

## REVIEW

# Roles of Human Papillomaviruses and p16 in Oral Cancer

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### Abstract

Head and neck cancer, including oral cancer, is the sixth most common cancer in humans worldwide. More than 90% of oral cancers are of squamous cell carcinoma type. Recent studies have shown a strong relationship between human papillomavirus (HPV) infection and head and neck cancer, especially oropharyngeal squamous cell carcinoma (OPSCC) and oral squamous cell carcinoma (OSCC). Moreover, the incidence of HPV-related OSCC appears to be on the rise while HPV-unrelated OSCC tends to have stabilized in the past decades. p16, a tumor suppressor gene, normally functions as a regulator of the cell cycle. Upon infection with high-risk types of HPV (HR-HPV), particularly types 16, 18, 31, 33, 34, 35, 39, 51, 52, 56, 58, 59, 66, 68, and 70, the expression of p16 is aberrantly overexpressed. Therefore, the expression of p16 is widely used as a surrogate marker for HPV infection in head and neck cancer.

**Keywords:** HPV - p16 - oral squamous cell carcinoma

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### Introduction

In general, the genetic and environmental factors are the combined causes of human cancers. The sum of many genetic alterations, influenced by a patient's genetic predisposition and by environmental factors for example tobacco uses, alcohol consumption, chronic inflammation, radiation, viral infections, etc. (Choi and Myers, 2008). Cancer development can be divided into three phases; (1) initiation, (2) promotion, and (3) progression. First, the initiation phase gets started when the genetic materials of the cell is damaged by either carcinogens, UV radiation, viruses, or unknown causes, leading to genetic mutations of the "initiated cell" (Devi, 2004). It was found that there is only a very small or no phenotypic changes in the initiation phase (Vincent and Gatenby, 2008). Secondly, the initiated cell is subsequently activated by promoters in several times such as consumption of alcohol, bringing about to cell proliferation and tissue overgrowth (Vincent and Gatenby, 2008). Thus many mutated cells are found in the promotion phase. Lastly, in the progression phase, the overgrowth tissue leads to anatomical limit such as lack of space and nutrients and hypoxia.

As the overgrowth tissue becomes hypoxic, the cancer cells survives by switching their metabolism into the glycolysis pathway resulting in an acidic environment. The localized acidosis environment is so toxic that the cancer cells cease proliferation and growth. In order to adapt to the acidic environment, the cancer cells become more resistant to acid-mediated toxicity. This phenotype is invasive because it generates an acidic environment

through the glycolysis pathway noxious to other cellular populations but not the cancer population (Choi and Myers, 2008). Taken together, the malignant cells invade normal tissue and metastasize to other organ systems causing clinical symptoms and life threatening conditions to the patients.

Cancer is a disease caused by accumulating nonlethal genetic damages, either by acquired environmental factors or inherited mutation of germ lines. There are four major classes of gene involved in carcinogenesis including the growth-promoting proto-oncogene, the growth-inhibiting tumor suppressor gene, gene that control apoptosis, and gene associated with DNA repair (Choi and Myers, 2008; Robbins et al., 2010). Upon stimulating the oncogene, derived from mutation of the proto-oncogene, cancer cells become autonomously proliferative. The proto-oncogene can be classified in five groups; 1) growth factors (such as int2, hst1, tgfa, hgf, etc.), 2) growth factor receptors (such as erbb1, ret, pdgfrb etc.), 3) proteins involved with signal transduction (such as kras, braf, abl etc.), 4) transcription factors (such as c-myc etc.), and 5) cell cycle regulators (such as cyclin-D etc.).

Tumor/cancer suppressor genes normally regulate proliferation and growth of cells. Inactivation of tumor suppressor genes is fundamental changes occurring during carcinogenesis. For example, mutations of p53 tumor suppressor gene allow cells with genetic damages to further proliferate without DNA repair. Moreover, collection of mutated cells may result not only from stimulation of the oncogenes or inactivation of the tumor suppressor genes but also from mutations of genes that

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regulate apoptosis. The apoptosis genes are divided into both pro-apoptotic genes such as bax and bak, etc. and anti-apoptotic genes such as bcl2 and bcl-xl, etc. Finally, mutations of the DNA-repair genes affect proliferation and survival of the transformed cells indirectly by manipulating the competency of the transformed cells to repair nonlethal damages caused by the oncogenes, tumor suppressor genes and apoptotic genes.

The multiple genetic alterations aforementioned create hallmark characteristics of the cancer cells differing from the normal counterpart. The hallmarks of cancer comprises six characteristics as follows, (Hanahan and Weinberg, 2000; Choi and Myers, 2008).

#### *Self sufficiency in growth signals*

Normal cells need extracellular signals to stimulate proliferation and growth. However, in the cancer cells, mutation of some oncogenes such as ras may lead to self stimulation independent of extracellular signals (Hanahan and Weinberg, 2000). Additionally, overexpression of some growth factors and growth factor receptors in the cancer cells results in abnormal cell cycle regulation and increased cell proliferation via the autocrine and/or paracrine fashions. For example, in OSCC, both transforming growth factor alpha (TGF- $\alpha$ ) and epidermal growth factor (EGF) and their common epidermal growth factor receptors (EGFR). Overexpression of EGFR and its ligands plays an important role in oral cancer progression. Upon binding its ligands, EGFR is dimerized resulting in activating many signal transduction pathways including ras/raf/mitogenactivated protein kinase (MAPK), phosphatidylinositol-3-kinase (PI3K)/Akt, janus kinase (JAK)/signal transducer an activator of transcription (STAT), and protein kinase C (PKC) pathways leading to cell proliferation, cell survival, angiogenesis, invasion and metastasis (Choi and Myers, 2008).

#### *Insensitivity to antigrowth signals*

In general, there are checkpoints in each step of the cell cycle in order to prevent DNA-damaged cell proliferation and promote DNA repairing or to promote cell apoptosis in incurable injured cells. The tumor suppressor genes have been found to function as molecular checkpoints of the cell cycle. The cells with mutations of the tumor suppressor genes are unable to undergo apoptosis and subsequently accumulate damaged genetic material resulting in malignant transformation. The mutations of tumor suppressor genes such as the retinoblastoma gene (Rb gene), p53 gene, p21 gene, and p16 gene have been frequently found in OSCC (Choi and Myers, 2008; Kumar et al., 2010).

#### *Evasion of apoptosis*

The capabilities of the cancer cells to maintain a high number are not only by increasing the rate of cell proliferation but also by declining the rate of cell apoptosis. As aforementioned, the tumor suppressor genes are mutated and nonfunctional resulting in evading the apoptosis of the cancer cells. For example, the anti-apoptotic proteins, Bcl2, and BclXL, are overexpressed and the pro-apoptotic proteins, Bak and Bax, are down-

regulated in OSCC (Choi and Myers, 2008).

#### *Limitless replicative potential*

The telomere is a repetitive DNA sequence, TTAGGG, binding with proteins at the end of each chromosome. The main function of the telomere is to protect DNA from deterioration. Interestingly, the telomere might also restrict the longevity of cells because it is shortened after each cell replication. Without the telomere, the cells undergo apoptosis. Nevertheless, the cancer cells can evade this mechanism by producing the enzyme telomerase, generally depleted in normal adult cells, to stabilize the length of the telomere resulting in immortalization of the cancer cells. Sustained production of telomerase has been observed in various cancers. It was found that 80-90% of head and neck squamous cell carcinoma (HNSCC) including OSCC were positive for telomerase activity. Furthermore, telomerase activity positively correlated with the degrees of tumor differentiation and clinical stages of OSCC (Sumida and Hamakawa, 2001).

#### *Sustained angiogenesis*

Blood supply is essential for every cell in normal tissues as well as the cancer cells. When the blood supply is limited, the tumor cannot grow beyond the volume of 1-3 mm<sup>3</sup> and becomes hypoxic. The hypoxic cancer cells then switch "on" the angiogenic genes and generate and release many pro-angiogenic factors for example vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), TGF- $\alpha$  and IL8, etc. to the host tissue. As a result, new blood formation occurs and brings in the oxygen and essential nutrients to the cancer cells. This process is called tumor angiogenesis or neovascularization. VEGF is not only produced by the cancer cells, but also the stromal cells and the inflammatory cells such as macrophages (Kumar et al., 2010). The overexpression of VEGF in OSCC has been demonstrated in many studies and suggested as a prognostic marker for patients with OSCC (Kapoor and Deshmukh, 2012; Zhao et al., 2013; Aggarwal et al., 2014; Kim et al., 2015). In HNSCC, a study of Cohen et al. found that IL-1 $\alpha$ , IL-1 $\beta$ , TGF- $\beta$ , EGF, TNF- $\alpha$  condition in a culture medium regulated HNSCC cell lines to produce IL8 to form new blood vessels (Cohen et al., 1995). The imbalance between pro-angiogenesis and anti-angiogenesis is also evident in cancer tissues since up-regulation of VEGF by both normal and cancer cells causes mutation of p53 gene leading to depletion of thrombospondin 1 acting as an inhibitor of angiogenesis (Hanahan and Weinberg, 2000). The role of tumor angiogenesis is not only for nutritional supply, but also growth factor supply to the tumor cells. In addition, tumor angiogenesis facilitates the cancer cells to metastasize to the distant organ systems.

#### *Tumor invasion and metastasis*

Tumor invasion and metastasis are involved with multiple steps in the tumor cells starting from cell-to-cell separation, cytoskeletal rearrangement, cell migration, basement membrane decomposition, entry into the blood vessels, survival in the blood circulation, extravasation to distant sites, and activation of angiogenesis (Choi and

Myers, 2008). E-cadherin is an important cell-to-cell adhesion molecule of the epithelial cells. As the epithelial cells undergo the epithelial mesenchymal transition (EMT), E-cadherin is minimally expressed so that the epithelial cells become detached from one another and increased cell motility. Evidently, down-regulation of E-cadherin has been found in many metastatic cancers including OSCC (Krisanaprakornkit and Iamaroon, 2012). Degradation of the basement membrane and extracellular matrix (ECM) is another important step for the cancer cells to migrate into the underlying tissue. Many proteinases are up-regulated to degrade the basement membrane and ECM during this process for example type IV collagenases/gelatinases (MMP2, MMP9), collagenases (MMP1 and MMP13), stromelysins (MMP3, MMP10 and MMP11), and membrane-type MMPs (MT1-MMP) (Tsantoulis et al., 2007). Furthermore, the integrins, the glycoproteins mediating cell-to-cell and cell-to-matrix interactions, also take part in cancer migration. In general, the cancer cells alter the expression and types of the integrins favoring to bind degraded ECM resulting in promoting cancer cell migration through ECM into the underlying tissue as well as the blood vessels (Hanahan and Weinberg, 2000). The cancer cells then circulate in the blood vessels and are likely to form in mass. The mass is formed by aggregating the cancer cells and host circulating cells especially the platelets. These cancer cells-platelets complexes support not only survival of the cancer cells in the blood circulation but also enable the cancer cells to implant into distant normal tissues by processes of extravasation through the capillary walls and degradation of the basement membrane. After those processes, the cancer cells can now metastasize to distant organ systems and induce angiogenesis as described above (Robbins et al., 2010).

## Oral squamous cell carcinoma

Head and neck cancer comprises cancer of the oral cavity, oropharynx, hypopharynx, and larynx. In general, oral cancer includes cancer of lip, buccal mucosa, tongue, gingiva, floor of mouth, soft palate and hard palate (Singhi and Westra, 2010; Chung et al., 2014). Oral cancer is the fifth most common type of cancer in the world and approximately 90% of all oral cancers are classified as squamous cell carcinoma (Iamaroon et al., 2004; Krisanaprakornkit and Iamaroon, 2012; Vargas-Ferreira F, 2012). OSCC is usually found twice more in men than in women. In the year 2014, 28,230 new cases of OSCC were estimated in the U.S.A. and 5,850 cases may die (American Cancer Society, 2014). It appears that in the near future new OSCC patients will occur more frequently in the developing world since it is predicted that in the year 2020, more than 60% of new OSCC cases will be diagnosed in the developing countries (Vargas-Ferreira F, 2012).

OSCC is a multiple etiologic disease. The main risk factors associated with classic patients with OSCC (the older group) are tobacco uses in various forms and alcohol consumption in the western population and areca nut/betel quid chewing in the south and southeast Asian populations (Johnson N, 2011; Vargas-Ferreira F, 2012;

Bixofis et al., 2014)

Recent data have shown that approximately 15 to 20% of patients of the western population with OSCC occur without those conventional risk factors and are related to high-risk genotypes of HPV infection (Johnson, 2011; Vargas-Ferreira, 2012; Bixofis et al., 2014). In some Asian populations, the incidence of HPV-related OSCC appears to be significantly higher (Zhu C, 2012). The general profiles of HPV-related OSCC are younger patients especially in white males with multiple partners and oral sexual behavior. Distinguishing OSCC patients with HPV infection is very crucial since HPV-related OSCC patients respond better to certain chemotherapy and radiotherapy. As a result, they have a better curable rate and prognosis. (Singhi and Westra, 2010; Johnson, 2011; Marklund and Hammarstedt, 2011; Vargas-Ferreira, 2012; Monsjou et al., 2013; Bixofis et al., 2014)

## Histopathologic features

Oral squamous cell carcinoma is histologically characterized by the invasion of the malignant epithelial cells through the mechanism of the EMT into the basement membrane and eventually the underlying connective tissue. Those invaded malignant cells are then arranged in islands, nests, or sheets and may produce keratin and form the keratin pearls (Krisanaprakornkit and Iamaroon, 2012). As OSCC progresses, the tumors cells are more invasive, destroy surrounding structures including the muscle and bone and eventually metastasize to the secondary organ systems.

The histologic grading of OSCC can be used for predicting the prognosis of patients. Traditionally, OSCC is histologically graded according to the degree of differentiation as the followings,

**Well differentiated tumor:** In this grade, the malignant cells are mature enough to resemble the normal epithelial cells. Keratin production is frequently presented, the so-called keratin pearls.

**Moderately differentiated tumor:** In this grade, the malignant cells are less differentiated and have less keratin production than in the previous grade. In addition, the nuclear pleomorphism, mitotic activity and abnormal mitoses of the malignant cells are present.

**Poorly differentiated tumor:** In this grade, the immature epithelial cells are predominately seen. Mitoses and abnormal mitoses are numerous without keratin production or only scarce keratin production.

Previous studies have revealed the correlation between histopathologic grading and prognostic parameters including lymph node involvement, metastases to neural tissues and other organs, and recurrences after treatment. For example, the poorer grading is associated with regional lymph node invasion, extracapsular spreading, and perineural invasion (Fang et al., 2009; Larsen et al., 2009).

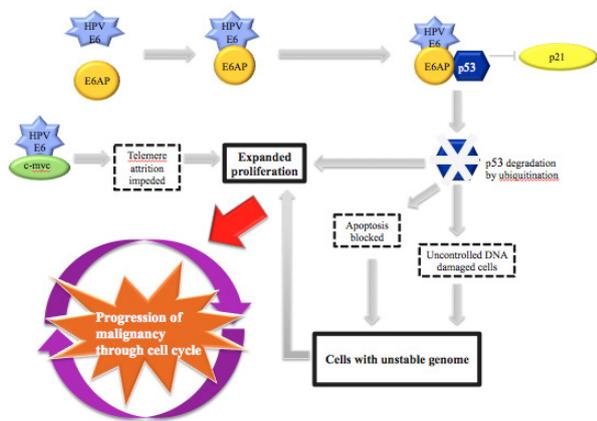
## Human papillomavirus

Human papillomavirus (HPV) is a small spherical double-stranded DNA virus. Nearly 150 types of HPV are

discovered and 120 HPV types are sequenced. The HPV types are grouped into the mucosal or cutaneous types depending on their specific sites. The mucosal HPV types are further divided into two types; low-risk and high-risk types. The high-risk types include types 16, 18, 31, 33, 34, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, and 70 (Marklund and Hammarstedt, 2011; Rautava and Syrjänen, 2012) and can transform the normal cell to the malignant cell particularly in the oropharyngeal carcinoma. (Rautava and Syrjänen, 2012).

The HPV genome consists of approximately 8,000 base pairs. The HPV genes are classified as the early genes (E) encoding E1, E2, E3, E4, E5, E6 and E7 proteins and the late genes (L) encoding the major (L1) and minor (L2) viral capsid proteins (Rautava and Syrjänen, 2012). E6 and E7 proteins are important viral components that can transform the normal cell into the malignant cell. E6 viral protein can attach to the ubiquitin ligase (E6AP) of the host cells and then transfer the ubiquitin to p53 protein resulting in p53 damage, thus, abrogation of p53 function. In addition, E6-E6AP-p53 complexes can block p21 protein leading to aberration of the cell cycle. E6 protein can also bind c-Myc protein resulting in the up-regulation of telomerase reverse transcriptase (hTERT), an enzyme that prevents telomere erosion (Figure 1).

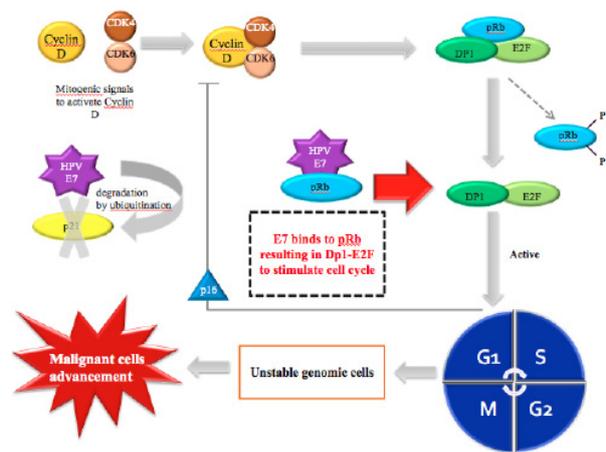
The HPV E7 protein can attach the retinoblastoma protein (pRb) preventing it to form a complex with the transcription factor E2F. As a result, E2F is available and can induce cell cycle progression. The HPV E7 protein



**Figure 1. Role of E6 Viral Protein.** E6 Protein Attach to E6AP Cause p53 Deterioration. E6 protein also activates hTERT that prevents telomerase erosion resulting in immortalization and evolution of cancer cells (Rautava and Syrjänen, 2012)

also inhibits the function of p21 protein, a potent cyclin-dependent kinase inhibitor. Moreover, loss of pRb-E2F complex causes the overexpression of the p16 protein (Marklund and Hammarstedt, 2011; Rautava and Syrjänen, 2012; Sanketh et al., 2014) (Figure 2). The mechanisms of p16 overexpression will be elaborated below.

HPV can be detected in 10-13% of normal oral mucosa. Many previous studies have revealed a strong association between HPV and HNSCC. In OPSCC, the prevalence of HPV ranges from 41% to 93% and from 27% to 74 % in OSCC (Table 1) (Shaw and Robinson, 2011; Syrjänen et al., 2011; Rautava and Syrjänen, 2012). Of all HPV types, HPV-16 is the most common type found in OSCC (Kreimer et al., 2005; Shaw and Robinson, 2011; Syrjänen et al., 2011). Recently, a meta-analysis of Chinese population by Zhu C et al. found a positive association between HPV and HPV-16 in OSCC revealing that were 58.0% and 47.5% which were significantly higher than in normal 10.4% and 7.1% respectively (Zhu C, 2012). In 2011, the International Agency of Research of



**Figure 2. Role of E7 Viral Protein.** Normally, pRb in a Hypo-Phosphorylated Structure Forms a Complex with E2F. E2F transcription factors then bind to DP1 protein as a heterodimer and control the cell cycle by regulating transcription of many genes. Upon activation by mitogenic signals, cyclin D forms a complex with CDK4/6. Subsequently, cyclin-CDK4/6 complexes promote a cycling series through the G1-S phase of the cell cycle resulting in phosphorylation of pRb. The phosphorylated pRb then releases E2F/DP1 from the DNA leading to entering further cell cycle process. Without any mitogenic signals, E7 viral protein can also bind pRb at the restriction point (R) causing a release of the E2F/DP1 complex, turning on an autonomous cell cycle process. Additionally, E7 viral protein can degrade p21 protein, a cell cycle regulator, via ubiquitination. (Rautava and Syrjänen, 2012)

**Table 1. Detection of HPV-16 and HPV-18 in OSCC from Different Ethnic Groups**

Authors	n	Ethnicity	HPV-16	HPV-18	Techniques
(Shima et al., 2000)	46	Japanese	20%	54%	PCR
(Minawaer et al., 2001)	45	Chinese	48.90%	15.60%	PCR
(Zhang et al., 2004)	73	Chinese	58.90%	24.70%	PCR
(Nemes et al., 2006)	79	Hungarian	34.20%	N/A	PCR
(Zhao et al., 2009)	52	Chinese	25%	11.50%	PCR
(Elango et al., 2011)	60	Indian	48.30%	N/A	PCR
(Akiba et al., 2014)	75	German	39%	N/A	PCR
(Gan et al., 2014)	200	Chinese	19.50%	7.50%	PCR

Cancer (IARC) announced that there is sufficient evidence that HPV-16 is associated with oral cancer (Rautava and Syrjänen, 2012).

The p16 protein, also known as p16INKa, is a member of INK4 family of CDK inhibitors functioning in cell cycle control (Fregonesi et al., 2003). In normal cells, p16 protein is expressed at a low level. However, due to the activity of E7 oncogene of HPV, p16 is highly expressed in cancer cells infected by HPV. E7 can bind to pRb and form a complex. The E7-pRb complex causes a release of the transcription factor E2F from the pRb-E2F complex, which normally restrains transcription of the p16 gene. Thus, upon infected with HPV, the targeted cells abundantly express p16 through the mechanism of interaction between pRb and E7 viral protein of HPV (Sanketh et al., 2014) (Figure 3).

Normally, p16 binds to CDK4/CDK6 to inhibit the interaction between cyclin D and CDK4, resulting in hypo-phosphorylated Rb and cell cycle restraint (Weinberger et al., 2004; Sanketh et al., 2014). In normal oral epithelium, p16 is detected merely in the basal and suprabasal cell layers where the cells are actively proliferative. In HPV-unrelated HNSCC, it is found that there is a lack of p16, hence, favoring cell cycle progression in cancer cells (Singhi and Westra, 2010; Pannone et al., 2012). Interestingly, many previous studies have, however, revealed the overexpression of p16 protein in HPV-related HNSCC due to the ability of the E7 viral protein to bind pRb causing aberration of p16 regulation (Table 2). For example, Singhi et al. found a correlation rate at 93% between HPV-16 and p16 by a technique of immunohistochemistry in HNSCC (Singhi and Westra, 2010). Pannone et al. showed 100% sensitivity of p16 immunostaining with no false negative in HPV-related OSCC cases. On the other hand, the specificity rate of p16 is only 74% with 26% false positive (p16 positive; HPV negative) cases of OSCC (Pannone et al., 2012). Although

many investigators have suggested that the expression of p16 be a surrogate marker for HPV-related HNSCC, the discordant results from many studies have revealed the imperfections of using p16 alone as a surrogate marker (Fregonesi et al., 2003; Weinberger et al., 2004; Singhi and Westra, 2010; Marklund and Hammarstedt, 2011; Pannone et al., 2012). Hence, p16 is suggested as a surrogate marker in a combination with other HPV-DNA molecular methods such as PCR, in situ hybridization, E6/E7 mRNA, etc. (Singhi and Westra, 2010; Marklund and Hammarstedt, 2011; Pannone et al., 2012). More recently, p16 is also suggested as a clinical prognosis marker for HNSCC patients since p16-positive cases had better treatment outcomes in comparison with those p16-negative (Chung et al., 2014). An example of the immunostaining of p16 in OSCC is shown in Figure 4.

## HPV vaccine

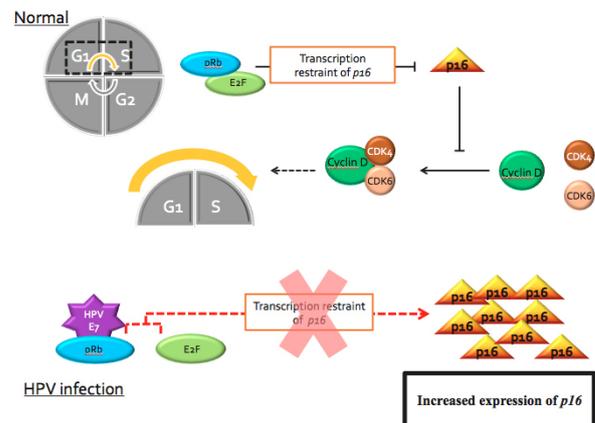
HPV is the primary cause of cervical cancer including its precancerous lesions and recently found to also play a role in head and neck cancers and non-cancerous lesions such as recurrent respiratory papillomatosis (D'Souza and Dempsey, 2011; Giral di et al., 2014; Wierzbicka et al., 2014). To prevent HPV infection particularly in cervical cancer, the HPV vaccination studies had initially

**Table 2. Prevalence of p16-positive OSCC Cases by Immunohistochemistry**

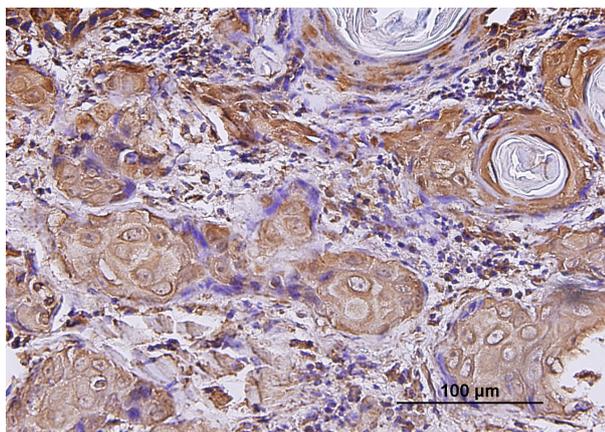
Authors	n	p16 positive
(Ramshankar et al., 2014)	156	15.4%
(Stephen et al., 2012)	20	20%
(Chandarana et al., 2013)	49	13%
(Dragomir et al., 2012)	34	64.7%
(Laco et al., 2012)	48	35%
(Muirhead, 2006)	45	13%
(González-Moles et al., 2002)	50	68%
(Pande et al., 1998)	35	37%

**Table 3. Correlation between HPV positive and p16 expression in cancer**

Authors	Sites	n	HPV positive	P16 positive	$\kappa$ index
(Kanao et al., 2004)	Cervical	25	21/25 (84%)	17/21(81%)	N/A
(Śnietura et al., 2010)	Oral/ oropharyngeal	59	9/59 (15.3%)	5/9(55.6%)	N/A
(Laco et al., 2012)	Oral	48	7/48 (15%)	17/48 (36%)	0.63
	oropharyngeal	44	35/44 (80%)	36/44 (82%)	0.62
(Geißler et al., 2013)	Head and neck	45	14/45 (31.1%)	13/14 (92.9%)	N/A
(Cao et al., 2014)	Esophageal	105	24/39 (64.1%)	39/105 (37.1%)	0.61
(Bussu et al., 2014)	Oropharyngeal	50	16/50 (32%)	N/A	0.62
(Antonsson et al., 2015)	Head and neck	248	50/248 (20%)	44/248 (19%)	0.72



**Figure 3. The Overexpression of p16 Protein in HPV-Infected Cells. (Sanketh et al., 2014).** Normally, the p16 protein blocks interaction between cyclin D and CDK4 resulting in hypo-phosphorylated pRb. On the other hand, the E7 viral protein causes uncontrolled transcription and translation of p16 gene by competing E2F to bind with pRb. As a result, there is a lack of E2F-Rb complexes to regulate p16 transcription and translation, hence, overexpression of p16



**Figure 4. Immunohistochemical Staining of p16 in the Tumor Islands of Oral Squamous Cell Carcinoma (Original Magnification, x400)**

been reported from the University of Rochester (USA), the National Cancer Institute, NIH (USA), Georgetown University (USA) and the University of Queensland (Australia) during 1991-1993 (Padmanabhan et al., 2010). At present, there are two HPV vaccines in the global market; quadrivalent HPV (qHPV) vaccine (Gardasil®, Merck, Rahway, NJ, USA) and bivalent HPV vaccine (Cervarix™, GSK, Middlesex, UK) (Kreimer, 2014). Subsequently, in 2006, the US Food and Drug Administration (FDA) approved the qHPV vaccine for preventing cervical, vaginal and vulvar cancers for 9-26 year-old females and in 2009, FDA also approved the bivalent HPV vaccine for preventing cervical cancer and expanded the qHPV vaccine licensure for preventing genital warts in both genders (D'Souza and Dempsey, 2011; Kim et al., 2014). Recent studies have however drawn a conclusion that universal vaccination may not be cost-effective in males, thus, the HPV vaccine program for males is only a policy in few countries such as Australia, New Zealand, USA, Canada, and Austria (Gibaldi et al., 2014; Kumar et al., 2015). On the other hand, some investigators have a different view that the actual cost-effectiveness of HPV vaccines may be underestimated because the vaccines are not indicated for head and neck cancers and other non-cancerous lesions such as recurrent respiratory papillomatosis. Moreover, the psychosocial cost and quality of life that HPV-infected patients have to deal with are not included in those studies (Wierzbicka et al., 2014; Kumar et al., 2015). In fact, recent data have shown that HPV vaccines can reduce the rate of HPV infection, oropharyngeal malignancies and related diseases in both sexes (Gibaldi et al., 2014; Kumar et al., 2015). Collectively, we would have to wait for more evidence to prove the efficacy and cost-effectiveness of HPV vaccines in preventing head and neck cancers and other HPV-associated lesions for the nationwide HPV vaccination policy.

## Conclusions

Oral squamous cell carcinoma is a devastating disease with high morbidity and mortality rates. Approximately

20-25% of OSCC are associated with HPV infection particularly HPV-16. p16 protein is a tumor suppressor, functioning as a cell cycle inhibitor. However, E7 protein in high-risk HPV subtypes can abrogate function of pRb protein resulting in aberrantly increased expression of p16 in HPV-related OSCC, hence, p16 is widely used as a surrogate biomarker for HPV infection. At present, two types of HPV vaccines are commercially available and are recommended mainly for preventing cervical cancer in young females. However, recent studies have revealed that HPV vaccines may also benefit for preventing head and neck cancers in both genders. Therefore, more investigations on HPV vaccines for preventing head and neck cancers are required prior to launch a vaccination program policy nationwide for males especially in the developing world.

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