridization and immunohistochemical staining against p16INK4A, indicating biologically active viral infection, revealed 17.5% positivity for high-risk HPVs in oral epithelial dysplasias (for discussion of p16INK4A immunohistochemistry see below). The presence of HPV seems to be significantly higher in men than in women. Furthermore, high-risk sexual behavior including oral-genital sex has been found to be associated with transmission of HPV infections between oral and genital sites. Immunodeficiency (e.g., HIV infection) and smoking seem to increase the risk for oral HPV infection, i.e., make infections more likely to persist. Initial studies suggest that most oral HPV infections are likely to be cleared within a year. Thus, persistence of the virus might be the critical factor for the development of HPV-related diseases.

Does HPV Play a Causal Role in OSCC Development?

In contrast to the clear picture in OPSCC, where the prognostic relevance of biologically active HPV infection is established, no such clear association can be found for OSCC. Contradictory studies exist, which are either in favor of a better survival probability for HPV-positive OSCC patients or of worse outcome, as well as supporting no effect on patient survival. A number of publications analyzing larger cohorts underline that HPV DNA and especially HPV16 is present in 10–25% of tumors of the oral cavity, which is higher than in the healthy control population but smaller than in OPSCC. In OSCC, HPV16 is followed less frequently by HPV18 (including occasional co-infections with HPV16). Types HPV31, HPV33 and other high-risk types are rarely found. However, in this respect it should be noted that several studies only tested for selected types namely HPV16 and HPV18 or used more general detection methods not determining the exact type. Beside the general term ‘oral cavity’ used in several studies, it is difficult to estimate the specific prevalence of HPV DNA for the individual sub-sites. Comprehensive studies testing larger numbers of patients and describing anatomic sub-sites as well as HPV DNA frequency per site indicate that HPV can be found in any of the sub-sites, (Table 1). Frequencies are varying between studies, but floor of mouth (9–42%) and the tongue (8–25%) seem to be predominantly infected by HPV. Whereas HPV DNA is present in a reasonable subgroup of OSCC, it has been shown that not all of HPV DNA positive tumors can be regarded as etiologically HPV-driven. OPSCC are in part truly HPV associated since active high-risk HPV-infection goes along with E6/E7 expression leading to deregulation of especially p53 / pRb and p16INK4A overexpression. Beside the fact that HPV DNA can be detected in a subset of OSCC, a series of recent reports presented clear data showing a discrepancy in HPV DNA positivity and oncogene activity. Studies analyzing expression of E6/E7 in HPV DNA positive OSCC could detect expression of viral oncoproteins in only 6–7% of cases, supporting the assumption that HPV is not biologically active in the majority of OSCC. In addition, most HPV-positive OSCC are negative for p16INK4A overexpression and enhanced p16INK4A levels can also be found in HPV-negative tumors. Moreover, there are HPV/p16INK4A positive tumors, in which viral oncogene expression could not be determined. Apparently, p16INK4A levels might not contribute to decipher active HPV status and other mechanisms controlling p16INK4A expression in this tumor entity might exist including mutations, deletion or methylation of CDKN2A (gene coding for p16INK4A). Results from the cervix uteri show that HPV-driven malignant transformation occurs at a distinct cell population of junctional cells harbouring a unique gene-expression profile that differs from squamous and columnar cells. It is tempting to speculate that similar gene expression profiles also exist at other sites where transition of squamous epithelium into glandular or reticular epithelium occurs (e.g., the oropharynx) and that these conditions influence HPV oncogene expression and rarely promote malignant transformation.

Conclusion

Several studies showed that HPV DNA is present in a considerable number of OSCC but still represents a distinct clinical entity with many unresolved issues. The lack of a clear molecular evidence raises the question if HPV DNA-positive OSCC are HPV-driven, since the presence of HPV-DNA does not mean the presence of a biologically active HPV per se. In spite of the fact that OSCC is the most common type of oral cancer representing approximately 90% of malignant tumors in this site, also other tumor entities exist in the oral cavity. Among the non-SCC are adenocarcinoma, adenoid cystic carcinoma.
from major or minor salivary gland, Kaposis Sarcoma, lymphoma, malignant melanoma and metastatic cancers from other head and neck sites. As discussed for nasopharyngeal carcinoma, malignant melanoma and metastatic cancers from major or minor salivary gland, Kaposi Sarcoma, lymphomas, a distinct subset of truly HPV-driven OSCC might emerge. Otolaryngol Clin North Am 2013; 46:567-20; PMID:23910467; http://dx.doi.org/10.1016/j.otc.2013.05.001.

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