Review Article :

Human Papilloma Virus in Oropharyngeal Carcinoma

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Abstract

Oropharyngeal cancer, the second most common of head and neck cancer patients in Siriraj hospital, is a challenging disease in head and neck squamous cell carcinoma (HNSCC). Although tobacco use is known the predominant risk factor in oropharyngeal oncogenesis. A very interesting etiological linkage between the human papillomavirus (HPV) and a subgroup of HNSCC has recently been established. At this time, in the year of 2011, we have more and more scientific data to support that tumor HPV status is a paramount prognostic biomarker for head and neck cancers in terms of tumor control, disease free survival and overall survival. In other words, the group of HPV positive patients has definitely better prognosis. In the near future, results from clinical trials on oropharyngeal cancer will inform us the better way how to treat these groups of patients without any compromise in survival and less toxicity.

Human Papilloma Virus

In 1935, the existence of papilloma viruses was first demonstrated by Shope[1], who used an ultrafiltrate of warts from rabbits to discover them. Later, papilloma viruses with an epithelial tropism have been demonstrated in humans that were detected in an invasive squamous cell carcinoma of head and neck by Southern blot hybridization in the first time[2]. Human Papilloma Virus (HPV), which is a member of the papillomavirus family of viruses that is capable of infecting humans, are encapsulated DNA viruses containing a double-stranded DNA genome of approximately 8,000 base pairs in size. After infecting a suitable epithelium, viral DNA replication takes place in the basal cells of the epidermis, where the HPV genome is stably retained in multiple copies, guaranteeing its persistence in the epithelium's proliferative cells. Some types of HPV don't affect humans, while other types can cause infection and cancer. Transcription from the HPV genome occurs in two waves: an early phase with seven to eight gene products (E1-E8) and a late phase with two gene products (L1-L2) (As Figure 1). The early phase of HPV infection plays a key role in the E5 oncoprotein. However, the ability of different high-risk HPVs to transform human epithelia has been primarily associated with the expression of two specific viral gene products, E6 and E7[3,4]. The E6 oncoprotein causes degradation of tumor suppressor protein p53, whereas the E7 oncoprotein results in loss of retinoblastoma (Rb) tumor suppressor protein. Thus, the E6 and E7 oncoproteins are the specific HPV-induced carcinogenesis that promotes tumor growth and malignant transformation[5,6].

Incidence of HPV in Oropharyngeal Cancer

The incidence of oropharyngeal cancer patients in 2008 was 80 patients who came to Siriraj Hospital, which was the second most common in head and

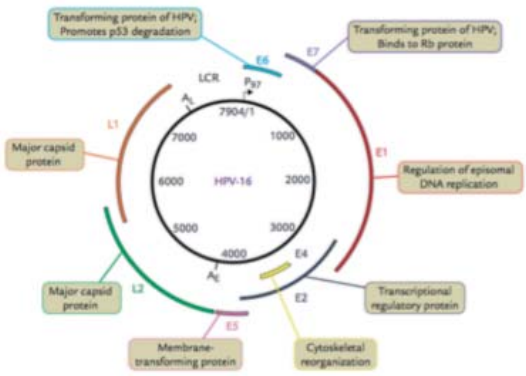

Figure 1 : Genome organization of human papillomavirus 16. (E1-E7 early genes, L1-L2 late genes : capsid)[4]

neck cancer in our hospital[7]. The Surveillance, Epidemiology, and End Results (SEER) database by Shiboski et al., studied the incidence of oral carcinoma among young adults that has been reported in the United States and Europe from 1973 to 2001. They found that there was a statistically significant increase in the incidence of oral tongue squamous cell carcinoma, base of tongue squamous cell carcinoma, and palatine tonsil squamous cell carcinoma (Annual percentage change = +2.1, +1.7, and +3.9, respectively)[8]. Thus, oropharyngeal cancer is a major cancer disease in head and neck squamous cell carcinoma (HNSCC), which is a disease largely attributed to the usage and exposure of tobacco and the consumption of alcohol[9,10]. However, Gillison ML, et al.[11] presented that a small number of HNSCCs, about 15-20%, demonstrates the presence of other risk factors. They hypothesized that HPV may be an etiologic agent for HNSCC; thus, they reviewed tumor tissues from 253 patients with newly diagnosed or recurrent HNSCC by testing for the presence of HPV genome with the usage of polymerase chain reaction (PCR)-based assays, Southern blot hybridization, and in situ hybridization. The result showed that HPV genomic DNA was detected in 62 (25%) of 253 cases (95% confidence interval [CI] = 19%–30%) and high- risk, tumorigenic type HPV16 was identified in 90% of the HPV-positive tumors. Moreover, poor tumor grade (odds ratio [OR] = 2.4; 95% CI = 1.2– 4.9) and oropharyngeal site (OR = 6.2; 95% CI = 3.1–12.1) independently increased the probability of HPV presence (As Table 1). They presented that HPV type 16 was the majority type in HPV positive group of oropharyngeal cancer patients. Nonsmokers were more frequent in the HPV-positive group (21%) than in the HPV-negative oropharynx group (4%), but this difference was not statistically significant.

Characteristic of HPV in Orophayrngeal cancer

Weinberger PM, et al.[12] proposed three-class models for oropharyngeal carcinogenesis. In the first model (class I), an excess alcohol or tobacco exposure induces mutations or epigenetic inactivations of p53, p16 and retinoblastoma (Rb) in a multistep progression that causes squamous cell carcinoma of the head and neck, which is called classic oncogenic. The second

Table 1 : Characteristics of the head neck cancer study population grouped by HPV status (adopted from Gillison ML, et al.[11])

Characteristic	HPV-positive group (n = 62 patients)	HPV-negative group (n = 191 patients)	Unadjusted OR (95% CI)
Primary site			
Oral cavity	10 (16%)	74 (39%)	1.0 (referent)
Oropharynx	34 (55%)	26 (14%)	9.7 (4.2-22)
Hypopharynx	2 (3%)	19 (10%)	0.78 (0.16-3.8)
Larynx	16 (26%)	70 (37%)	1.7 (0.72-4.0)
Nasopharynx	0 (0%)	2 (1%)	-
Tumor grade			
Well	8 (13%)	33 (17%)	1.0 (referent)
Moderate	26 (42%)	115 (60%)	0.93 (0.39-2.3)
Poor	27 (44%)	32 (17%)	3.5 (1.4-8.8)
Unknown	1 (2%)	11 (6%)	-
Multivariate analysis	Adjusted OR	95% CI	*p* value
Oropharynx	6.2	3.1-12.1	<0.001
Poor tumor grade	2.4	1.2-4.9	0.01

model (class II) describes a novel class, possibly formed when tobacco/alcohol-related tumors are subsequently infected by high risk HPVs, which was multifactorial in origin. In the third model (class III), high-risk human papillomavirus (HPV) may also lead to tumor formation, in a similar fashion to its role in cervical cancer, which oncogenic HPV E6 and E7 proteins act to inactivate p53 and Rb pathways, with subsequent upregulation of p16 expression through loss of feedback inhibition. (As Figure 2)

Moreover, Pernille Lassan[13] concluded the characteristics of HPV-positive and HPV-negative HNSCC on a molecular, epidemiological, and clinical basis (As Table 2). However, there are some issues that still are controversial such as smoke. Kumar B, et al.[14] suggested that smoking was not a significant prognostic factor after accounting for HPV status, whereas another found HPV status had a minimal effect on survival outcomes among smokers, but the smoking status had an important impact among HPV-positives and concluded that HPV-positive nonsmokers had the best outcome[15].

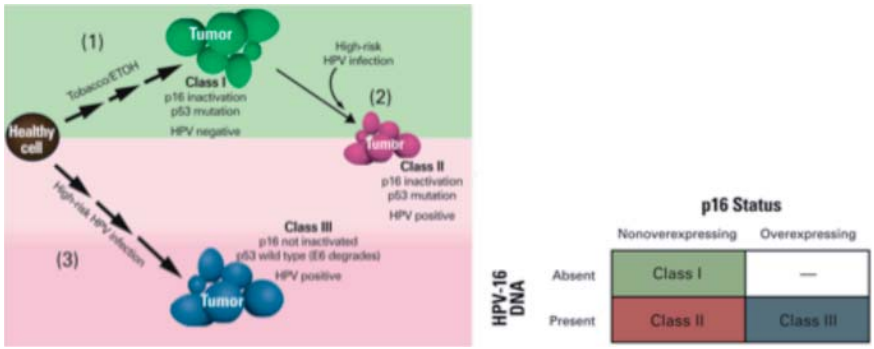

Figure 2 : three-class model for oropharyngeal carcinogenesis [12]

Table 2 : Distinct differences between HPV negative and positive HNSCC[13]

	HPV-positive	HPV-negative
Clinical factors		
Anatomic site	Predominantly oropharynx	All sites
Pathology	Poorly differentiated	All types
Age	Younger	Older
Performance status	Good	Poor
T-classification	T1–2	All
N-classification	N+	All
Stage	III–IV	All
Prognosis	Improved	Worse
Epidemiological factors		
Tobacco use	Never smoker	Heavy smoking
Alcohol use	Mild/moderate	Heavy
Sexual behaviour	Associated	Not associated
Incidence	Increasing	Decreasing
Molecular biology		
p53 mutations	Infrequent	Common
p16 expression	High	Low
High EGFR expression	Infrequent	Common

Specific characteristics of head and neck cancer patients who have HPV+ are described scientifically, such as younger age (5-10 year, compared with HPV-), anatomic sites as tonsil and base of tongue, histology as poorly differentiated nonkeratinizing with basaloid features, and high number of vaginal and oral sex partners.

HPV as Prognostic Factor in Oropharyngeal Cancer

There were many studies that presented that HPV was a prognostic factor for treatment in oropharyngeal cancer. The Gillison ML, et al.[11], demonstrated that the HPV positive group showed better improvement than the HPV negative group in disease specific free survival (hazard ratio [HR] = 0.26; 95% CI = 0.07–0.98). In addition, the authors adjusted the data that showed the presence of lymph node disease (HR = 2.3; 95% CI = 1.4– 3.8), heavy alcohol consumption (HR = 2.6; 95% CI = 1.4–4.7), and age greater than 60 years (HR = 1.4; 95% CI = 0.8–2.3), all patients with HPV-positive tumors had a 59% reduction in risk of death from cancer when compared with HPV-negative HNSCC patients (HR = 0.41; 95% CI = 0.20–0.88). Thus, they concluded that there is a high probability that HPV-positive oropharyngeal cancer is associated with HPV infection and has a markedly improve prognosis.

In 2007, Ragin CC, et al.[16] reviewed many case-series as a recent meta-analysis, the reduction in risk of death of patients with HPV-positive head and neck squamous cell carcinomas was 18% (hazard ratio (HR) 0.85, 95% confidence interval (CI) 0.7–1.0) and the reduction in risk of disease failure was 38% (HR 0.62, 95% CI 0.5–0.8) when compared to the HPV-negative patient. Especially in oropharyngeal cancers, they showed that HPV-positive have a 28% (HR 0.72, 95% CI 0.5–1.0) reduced risk of death and a 49% (HR 0.51, 95% CI 0.4–0.7) reduced risk of disease-failure when compared to patients with HPV-negative oropharyngeal cancers. However, this meta-analysis had some limitations, as the estimates were derived from un- adjusted hazards and were not based on individual patient data.

Last year, 2010, Dayyani et al.[17] included 5681 patients from 34 published articles in meta-analysis. The hazard ration for death in HPV+ patients was 0.42 (95%CI = 0.27 to 0.57). Other than prognosis and treatment response, the authors also proved that the HPV16+ patients, compared with HPV16- patients, has higher risk to develop head and neck cancer (Odds Ratio = 4.44 with 95% CI = 2.87 to 6.02)

In 2008, a phase II trial of investigational therapy in patients with oropharyngeal and laryngeal cancers, ECOG 2399[18] was established. In this trial, patients were treated with paclitaxel and carboplatin induction followed by radiation concurrently administered with weekly paclitaxel. There was 40% HPV-positive of all cancers and 63% HPV-positive of oropharyngeal cancers via a combination of HPV in situ hybridization and polymerase chain reaction (PCR). After a median survival of 39 months, patients with HPV- positive tumors had an improved overall survival and after adjustment for age, tumor stage and ECOG performance status, had a 73% (HR 0.27, 95% CI 0.10–0.75) reduction in risk of progression and 64% (HR 0.36, 95% CI 0.15–0.85) reduction in risk of death when compared to the HPV-negative patients.

From a recent analysis of over 47,000 incident cases of oral cancer reported to SEER[19] program of the National Cancer Institute, USA. In the US, during the period from 1973 through to 2003, the incidence rate for cancers at sites etiologically related to HPV infection significantly increased, whereas significant declines in incidence were observed for oral cancers not etiologically related to HPV. In addition, they presented that there was a significant improvement in absolute 2-year overall survivals of patients treated with radiation therapy with local (~10.1 versus 5.3%) or regionally (~23.1 versus 3.1% increase) advanced HPV-related cancers, but not for patients with HPV-unrelated cancers, respectively.

In 2010, Lassen P, et al.[13] reviewed and provided a summary of the current understanding of the role of HPV in head and neck cancer. A researcher found that there were five studies about the effect of tumor HPV status on response to conventionally fractionated radiotherapy. These studies showed that there were about 15-50% HPV-positive of oropharyngeal cancers in each study and most studies established that HPV-positive tumor patients had a significant improvement in local control, disease free survival, and overall survival. Moreover, one study demonstrated that p16 expression was the strongest independent determinant of them,

too. In addition, this study focused on the effect of HPV on accelerated fractionated radiotherapy in HNSCC. This study concluded two studies (DAHANCA 6 and 7[20], and RTOG 129[21]). DAHANCA 6 and 7 established that tumor p16 status was the strong independent prognostic impact of HPV- associated p16 expression on outcome of radiotherapy in terms of tumor control, disease specific survival, and overall survival. However, RTOG 129 showed a statistical significance in HPV-positive when compared with HPV-negative tumors in terms of overall survival and progression-free survival (3-year rate: overall survival = 82.4% vs 57.1%, progression-free survival = 73.3% vs 43.3%). In concurrent chemoradiotherapy (CCRT) in HNSCC, there were seven studies that were included in this study. The result of this study showed that tumor HPV status was a significant prognostic factor for outcome after CCRT.

DAHANCA 5[22] focused on the effect of HPV on response to hypoxic modification in radiotherapy in HNSCC. They assessed by using p16-status as a retrospective stratification parameter and evaluated the influence of p16-expression on the response to nimorazole in HNSCC. The result showed that positive expression of p16 also significantly improved the outcome after radiotherapy. In the subgroup of patients with p16- negative tumours, locoregional failure was more frequent in the placebo group than in the nimorazole group. However, in the p16-positive group, patients treated with nimorazole had a loco-regional control rate similar to patients given placebo. Thus, they concluded that HPV/p16-expression significantly improved outcome after radiotherapy in HNSCC. Hypoxic modification improved the outcome in HPV/p16-negative tumors but was of no significant benefit in HPV/ p16-positive tumors.

In summary, at this time data support the conclusion that especially oropharyngeal cancer, tumor HPV status is an important prognostic biomarker for head and neck cancers. Moreover, in advanced disease due to nodal involvement, patients with HPV-positive tumors have apparently a superior outcome when compared to the HPV-negative patients in terms of tumor control and survival. In addition, the characteristic genotype and molecular biological profile of the HPV-associated HNSCC may be an important factor of tumor response to radiotherapy; therefore, in the near future, the attenuated treatment, such as reduced dose of radiotherapy or chemotherapy might be proved to have not only the same clinical outcome but also less toxicity.

Effect of HPV Vaccines in treatment of Oropharyngeal Cancer

There are many studies that have shown that HPV-positive tumor have an effect on the outcome of treatment for oropharyngeal cancer. Thus, some researchers believe that HPV vaccine may reduce the incidence of HPV infection and treat oropharyngeal cancer. At this time, there are three approaches to HPV vaccine development (As Figure 3)[23].

- Prophylactic HPV Vaccines
  - HPV L1 capsid protein-based vaccines (VLPs*)
  - HPV L2 capsid protein-based vaccines
- Therapeutic HPV Vaccines
  - Viral vector vaccines
    - Vaccinia virus vaccines
    - Adenovirus and adeno-associated virus vaccines
    - Alphavirus vaccines
  - Bacterial vector vaccines
    - Listeria vaccines
    - Other bacterial vaccines (Salmonella, BCG)
  - Peptide/protein vaccines
    - Peptide vaccines
    - Protein vaccines
  - Nucleic acid vaccines
    - DNA vaccines
    - RNA replicon vaccines
  - Cell-based vaccines
    - Dendritic cell-based vaccines
    - Tumor cell-based vaccines
- Combined Prophylactic and Therapeutic Vaccines
  - HPV chimeric VLPs*
  - HPV pseudovirion vaccines

*VLPs: virus-like particles.

Figure 3 : HPV vaccine strategies [23]

Nowadays, the prophylactic vaccine is a well-known vaccine because in a recent proof-of-principle efficacy trial, systemic immunization with a prophylactic HPV16 vaccine was highly effective in preventing a persistent HPV16 infection in the female genital tract[24]. However, it is not known whether such a vaccine will also alter the carriage rate of oropharyngeal HPV16; thus, we still suspect successfully targets genital HPV16 infection that might also reduce the incidence of HPV16-associated disease outside the anogenital tract, including HNSCC. The current strategy in preventive vaccines utilizes the capsid proteins L1 and L2 as target

antigens, inducing antibodies to neutralize and prevent entry of HPV into cells.

On the other hand, the therapeutic vaccines have been developed for the usage in humans. A number of therapeutic vaccines have been targeted of E6 and E7 oncoproteins[25]. Up to now, delivery systems tested clinically have included fusion proteins used alone and with adjuvant. Clinical trials that have been done up to now have been moderately successful in eliciting cell-mediated immune responses to HPV E6 and E7 in patients with a spectrum of HPV-associated disease[26]. However, for preclinical data and evidence of therapeutic benefit from induced T-cell response in humans has been limited.

Conclusion

In conclusion, in the future, clinical trials on oropharyngeal cancer have to take HPV-status in consideration. Thus, the future direction of HPV in treatment of HNSCC has three aspects. Firstly, HPV-positive in HNSCC is the prognostic significance; thus, the Radiation Therapy Oncology Group (RTOG) conducts whether less intensive therapy (radiation alone) is as effective in HPV-positive HNSCC as more intense therapy (concurrent chemo-radiotherapy). Secondly, the similar risk factors for exposure between cervical and oropharyngeal HPV cancer are of great importance to be studied of whether preventive HPV vaccines should be available to men. Finally, there are several phase II trials of therapeutic vaccine for HPV-related HNSCC that are ongoing with results available within a few years[27].

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