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Review

Impact of Human Papillomavirus Infection on Radiosensitivity of Head and Neck Cancers

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Tumor volume is reportedly the most important factor influencing the outcome of radiotherapy for various types of tumors, including head and neck squamous cell carcinomas (HNSCCs). However, other factors have also been found to affect radiosensitivity, including the primary site and high-risk human papillomavirus (HPV) infection; the latter may increase radiosensitivity. Paradoxically, molecular biological studies have revealed that the oncogenic HPV E6 and E7 proteins possess anti-apoptotic or immune-evading functions. In this review, the impact of HPV infection in HNSCC is discussed, including molecular mechanisms of carcinogenesis, the prevalence of HPV infection in HNSCCs, and HPV's effects on radiosensitivity and prognosis.

Key words: human papillomavirus, head and neck squamous cell carcinoma, radiotherapy, viral oncogenes

Structure and biological features of human papillomavirus (HPV)

Papillomaviruses (PVs) have been isolated from many vertebrates, including reptiles. Although PV infections are species-specific, the viruses share several features such as a double-stranded circular DNA genome, an icosahedral capsid, and a non-enveloped virion structure. They are classified according to the host species, with distinct types determined by DNA sequence homology in the L1 region. More than 180 types of HPVs have been identified to date. They are clustered in five genera: alpha, beta, gamma, mu, and nu. Most alpha papillomaviruses infect genital and nongenital mucosae and are closely associated with mucosal lesions. Therefore, they are referred to as genital–mucosal HPVs. The beta, gamma, mu, and nu viruses infect nongenital skin[1-3].

HPVs have a specific tropism for squamous epithelial cells. The viral life cycle is tightly linked with the differentiation status of the infected host cells. Because only basal cells are capable of dividing, persistent HPV infection requires infection of the squamous epithelial basal cells. Several HPV receptors, including alpha-6-beta-4 integrin, syndecan-1, and heparan sulfate proteoglycan, are highly expressed on basal cells or the basement membrane[4-6]. HPV is maintained at a low copy number in the nuclei of basal cells. Vegetative DNA synthesis, capsid protein synthesis, and virion assembly exclusively occur in differentiated squamous epithelial cells. Because the replicative phase of the HPV life cycle is tightly linked to terminally differentiated epithelium, it has been difficult to establish an in vitro HPV

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Table 1. Human papillomavirus infection in head and neck squamous cell carcinomas in Japan
OPC: Oropharyngeal cancer, LC: Laryngeal cancer, ND: Not done

First Author	HPV (+) cases	HPV (+): OPC	HPV (+): Lrx
Deng	30.1% (63/209)	47.2% (25/53)	16.7% (4/24)
Fujita	41.7% (58/139)	64.6% (42/65)	36.3% (8/22)
Hanamoto	37.0.0% (11/30)	37.0% (11/30)	ND
Hasegawa	37.9% (11/29)	37.9% (11/29)	ND
Hassani	41.7% (10/24)	41.7% (10/24)	ND
Hatakeyama	29.1% (23/79)	29.1% (23/79)	ND
Inohara	30.2% (13/43)	30.2% (13/43)	ND
Kawakami	38.5% (40/104)	38.5% (40/104)	ND
Kouketsu	54.1% (13/24)	54.1% (13/24)	ND
Maruyama	13.8% (68/493)	34.4% (56/163)	3.9% (3/77)
Matsumoto	23.3% (7/30)	23.3% (7/30)	ND
Mizumachi	32.4% (23/71)	32.4% (23/71)	ND
Nakano	53.3% (56/105)	53.3% (56/105)	ND
Nomura	41.6% (32/77)	41.6% (32/77)	ND
Saito	34.0% (51/150)	34.0% (51/150)	ND
Urashima	10.9% (27/248)	ND	ND
Wakisaka	41.5% (22/53)	41.5% (22/53)	ND
Yamashita	25.0% (4/16)	ND ND	ND
Yasui	51.4% (19/37)	51.4% (19/37)	ND
Yoshida	41.5% (22/53)	41.5% (22/53)	ND

replication model. Historically, bovine papillomavirus 1 has been investigated as a prototypical PV. The genomic organization of PVs is remarkably similar. There are approximately 10 open reading frames (ORFs) classified as early (E) or late (L) ORFs. The early genes (E1–E8) are expressed in nonproductively infected and transformed cells, whereas the late genes (L1 and L2) encode the viral capsid proteins and are expressed in productively infected cells[7].

PV DNA replication occurs in three modes. The first type occurs during initial infection of basal cells, with approximately 50–100 copies of viral genome detected in these cells. The second type is observed in dividing basal cells as a stable multicopy plasmid. During this phase, the viral genome replicates along with the host chromosome once per cell cycle in the S phase. Persistent and latent PV infections are maintained by this type of replication. The third type, vegetative replication of viral DNA, occurs in differentiated non-dividing epithelial cells[8].

Most PVs, including beta and gamma HPVs, produce only chronic, inapparent infections. Virions are produced from the surface of infected epithelial cells without apparent detriment to the host. However, a subset of HPVs is clearly implicated in the development of cervical cancers[9-12]. Most cervical cancers occur in the transformation zone, where columnar cells form a junction with stratified squamous epithelial cells, although the reason is unknown. In this area, columnar epithelial cells are transformed into squamous cells. The major route of HPV infection in this region of the cervix is via sexual contact. Therefore, HPV prevalence is correlated with the number of sexual exposures[13]. The estimated prevalence of genital HPV infection varies with the age of the population and sensitivity of the detection modality. However, the prevalence decreases with increasing age, with a peak in <25-year-old females (14). Wallin *et al*. reported that 30% of 118 HPV-positive women with normal Pap smears developed invasive cervical cancer, whereas only 3 of 118 (3%) women in the control group were HPV-positive[15]. On the other hand, there is evidence that most HPV infections are transient and spontaneously clear within 12–24 months following detection[16]. However, HPV infection alone may not be sufficient to cause cervical cancer; other factors associated with increased risk include high parity, smoking, long-term oral contraceptive use, sexual behavior, genetic factors, and coinfection with other sexually transmitted infectious agents such as *Chlamydia trachomatis*[17].

Among the alpha genus of HPVs, 15 types are classified as conferring a high risk of cancer (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82). While three are considered as probable high-risk types (26, 53, and 66), 12 are classified as low-risk types (6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, and CP6108)[18].

HPV detection in head and neck squamous cell carcinoma (HNSCC)

A subset of HNSCC has been attributed to HPV infection. In a systematic review of 5,046 cases of HNSCC from 60 studies, Kreimer *et al*. found an overall HPV prevalence of 25.9%. HPV positivity was significantly higher in

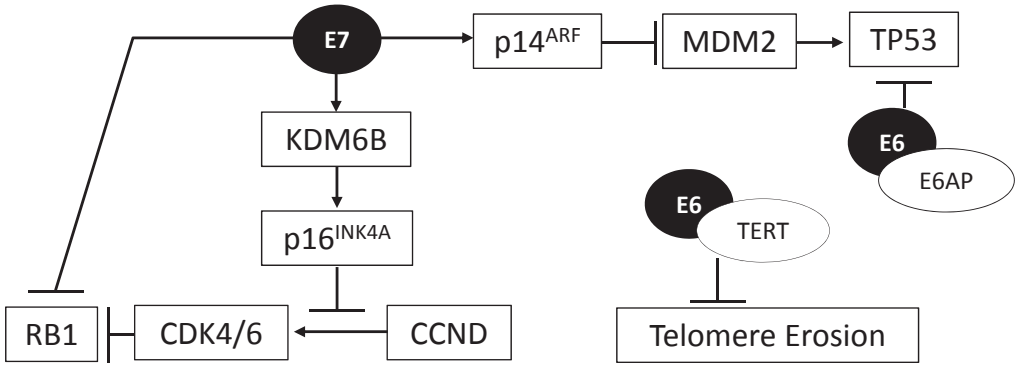

Fig. 1. HPV carcinogenesis
HPVE7 expression triggers a p16INK4A-mediated cellular defense response. p16INK4A expression is triggered by removal of repressive trimethyl marks on lysine 27 of histone H3 (H3K27me3) by the KDM6B histone demethylase. High-risk HPVE7 proteins have evolved to degrade retinoblastoma tumor suppressor protein (RB1) to avoid the p16INK4A-mediated activation of RB1 tumor suppressor activity that is designed to trigger G1 cell cycle arrest and senescence). p16INK4A also inhibits CDK4/6 activity by abrogating complex formation with D-type cyclins (CCND). CDK4/6 inhibition causes accumulation of hypophosphorylated RB1 tumor suppressor, which triggers G1 cell cycle arrest. E7 protein initiates the activation of E2F transcription factors causes TP53 activation, a cellular defense response that results in G1 cell cycle arrest and/or apoptosis. To evade this response, the E6/ E6AP ubiquitin ligase complex targets TP53 for ubiquitylation and proteasomal degradation. To counteract telomere erosion, E6 also activates telomerase (TERT), which results in unlimited-aberrant cellular proliferation.

oropharyngeal cancers (35.6% of 969 cases) than in oral (23.5% of 2,642 cases) or laryngeal (24.0% of 1,435 cases) cancers[19]. Ndiaye *et al*. reported an HPV DNA detection rate of 31.5% (3,837 of 12,163 cases) in HNSCCs from 148 studies in 44 countries[20]. Among these reports, HPV prevalence of oropharyngeal cancer versus laryngeal cancer samples were not consistent (46.3% vs. 38.2%: Kreimer *et al*., 45.8% vs. 22.1%: Ndiaye *et al*.). From 2013 to 2016, there were at least 20 studies of HPV infection of head and neck cancer in Japan, with 522 of 1577 cases (33.1%) being positive for HPV (Table 1)[21-39]. Among HNSCCs, oropharyngeal cancer had a relatively higher HPV positivity rate (40.0% vs. 12.2% for laryngeal cancer). It seems likely that there is a geographical difference in an HPV prevalence in laryngeal cancer. However, HPV positivity in oropharyngeal cancers is obviously higher than in laryngeal cancers.

A case-control study performed by D'Souza *et al*. concluded that a high lifetime number of vaginal or oral sex partners was independently associated with a risk of oropharyngeal cancer, regardless of tobacco or alcohol use. In addition, they noted annual increases in the incidences of tonsillar and base-of-tongue cancers in the United States since 1973, possibly implicating widespread oral sex practices among adolescents[40]. In these cases, the presence of oral HPV-16 infection was strongly associated with oropharyngeal cancer.

HPV-dependent phenotypes and HNSCC radiosensitivity

A PubMed search retrieved 242 English publications using the key words "HPV," "oropharyngeal," "prognosis," and "radiotherapy." Most of these papers suggested that HPV positivity was a favorable prognostic factor. From 2008 to 2017, at least 120 publications concluded that HPV infection is associated with a better prognosis. Ang *et al*. presented evidence that HPV-positive oropharyngeal cancer represents a distinct clinicopathologic entity associated with a better prognosis than does HPV-negative disease[41]. They performed a post-hoc analysis of a randomized trial wherein patients with HNSCC had been randomly assigned to concurrently receive high-dose cisplatin with accelerated-fractionation radiotherapy (with acceleration provided by concomitant boost radiotherapy) or standard-fractionation radiotherapy. Among 323 patients with oropharyngeal cancer whose tumors were tested for HPV, 206 tumors (64%) were HPV-positive, and these patients had a significantly better 3-year overall survival than those with HPV-negative tumors (82.4% vs. 57.1%, $P < 0.001$). After adjustment for age, race, tumor and nodal stage, tobacco exposure, and treatment assignment, HPV positivity was associated with a 58% reduction in the risk of death.

As shown in Figure 1, after a successful persistent infection of HPV, carcinogenesis is accomplished through several routes. HPV-infected cancer cells, particularly after integration of the virus into the host genome, express a large amount of E6 and E7 oncoproteins. These proteins from high-risk HPVs interfere with normal cell cycle regulation, preventing apoptosis as a result of unscheduled DNA replication. In contrast to low-risk HPV types, the high-risk E6 and E7 proteins drive cell

cycle entry in the upper epithelial layers and stimulate proliferation of infected basal cells, leading to neoplasia. E6 oncoproteins trigger proteasomal degradation of p53 by forming a trimer of E6, ubiquitin ligase, and p53[42, 43]. E6 also represses the transactivation function of p53 by association with CBP/p300[44, 45], a coactivator of p53. Cellular p53-dependent apoptotic cascades are severely impaired by high-risk E6 proteins[46]. p53 mutations are reportedly uncommon in HPV-positive HNSCCs but are common in HPV-negative tumors[47]. Maruyama *et al*., studying oropharyngeal cancers, reported that 44.2% (23 of 52 cases) of HPV-negative tumors harbored p53 mutations versus only 6.7% (2 of 30) of HPV-positive cancers[30]. Tinhofer *et al*. also demonstrated that HPV-negative tumors had an increased frequency of p53 gene mutations compared with HPV-positive cancers (67% vs. 4%)[48].

E7 oncoproteins inhibit the association between the E2F transcription factor and retinoblastoma (Rb) protein, another major anti-oncogenic factor. E7s also stimulate cell cycle progression by inhibiting the function of two G1-checkpoint cyclin-dependent kinase (CDK) inhibitors, p21[49, 50] and p27. HPV-16 E6 activates *TERT* gene transcription by inducing c-Myc and reducing upstream stimulatory factor-mediated repression[51, 52]. It is widely accepted that persistent HPV infection and sustained high E6 and E7 expressions are major factors in the accumulation of genetic mutations. The function of both proteins is required for the maintenance of malignant phenotypes by influencing cell proliferation and survival. However, the better survival rate among patients with HPV-positive cancer has been ascribed in part as due to greater locoregional control (LRC) of the disease, reflecting a higher intrinsic sensitivity to chemoradiation therapy. These conflicting findings raise the question as to how the virus can increase malignant behavior and yet also improve response to treatment such that HPV-positive HNSCC has a relatively favorable prognosis.

Fischer *et al*. reported that survival benefit of patients with oropharyngeal cancer positive for the tumor suppressor p16 protein was independent of treatment modality[53]. It is likely that HPV-positive tumors that overexpress p16/Ink4a are inherently less malignant than HPV-negative cancers. p16/Ink4a, the principal member of the Ink4 family of CDK inhibitors, is considered a surrogate marker for HPV infection. It is a product of the *CDKN2A* gene located on chromosome 9p21 within the INK4a/ARF locus. p16/Ink4a has antiproliferative effects[54]. During immortalization of cancer cells, the p16/Ink4a-Rb pathway, critically important in preventing inappropriate cell proliferation, is often targeted by viral oncoproteins. E7-dependent Rb inactivation releases p16/Ink4a from negative feedback control, causing an increase in the levels of this protein[55].

Because a robust vascular supply is required for oxygen and nutrient delivery to a rapidly growing cancer, both metastasis and aggressive tumor progression are dependent on angiogenesis[56-58]. Al-Ansari *et al*. demonstrated a possible inhibitory function of p16/Ink4a on migration and invasion of tumor cells. p16/Ink4A also represses the expression and secretion of the pro-angiogenesis protein VEGF-A[59].

Although the expression of HPV E6 and E7 oncogenes markedly inhibits the activity of certain tumor suppressors, these suppressor pathways are often inactivated to a greater degree in HPV-negative tumors, perhaps by genetic or epigenetic mechanisms. It is plausible that an expression level of E6 causing accelerated degradation of p53 is saturated under normal conditions. Therefore, robust expression of p53 under genotoxic conditions, such as that induced by gamma irradiation, may overcome the degradative capacity of E6. In contrast, p53 mutations in HPV-negative tumors may result in the synthesis of a p53 protein that not only fails to function as a tumor suppressor but can also inactivate any remaining wild-type p53, thereby increasing the malignant phenotype. The other possible explanation of the apparently paradoxical benefit of HPV positivity is that downregulation of E6 and E7 expression with standard chemotherapeutic agents reactivates tumor-suppressor pathways.

Tumor volume has a significant influence on LRC in HNSCCs, with small tumors significantly more likely to be associated with better LRC compared with large tumors at the same radiation dose. However, the impact of tumor volume may also depend on the tumor site. Mendenhall *et al*. showed that tumor volume has a more significant influence on laryngeal than on oropharyngeal cancers[60]. As described earlier, HPV positivity in laryngeal cancers is lower than in oropharyngeal cancers. The higher rate of HPV positivity in oropharyngeal tumors may explain this finding. Linge *et al*. demonstrated that cancer stem sell (CSC) marker expression (CD44 mRNA, CD44 protein, and SLC3A2) was on average lower in HPV-positive HNSCC samples compared with that in HPV-negative tumors[61]. It seems likely that the absolute number of CSCs would be higher with a larger tumor total volume, predicting a poorer prognosis. Therefore, if HPVs have an adverse effect on CSCs, as suggested by reduced expression of CSC markers, HPV positivity in a tumor should indicate a more favorable prognosis. This would contrast with the possible mechanism in cervical cancer reported by Tyagi *et al*., suggesting that HPV E6 induces and maintains stem-like characteristics in cervical cancer cells[62].

The immunogenicity of viral proteins may also partially restrict the malignant behavior of HPV-positive cancers. The biologic and immunologic properties of HPV-

positive tumors may contribute to their better response to treatment with radiation and chemotherapy. The presence of an immune response against E6 and E7 proteins may be associated with a better prognosis. In an in vivo murine model, Spanos *et al*. demonstrated that HPV-positive tumors were more sensitive to radiation, with complete clearance at 20 Gy, compared with HPV-negative tumors. However, this response was not observed in immune-incompetent mice[63]. Overexpression of E6 and E7 proteins is usually found in the nucleus of HPV-positive transformed cells[64, 65]. E7 oncoprotein reportedly represses the major histocompatibility complex class I heavy chain promoter[66]. Therefore, E6 and E7 proteins may rarely be exposed to immune-surveillance cells. However, radiochemotherapy-induced cell damage might unmask these proteins to the immune system. It has been hypothesized that radiation increases the percentage of activated circulating $CD8^+$ and $CD4^+$ lymphocytes, which is correlated with improved survival[67].

Over the past decades, it has been well recognized that HPV infection and p16 positivity significantly improve the prognosis in HNSCCs. However, there are still apparent inconsistencies such as viral integration into the cellular genome, HPV genome copy numbers, and the expression level of HPV oncoproteins. Ongoing research is warranted to further elucidate the mechanisms by which HPV infection influences treatment response and outcome of HNSCCs.

Conflict of Interest Disclosure

The author has no conflicts of interest directly relevant to the content of this article.

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