

Possible Oncogenic Viruses Associated with Lung Cancer

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Abstract: Lung cancer is the most common cause of cancer death worldwide. Tobacco smoking is the most predominant etiology for lung cancer. However, only a small percentage of heavy smokers develop lung cancer, which suggests that other cofactors are required for lung carcinogenesis. Viruses have been central to modern cancer research and provide profound insights into cancer causes. Nevertheless, the role of virus in lung cancer is still unclear. In this article, we reviewed the possible oncogenic viruses associated with lung cancer.

Keywords: oncogenic virus, lung cancer, human papillomavirus, Merkel cell polyomavirus, Epstein–Barr virus, jaagsiekte sheep virus, John Cunningham virus

Introduction

Lung cancer is the most common cause of cancer death worldwide, with an estimated 1.8 million deaths each year.¹ Lung cancer is divided into two main categories: non-small-cell lung cancer (NSCLC) that comprises approximately 85% of lung cancer cases and small-cell lung cancer (SCLC) that comprises about 15%.² Tobacco smoking is the most predominant etiology for lung cancer, accounting for more than 80% of cases in the US and other countries where cigarette smoking is common.³ Lung cancer in nonsmokers is more common in women and in Asia and is a different disease with molecular characteristics that differ from lung cancer in smokers.⁴ This suggests that other factors are required for lung carcinogenesis, which include inherited genetic susceptibility and infectious agents, such as virus.³

Since Rous's initial experiments suggesting virus as a possible transmissible agent for cancer, the virus-associated cancer research field has witnessed a roaring progress over the last century.⁵ Until now, seven human viruses have been discovered to cause 10–15% of human cancers worldwide, including Epstein–Barr virus (EBV), hepatitis B virus (HBV) or hepatitis C virus (HCV), human T-lymphotropic virus-1 (HTLV-1), human papillomavirus (HPV), Kaposi's sarcoma herpesvirus (KSHV), and Merkel cell polyomavirus (MCPyV).^{5,6} Mounting evidences point to a potential role of several viruses for lung cancer (Figure 1.), such as HPV,^{7–9} MCPyV^{10–12} and EBV.^{13–15} It has been reported that Jaagsiekte sheep retrovirus (JSRV) spontaneously induces a transmissible lung adenocarcinoma through the viral envelope protein in sheep.¹⁶ However, human lung cancer shows no cogent evidence of being associated with oncogenic viruses so far.¹⁷ In this article, getting more insight into viral etiology for lung cancer, we thoroughly reviewed the possible oncogenic viruses associated with lung cancer through systematic literature searching.

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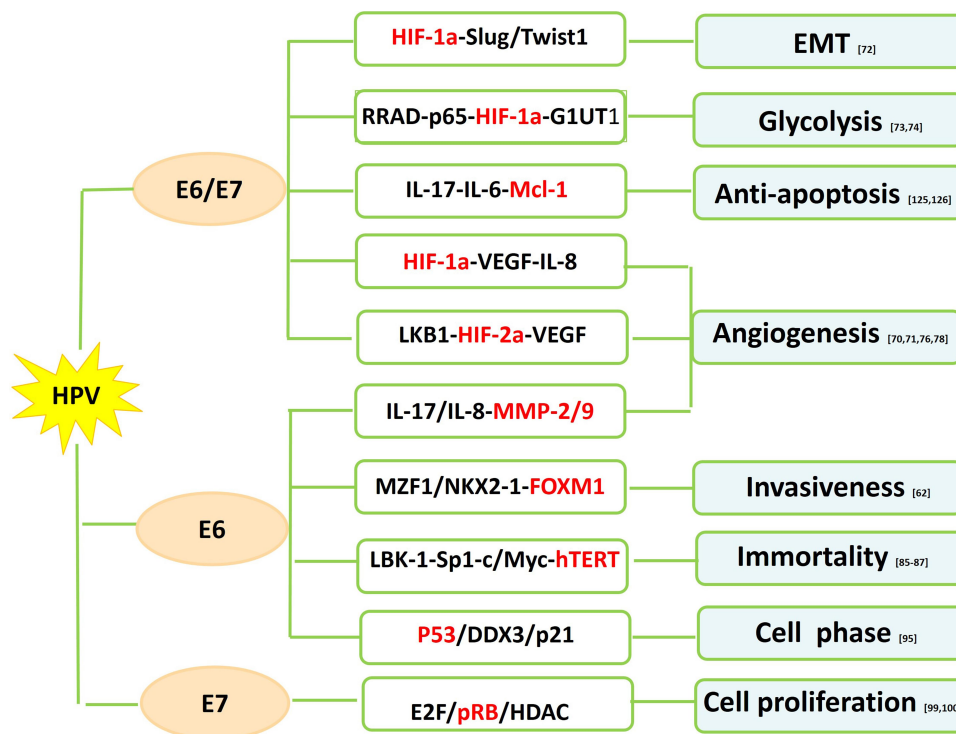


Figure 1 HPV-mediated oncogenic mechanisms in NSCLC.

HPV

HPV Detection in Lung Cancer Tissues

Since Syrjanen's suggestion of HPV involvement in bronchial squamous carcinoma reported in 1979,^{18,19} several studies have explored this relationship between HPV infection and lung cancer occurrence. However, the results of these studies have been inconsistent, with some authors in favor of this association^{20–36} and the others not^{37–43} (Table 1). HPV infection is the most common sexually transmitted infection, with estimates for the probability of infection with the virus exceeding 80% for women and 90% for men across their lifetime.⁴⁴ The HPV DNA is detected in lung cancer tissue, but also detected in peripheral blood, bronchial brushing, and the exhaled breath condensate of patients with lung cancer.^{45–47} HPV 16 and 18 are the two most common genotypes detected in lung cancer worldwide.⁴⁸ The other frequently detected high-risk subtypes are HPV 31 and 33 and the most prevalent low-risk subtypes are HPV 6 and 11.¹⁹ Recently, Xiong et al conducted a meta-analysis comparing HPV infection rates in lung cancer tissues (19.8% for HPV 16 and 18.59% for HPV 18) vs noncancer controls (5.84% for HPV 16 and 4.29% for HPV 18) and found that HPV infection was a risk factor of lung cancer.⁷

Several studies have reported a higher prevalence of HPV infection in lung cancer tissues derived from patients in Asian than other continents.^{7,9,19,20,25,26,32} Syrjanen shows that the average HPV infection rate in lung cancer tissues worldwide is 26.5%, the highest in China (37.7%), the lowest in North America (12.5%), with Australia, Europe, South America, and other Asian regions (18.5%, 16.9%, 23.9%, and 17.2%), respectively.¹⁹ Moreover, HPV infection rate in squamous cell carcinoma (25.1%) is found to be higher than that in adenocarcinoma (15.1%). This result is consistent with that of the study by Xiong et al, which may be explained by the high affinity of HPV to squamous epithelial cells.⁷ They further explored that the reported wide variability in HPV infection rates of lung cancer tissues was not majorly owing to the HPV detection methods, but was better explained by the geographical origin of the study and the histological type of lung cancer.¹⁹

Transmission Routes of HPV into Lung

HPV can be transmitted through physical contact as well as vertically from the HPV-positive mother to her newborn and cause subclinical or clinical infection.⁴⁹ How HPV is transmitted into the lung, however, remains unidentified. Several studies suggested that HPV might be transmitted

Table 1 Key Findings of Possible Oncogenic Viruses Associated with Lung Cancer

Virus	Whether Lung Cells are Infected and Transformed	Animal Models of Virus-induced Lung Cancer	Receptors Expressed in Lung Cells	Specific Type of Lung Cancer Related to the Virus	Detection of Viruses in Lung Cancer Tissues				Epidemiology	Viral Genome		Oncogenic Mechanisms
					NGS	PCR	ISH	IHC		Integration		
HPV	NA	HPV-induced SCLC transgenic murine model ³²	HSPG ^{58,61}	NA	NA	From 0 to 81.8%	From 0 to 44.1%	NA	NA	Integrated ³⁶	See Figure 1	
						20-24,26-31,34,36-38,40,43	E6/E7/LI casid protein + 23,24,30,33,34,36					
MCPyV	NA	NA	Sulfated glycosaminoglycans ¹⁵⁴	NA	NA	From 0 to 40.7%	NA	NA	NA	Integrated ¹⁰	Deregulation of BRAF and Bcl-2 ¹⁴⁹ RASSF1A tumor suppressor hypermethylation ¹⁴⁶	
						10,11,145-151,153	LT antigen + ^{10,152}					
EBV	NA	NA	CD21 ^{168,169}	PLELc ^{13,161-164}	Genomic diversity ¹⁵	NA	EBER 100% for PLELC 13,161-163	EBV DNA detected in serum ^{14,170,171}	NA	NA	NA	
JSRV	JSRV Env transforms human lung epithelial cells in vitro ^{174,182}	Animal models of OPA ¹⁷²	Hyal ¹⁷⁵	Lepidic adenocarcinoma 172,173	Negative ¹⁸³	JSRV DNA/RNA ± 177,178,180,181	NA	JSRV Env and Gag proteins ± 176,177,182	JSRV DNA detected in blood ¹⁷⁹	NA	JSRV envelope ¹⁶	
JCV	NA	JCV T-antigen induced murine model ¹⁹¹	Terminal α2,6-linked sialic acid ¹⁸⁸	NA	NA	JCV T-antigen sequences found ^{184,185,188,190}	T-antigen + ¹⁸⁸	NA	NA	NA	Inactivation of p53 and pRb and deregulation of Wnt signaling pathway ¹⁸⁷	

Abbreviations: NA, not available; HPV, human papillomavirus; MCPyV, Merkel cell polyomavirus; EBV, Epstein-Barr virus; JSRV, Jaagsiekte sheep retrovirus; JCV, John Cunningham virus; SCLC, small-cell lung cancer; OPA, ovine pulmonary adenocarcinoma; HSPG, heparan sulfate proteoglycans; Hyal2, hyaluronidase-2; PLELC, pulmonary lymphoepithelioma-like carcinoma; NGS, next-generation sequencing; ISH, in situ hybridization; EBER, EBV-encoded RNA; IHC, immunohistochemistry.

to the lung from the aerodigestive tract, for instance, oral mucosa, esophagus, larynx, or sinonasal mucosa.^{50–52} In addition, the findings of higher risk of developing lung cancer in female patients with anogenital malignancies than those without^{53,54} and morphological resemblance of HPV-infected bronchial squamous cell carcinoma to HPV-infected genital warts¹⁸ indicate that HPV may be transmitted to the lung from the genital tract.⁵⁵

Blood circulation may be another transmission route for HPV infection in the lung. Chiou et al found that HPV 16 DNA in blood circulation was significantly associated with that in lung cancer tissue.⁴⁶ They proposed that peripheral lymphocytes could harbor HPV particles and might be involved in the spreading of HPV viral particles.

Several studies have confirmed the presence of HPV DNA in surgical smoke and previous cases of treating gynecologist developing laryngeal HPV-associated neoplasm after performing laser therapy have been reported as well.⁵⁶ In addition, Carpagnano et al found the presence of HPV in the exhaled breath condensate of patients with lung cancer.⁴⁵ This evidence suggests another possible means of HPV transmission through inhalation.

Interplay of HPV with Lung Cell Receptors and Cell Entry

Cell entry is a fundamental process of the infectivity of any virus into host cell.⁵⁷ Host cell entry of HPV is initiated by binding of the virus particle to cell surface receptors. Heparan sulfate proteoglycans (HSPG) is suggested as the initial attachment receptor for HPV and is ubiquitously expressed in the extracellular matrix and on the surface of most cells, including baseline membrane of type 1 alveolar epithelial cells and endothelial cell and endothelial cell surface.⁵⁸ HPV can specifically attach to exposed basement membrane HSPG, followed by a series of conformational changes and, ultimately transfer of encapsidated plasmid DNA into the host cells.⁵⁹ Although evidence of infectivity of human lung cell lines by HPV is lacking, the presence of HSPG in lung cells indicates that HPV may interact with these receptors and enter into lung cells. Moreover, mutation and modification in HSPG chains/sulfation patterns on a variety of solid tumors has been demonstrated.⁶⁰ In particular, a recent study showed HPV capsids preferentially bind and infect lung cancer cells in vitro and in vivo, at least supporting HPV as a cofactor in the process of lung cancer carcinogenesis.⁶¹

HPV and Survival

Several studies have explored the impact of HPV infection on lung cancer prognosis.^{23,34,37,62–66} However, the results of these studies have been inconsistent, with some authors supporting the prognostic role of HPV infection^{34,64–66} and others supporting no association.^{23,37,62,63} Among them, Miyagi et al suggested that high intratumor infiltration of Langerhans cell might be responsible for better prognosis of HPV-infected lung cancer.⁶⁶ Wang et al demonstrated the prognostic value of HPV status in lung adenocarcinoma, showing that patients with HPV-infected lung adenocarcinoma had a better prognosis than those without, with a 32% reduction in mortality.³⁴ Guo et al further conducted a meta-analysis confirming the association between HPV infection and improved survival for lung adenocarcinoma patients, but not for squamous cell carcinoma patients.⁶⁷ It seems mutually conflicting when it comes to HPV with pro-oncogenic potential seeming to improve survival of patients with lung cancer. We can only hypothesize that the presence of HPV may attract more immune cells including Langerhans cells in the tumor microenvironment and trigger stronger antitumor immune responses, leading to better prognosis. Without doubt, more evidence is needed to explain this phenomenon.

Oncogenic Mechanisms of HPV-associated NSCLC

HPV-16 E6/7 and HIF-1 α /HIF-2 α

HIF-1 α is a transcription factor involved in the regulation of angiogenesis, which plays a vital role in tumor progression, metastasis, and drug resistance.⁶⁸ VEGF, one of the key downstream targets of HIF pathway, regulates vessel formation through their effect on endothelial cell migration, proliferation, permeability and survival.⁶⁹ HIF-1 α expression tends to be higher in HPV-infected NSCLC than HPV-negative NSCLC. HPV-16 E6/7 oncoproteins significantly potentiate angiogenic phenotype of NSCLC cells in vitro and in vivo by upregulating the expression of HIF-1 α , VEGF and IL-8^{70,71} (Figure 1). PI3K/Akt and c-Jun signaling pathway might be responsible for HIF-1 α /VEGF-mediated angiogenesis triggered by HPV-16 oncoproteins.⁷⁰ HPV-16 E6/7 also promote NSCLC progression by facilitating epithelial-mesenchymal transition (EMT) process through their effect on EMT-related transcription factors including ZEB1, Snail1, Slug and Twist1.⁷² Moreover, upregulated expression of HPV-16 E6/7 enhanced GLUT1 expression

in NSCLC cells through the inhibition of RRAD and translocation of p65,⁷³ suggesting HPV oncoproteins involved in the regulation of the Warburg effect.⁷⁴

HIF-2a, however, possesses similarly proangiogenic functions as to HIF-1a via the activation of downstream effectors including VEGF.⁷⁵ HPV-16 E6/7 stimulates the expression of HIF-2a and subsequent VEGF in several NSCLC cell lines by suppressing LKB1.⁷⁶

HPV E6 and MMPs/TIMP-3

MMPs are enzymes that degrade protein and collagen in the extracellular matrix (ECM) and are implicated in tumor invasion and metastasis.⁷⁷ An *in vitro* study has shown that HPV-16 E6 enhances the expression of MMP-2 and MMP-9 by stimulating IL-8 expression in lung adenocarcinoma cells.⁷⁸ Tissue inhibitor of metalloproteinase (TIMP), rather, acts as a MMP inhibitor to reduce proteolytic destruction of the cell matrix to decrease cancer metastasis and improve prognosis.⁷⁹ Loss of heterozygosity of TIMP-3 has been involved in several cancer types.^{80–82} Frequency of TIMP-3 loss by LOH and/or promoter hypermethylation is higher in HPV-16/18 infected NSCLC than HPV-16/18 negative NSCLC.⁶³ Loss of TIMP-3 potentiates malignant behaviors and poor survival of HPV-infected NSCLC by elevating IL-6 production via the tumor necrosis factor α /nuclear factor κ B axis.⁶³

HPV E6 and hTERT

The activation of human telomerase reverse transcriptase (hTERT), a catalytic subunit of the enzyme telomerase, is implicated in the process of human cell immortality and malignant transformation.⁸³ The hTERT expression is found to be elevated in NSCLC including preneoplastic lesions, suggesting its role in the early stage of lung cancer development.⁸⁴ HPV E6 seems to activate hTERT overexpression in HPV related lung cancer.⁸⁵ Cheng et al further explored that Sp1 cooperated with c-Myc to activate hTERT transcription in HPV E6-positive lung cancer cells under the context of c-Myc induced by E6 promoting its binding onto hTERT promoter.⁸⁵ However, p53 has been reported to inhibit hTERT expression by binding to Sp1 and preventing its access to the hTERT promoter.⁸⁶ Moreover, LBK1 inhibition and subsequent Sp1 upregulation are required for the HPV E6-mediated hTERT upregulation in lung carcinogenesis.⁸⁷

HPV E6 and p53

p53 has been described as “the guardian of the genome” because of its role in conserving the integrity of the genome by inducing cell cycle arrest or apoptosis on DNA damage. Inactivation of tumor suppressor p53 has been found to occur in most cancers including lung cancer. The classic function of oncogene protein E6 is to induce p53 degradation through its binding to the LxxLL motif of the cellular ubiquitin ligase E6AP.^{88,89} E6-mediated p53 inactivation results in chromosomal instability and increased potential of HPV-infected cells becoming malignant.⁸⁹ Transcription of p21^{WAF1/CIP1} and mdm-2, two downstream targets of p53, are inhibited by E6 in lung tumors. p21^{WAF1/CIP1}, a cyclin-dependent kinase (CDK) inhibitor, acts on cyclin E/cdk2 complexes and inhibits the phosphorylation of the pRb protein, thus preventing S phase entry.⁹⁰ Induction of p21 is fulfilled through p53-dependent⁹¹ or p53-independent⁹² pathways. mdm-2, a cellular oncogene product, regulates the activity of p53 protein, which in turn modulates the transcription of mdm-2 gene.⁹³ The human dead-box RNA helicase (DDX3), which plays a role in the regulation of gene expression via RNA metabolism,⁹⁴ has been implicated in the development of viral-associated cancers.⁹⁵ DDX3 transcription is directly regulated by p53 and DDX3 synergistically promotes p53-activated p21 transcription via increased Sp1 binding affinity onto the p21 promoter in NSCLC cells.⁹⁵ p21 reduction by the E6-inactivated p53 pathway contributes to tumor progression and a poor relapse-free survival in lung cancer patients.⁹⁵

HPV E7 and pRb

Oncoprotein E7 targets retinoblastoma suppressor protein (pRb) to induce its degradation, allowing the dissociation of the E2F/pRb/histone deacetylase (HDAC) complex and deregulation of cell proliferation.⁹⁶ E7 also target and degrade the “pocket proteins” p107 and p130, both of which are E2F regulators.⁹⁶ p16^{INK4A}, an inhibitor cyclin-dependent kinase, is mapped to a critical region at chromosome 9p21 and hypermethylation of p16^{INK4A} in the CpG-rich promoter regions occurs frequently in NSCLC.^{97,98} E7-mediated pRb degradation leads to the release of HDAC to enhance p16^{INK4} hypermethylation through chromatin remodeling by HDAC in HPV-infected tumors.^{99,100} Wu et al confirmed the potential correlation between p16^{INK4A} hypermethylation and HPV infection in nonsmoking female patients with NSCLC with

the finding that p16^{INK4A} hypermethylation frequency was as high as 70% with HPV infection as compared to those without HPV infection.¹⁰⁰ Reports from the same research group further indicated the linking of expression of DNA methyltransferase 3 (DNMT3) protein and HPV infection.¹⁰¹ They argued that, HPV infection upregulated DNMT3 protein expression, which subsequently increased p16^{INK4A} hypermethylation.

HPV and FHIT LOSS

The fragile histidine triad (*FHIT*) gene at chromosome 3p14.2 is altered by loss of heterozygosity (LOH) and occasional homozygous deletions in various human cancers including lung cancer.^{102,103} Allelic deletion of *FHIT* plays an important role in lung tumorigenesis¹⁰⁴ and can be used as a negative prognostic marker.¹⁰⁵ After HPV infection, HPV DNA integration into the fragile site FRA3B adjacent to *FHIT* occurs to cause allele loss of the gene.¹⁰⁶ A study from Taiwan reported a high frequency of *FHIT* LOH in HPV-positive nonsmoking female lung cancer patients, suggesting its possible role in HPV-infected lung carcinogenesis.¹⁰³ Carpagnano et al found microsatellite alterations (MA) at chromosome 3p existed in 100% of HPV-positive NSCLC patients enrolled in their study.¹⁰⁷ Yu et al further suggested *FHIT* loss and p53 mutation might synergistically exerted on HPV-infected lung carcinogenesis.¹⁰⁸ Verri et al found that different mechanisms as promoter methylation and LOH interplay to inactivate *FHIT* expression.¹⁰⁹

HPV E6 and EGFR Mutation

EGFR somatic mutation is associated with HPV presence in NSCLC.¹¹⁰ A meta-analysis including four studies with a total of 498 Asian patients showed the presence of EGFR somatic mutation was significantly higher in HPV-positive patients compared with HPV-negative counterpart ($P=0.012$).¹¹⁰ Several studies reported that HPV infection in NSCLC denoted a better overall survival and better response to EGFR-TKI therapy.^{111,112} This observation could be explained that HPV-positive NSCLC patients are more likely to exhibit EGFR somatic mutation, thus having a better response to EGFR-TKI and better survival. However, the association between HPV infection and EGFR mutation or response to EGFR-TKI seems to be limited by geographical origin since Marquez-Medina et al reported the negative outcomes obtained from western patients.¹¹³ The underlying mechanism of this relationship remains unknown.

Inhibitors of antiapoptosis proteins (IAP), including cIAP1, cIAP2 and XIAP, are a family of caspase inhibitors that block cell apoptosis and are considered as a therapeutic target in lung cancer.¹¹⁴ Wu et al indicated that HPV-16 E6 led to cIAP2 upregulation through phosphorylation of cAMP response element-binding protein (CREB) via EGFR/PI3K/AKT pathway and cIAP2 expression correlated with EGFR mutation.¹¹⁵ Inflammatory-induced oxidative stress is implicated in the development of lung adenocarcinoma and the level of 8-hydroxy-2'-deoxyguanosine (8-OH-dG), an oxidative stress biomarker, is closely associated with EGFR mutation in lung cancer.¹¹⁶ Tung et al found that HPV16/18 E6 elevated 8-OH-dG through increased ROS production, which in turn cooperated with HPV16/18 E6 to contribute to EGFR mutation in NSCLC.³³

HPV and Smoking Exposures

Tobacco smoking is one of the well-known risk factors for developing lung cancer. However, only a small percentage of heavy smokers develop lung cancer. This phenomenon suggests that other cofactors are required for lung carcinogenesis. Whether HPV infection has a synergistic effect with smoking on lung carcinogenesis remains unknown. Munoz et al demonstrated that the proliferative rate and anchorage-independent growth of HPV16 E6/7 transfected lung epithelial cells were significantly elevated when exposed to cigarette smoke components (CSC), suggesting that the functional interaction between cigarette smoking and HPV infection promoted the possibility of lung carcinogenesis.¹¹⁷ Pena et al further showed that CSC activated HPV16 p97 promoter through their effects on the long control region (LCR) in lung epithelial cells.¹¹⁸ Moreover, HPV16 E6/7 was able to increase oxidative DNA damage induced by CSC.¹¹⁸

Benzo[a]pyrene (B[a]P), a major constituent of cigarette smoke, is associated with lung cancer development.¹¹⁹ B[a]P can increase the number of virions and genomes of HPV.¹²⁰ B[a]P treatment contributes to gene promoter hypermethylation, which is the main pathway implicated in repair gene inactivation.¹²¹ In addition, combination of inactivation of repair genes and exposure to B[a]P facilitates DNA damage.¹²² Interestingly, HPV acts synergistically with B[a]P to induce DNA damage in NSCLC cells, promoting lung carcinogenesis, especially in female patients.¹²³

Others

HPV E6/7 and Mcl-1

Myeloid cell leukemia (Mcl)-1 is an antiapoptotic member of the Bcl-2 family that contributes to the control of cancer development.¹²⁴ The presence of Mcl-1 is implicated in cancer cell growth and evasion of apoptosis in various cancer types, including lung cancer.¹²⁴ Concomitant expression of IL-6 or IL-17 and Mcl-1 is colocalized with HPV DNA in NSCLC.¹²⁵ HPV E6/7 leads to upregulated expression of IL-6 or IL-17 and Mcl-1 through phosphatidylinositol-3-OH kinase pathway.¹²⁶ The micro-environmental inflammation manifested by high level of IL-6 and IL-17 secreted by lung cancer cells in response to HPV stimuli is, therefore, likely to be responsible for HPV-infected lung tumorigenesis.^{125,126}

HPV E6/7 and FOXM1

Increased expression of Foxhead box M1 (FOXM1) is associated with tumor progression and poor prognosis in various cancer types including NSCLC.¹²⁷ FOXM1 is upregulated by E2F released by Rb phosphorylation through p53 inactivation and interacts with HPV-16 E7 to enhance the transformation potential of rat embryo fibroblasts.¹²⁸ Chen et al failed to identify E7-triggered FOXM1 upregulation in HPV-positive cancer cells including lung cancer cells.⁶² However, elevated FOXM1 expression was triggered by E6 through the MZF1/NKX2-1 axis, which activated beta-catenin nuclear translocation and subsequently potentiated cell invasiveness and stemness in HPV-positive NSCLC.⁶²

HPV E6 and miR-30c-2/MTA-1

miR-30c-2 is one of tumor suppressor microRNAs which are implicated in tumor development. The downregulation of miR-30c-2 promotes the invasion of NSCLC by targeting metastasis-associated protein-1 (MTA-1).¹²⁹ Wu et al demonstrated that HPV-16/18 E6 negatively correlated with miR-30c-2 expression and positively with MTA-1 in NSCLC tissues and expression levels of miR-30c-2 and MTA-1 could predict prognosis and therapeutic response to chemotherapy of patients with NSCLC.¹³⁰

Experimental Models of HPV-Associated Lung SCLC

In vitro and in vivo animal models are widely used in HPV research.¹³¹ No data are available concerning experimental models used to investigate how HPV infects and

transforms lung cells. Importantly, Carraresi et al initially established a transgenic mouse model of SCLC induced by HPV-16 E6/E7 oncoproteins under the control of the cytokeratin 5 gene promoter.¹³² Furthermore, they developed two murine cell lines derived from transgenic lung SCLC, both of which showed absence of p53 and pRB and sustain tumor formation after subcutaneous injection in syngenic mice.¹³³ These findings provide more direct evidence for ability of HPV to induce SCLC, possibly through the inactivation of p53 and pRB.

Possible Connection of HPV Vaccination and Lung Cancer

Prophylactic HPV vaccination, mainly covering girls and women under 25, is currently included into national vaccination programs in 60 countries worldwide.¹³⁴ It aims at the formation of virus-neutralized antibodies and expected to protect from developing cervical cancer based on the established association between HPV and the progression of cervical neoplasia. The possible involvement of HPV in lung cancer may necessitate the introduction of prophylactic vaccination in both boys and girls. Undoubtedly, more lines of evidence are warranted to establish definitive evidence of HPV as an oncogenic factor of human lung cancer and to verify whether there is an impact on the lung cancer incidence of HPV-directed vaccine meant to prevent cervical cancer.

Merkel Cell Polyomavirus (MCPyV)

With the discovery by Feng et al in 2008 of Merkel cell polyomavirus (MCPyV) as a causative agent of Merkel cell carcinoma (MCC),¹³⁵ several authors have investigated the association between the presence of MCPyV in several human tumors including lung cancer.¹³⁶⁻¹³⁹ MCPyV infections are widespread in the human population with MCPyV continuously shed from healthy skin.¹⁴⁰ Moreover, MCPyV DNA fragments have been detected in a wide variety of anatomical locations, including lower respiratory tract.¹⁴¹ A recent study investigating MCPyV presence in 10 autopsies with an extensive organ sampling revealed a high prevalence of MCPyV was found in lung samples as well as in blood and brain samples.¹⁴² Persistent presence of MCPyV in the respiratory tract may facilitate the development of lung cancer.

MCPyV and Lung Cancer

Since SCLC harbors the histological similarities towards MCC,^{143,144} whether MCPyV leads to the development of

SCLC has caught the attention of researchers. Studies have detected viral DNA sequences in SCLC tissues.^{145,146} However, there is evidence indicating no role of MCPyV in SCLC.^{147,148} Furthermore, the prevalence of MCPyV in NSCLC has been investigated as well. Researchers found varying degrees of MCPyV infection positivity in NSCLC at the DNA, RNA and protein levels.^{11,149–153} Hashida et al provided the first evidence of not only the detection of MCPyV DNA but also the expressions of both LT RNA transcripts and LT antigen in NSCLCs.¹⁰ They revealed that nine out of 32 SCC, nine out of 45 AC, one out of 32 large-cell carcinoma, and one out of three pleomorphic carcinomas were positive for MCPyV DNA.¹⁰ Another study showed statistically significant difference between stages of NSCLC and MCPyV LT-Ag DNA load, which suggested viral load may be increased with tumor progression.¹¹

Possible NSCLC-Specific Oncogenic Mechanisms

Currently, neither human cell lines nor animal models are available to explore lung carcinogenesis by MCPyV. Like HPV, cell entry of MCPyV follows a two-step attachment-and-entry process¹⁵⁴ and abundant expression of sulfated glycosaminoglycans in lung cells provides a basis for MCPyV to enter lung cells.

Integration of MCPyV DNA into the host cell genome is thought to cause MCC through the constitutive expression of the transforming large T (LT) and small T (ST) proteins with distinct mechanisms.¹⁵⁵ However, the pathogenesis of MCPyV-induced NSCLC remains to be determined. It has been suggested that mutant BRAF drives the development of lung adenocarcinoma.¹⁵⁶ Heterodimer of Bax/Bcl-2 induces a neutralization of Bax and a loss of apoptosis.¹⁵⁷ Lasithiotaki et al revealed increased expression of BRAF as well as the downregulation of Bcl-2 in MCPyV-positive NSCLC samples as compared to MCPyV-negative NSCLC samples, suggesting a role of MCPyV in NSCLC through deregulation of BRAF and Bcl-2.¹⁴⁹ They further demonstrated that the expression of NSCLC-associated microRNAs (miR-21, miR-376, and miR-145) and their corresponding target genes were influenced by the presence of MCPyV in lung cancer tissues, providing evidence of a MCPyV-associated epigenetic mechanism in NSCLC.¹² Xu et al found a significant correlation between MCPyV infection and EGFR mutations by screening 189 NSCLC

samples.¹⁵² Their finding suggested MCPyV infection might induce EGFR mutations in NSCLC. If this were the case, it would explain the phenomenon provided by Lasithiotaki et al of higher expression of BRAF in MCPyV-positive samples since BRAF is a downstream target of EGFR pathway.¹⁴⁹ Hashida et al identified two MCPyV integration sites (5q23.1 and 11q25) in NSCLC patients.¹⁰ However, both integration sites were not close to the EGFR gene location (7p12).¹⁰ Therefore, whether MCPyV infection induces EGFR mutations in NSCLC warrants further investigations.

Epstein–Barr Virus (EBV)

EBV is a lymphotropic gamma herpes virus with oncogenic properties infecting more than 90% of adults worldwide. It is directly involved in the pathogenesis of a variety of lymphoproliferative and neoplastic disorders, including undifferentiated nasopharyngeal carcinoma and lymphoepithelioma-like carcinoma (LELC) at various sites.^{158,159} The association of EBV and lung cancer presents significant differences according to tumor histotype and geographical site.^{15,160} The EBV is often detected in pulmonary LELC occurring in patients from east and southeast Asia where nasopharyngeal carcinoma is highly prevalent,^{13,161–164} but rarely detected or even undetected in other types of lung cancer such as adenocarcinoma, squamous cell carcinoma, and SCLC.^{42,165,166} Recently, Wang et al explored the EBV genomic variations in lung carcinoma and reported four newly sequenced EBV genomes isolated from primary lung carcinomas with apparent genomic diversity among these EBV genomes.¹⁵ However, whether EBV genomic variations contribute to lung carcinogenesis remains unknown and deserves further investigation.

It is well-known that cell entry of EBV is initiated by the interaction of the viral envelope glycoprotein gp350 with the cellular surface receptor CD21 of B cells and epithelial cells.¹⁶⁷ Several studies have demonstrated the expression of EBV receptor CD21 in human bronchial epithelium,¹⁶⁸ and more specifically in type 2 alveolar epithelial cells,¹⁶⁹ implying possibility of EBV to infect and possibly transform lung cells in a CD-21 dependent manner. Yet, it needs further investigations.

Previous studies have measured circulating EBV DNA in the plasma of patients with pulmonary LELC and suggested its role for monitoring response to therapy.^{170,171} Recently, Xie et al performed a prospective multicenter study in Southern China investigating the association

between baseline EBV DNA and OS and disease-free survival (DFS) in a total of 429 patients with pulmonary LELC and showed that baseline EBV DNA copy of at least 4000 copies/mL predicted disease recurrence and poorer survival among patients with early- or advanced-stage pulmonary LELC.¹⁴ Through sequential blood draw, they found that plasma EBV DNA frequently preceded disease progression during posttherapy follow-up. Moreover, patients with persistently detectable plasma EBV DNA after radical resection had significantly worse OS and DFS than did those with EBV DNA after surgery.¹⁴ The above findings further supported an oncogenic role of EBV in a fraction of Asian patients of pulmonary LELC. Nevertheless, neither in vitro cellular nor animal models exist currently verifying EBV's ability to infect and transform malignantly human lung cells. Therefore, the possible oncogenic mechanism in EBV-associated lung cancer remains unknown.

Jaagsiekte Sheep Retrovirus (JSRV)

JSRV is a known betaretrovirus capable of inducing the formation of transmissible lung cancer in sheep called ovine pulmonary adenocarcinoma (OPA).¹⁷² OPA is characterized by the multifocal mixed presentation of adenocarcinoma, with its early lesions having similarity to lepidic-predominant adenocarcinoma and its advanced lesions resembling adenocarcinoma with papillary or acinar characteristics.¹⁷³ JSRV infects and transforms bronchiolar and alveolar epithelial cells, namely type II pneumocytes and club cells.¹⁷⁴ The cellular receptor for JSRV is hyaluronidase-2 (Hyal2) and Hyal2 has been shown to mediate human cell entry of JSRV Env pseudotyped retroviral particles.¹⁷⁵ The envelope (Env) protein of JSRV is an oncogenic protein and its carcinogenic property has been demonstrated in sheep and mice in vivo and in various cell lines in vitro, including human bronchial epithelial cells.¹⁶

Given that the capacity of JSRV to induce OPA and histological similarities between OPA and human adenocarcinoma, as well as due to the findings that human bronchial epithelial cells express Hyal2 for JSRV entry and JSRV Env protein transforms human lung epithelial cells in vitro, numerous studies have explored the role of JSRV in the induction of human lung cancer.^{176–183} By immunohistochemical analysis on human lung tissues using an antiserum to JSRV capsid protein, De las Heras et al revealed a positive reaction in 30% of 129 human lepidic adenocarcinomas, 26% of 65 other adenocarcinomas, and two of seven large cell

carcinomas.¹⁷⁶ By human lung cancer tissue arrays, Linnerth-Petrik et al further found the presence of JSRV Env protein by immunostaining and JSRV Env and Gag sequences by PCR in a subset of human adenocarcinomas.¹⁷⁷ In addition, JSRV related DNA sequences have been detected in paraffin sections of lepidic adenocarcinoma specimens from lung cancer patients in Sardinia¹⁷⁸ and in blood of Africans from Nigeria and Cameroon.¹⁷⁹ Nonetheless, other groups failed to find evidence for JSRV Env and Gag proteins by immunostaining¹⁸² or for JSRV DNA or RNA by PCR in human adenocarcinoma.^{180,181} Recently, a more definitive method using a high-throughput sequencing approach was also unable to find evidence for JSRV sequences in five lepidic adenocarcinomas.¹⁸³ Until now, no conclusive evidence exists regarding the link between JSRV and the development of lung adenocarcinomas in humans and much needs to be done.

John Cunningham Virus (JCV)

JCV is a member of polyomavirus family infecting a large proportion of the population worldwide and 80% to 90% of adults are seropositive.¹⁸⁴ Recent literature reports the presence of JCV in various types of human neoplasm, including lung cancer.¹⁸⁵ The JCV T-antigen is considered to play an important role in JCV-associated carcinogenesis.¹⁸⁶ The T-antigen can inactivate tumor suppressor proteins, p53 and pRb, and deregulate the Wnt signaling pathway through promoting the stability and accumulation of beta-catenin via direct binding, which culminating in uncontrolled proliferation and immortal survival.¹⁸⁷ Giuliani et al, for the first time, suggested the presence of JCV in lung tumors by showing JCV sequences were amplified in one lung carcinoma only.¹⁸⁴ Zheng et al examined the JCV by targeting JCV T-antigen and expression of Ki-67, caspase-3, beta-catenin, p53, and pRb in 103 lung carcinomas and 18 normal lung tissues.¹⁸⁸ In their study, the detection rate and copy number of JCV was higher in lung carcinoma than in normal lung tissue. JCV copies correlated positively with expression of Ki-67 and negatively with membrane beta-catenin expression, which suggested that lung carcinoma with high JCV copies exhibited high proliferation and downregulation of cell adhesion mediated by membrane beta-catenin. Moreover, JCV T-antigen was found in the nuclei of lung carcinoma cells and adjacent alveolar epithelial cells.¹⁸⁸ These above findings along with previous report indicating that terminal α 2,6-linked sialic acid is a critical component of the JCV receptor, which is

abundantly expressed in normal lungs,¹⁸⁹ suggested that JCV might be implicated in the malignant transformation of pulmonary epithelial cells and supported the notion that the respiratory tract might be a portal of entry for JCV infection.¹⁸⁹ Abdel-Aziz et al explored the presence of JCV genome in 62 lung cancer and 23 normal lung tissue by targeting the T-antigen, VP, and agnoprotein.¹⁹⁰ The JCV genome was detected in approximately half of lung cancer cases and JCV T-antigen correlated significantly with p53 and nuclear staining of beta-catenin. Sinagra et al recently investigated JCV gene sequences by targeting T-antigen in lung adenocarcinoma and its surrounding normal lung tissue.¹⁸⁵ JCV positivity was observed in seven of 13 lung cancer tissues and none were JCV-positive for surrounding normal lung tissues. Noguchi et al generated transgenic (TG) mice with a transgene including the K-19 promoter, which was specific to bronchial and digestive epithelium and the JCV T-antigen and found pulmonary tumors in two out of 15 TG mice (13.3%) without any metastasis, suggesting possible association of JCV with bronchial tumorigenesis in experimental animals.¹⁹¹

Challenges in Virus-associated Cancer Research and Future Perspectives

The global health burden of viral infection in cancer is high but underappreciated. Infectious agents are estimated to be blamed for 15.4% of cancers worldwide, with most being viruses.¹⁹² Viruses have had a chequered history in cancer biology throughout the past century.⁵ There are several inherent characteristics of viral biology making it difficult to identify viral agents as causative factors for human cancers.¹⁹³ First, most of the viruses are ubiquitous in nature, but only a small percentage of infected individuals develop cancers. Second, no human cancer arises as the acute consequence of infection. The latency period between infection and the development of a cancer make exposure markers difficult to assess along the carcinogenic process. Third, viral agents might act as indirect carcinogens, without persistence of their genes within the respective cancer cells. It remains unclear whether these viruses cause cancer solely through sustaining mature tumor cells by viral products or chronic infection and inflammation or directly through contributing to cancer cell transformation by viral oncogenes. Viral etiology research may be influenced by “hit and run” hypothesis, where the viral genes are lost as the tumor begins to mature. Fourth, species-

specific barriers often limits the use of animals as surrogate hosts to study human tumor viruses.

Even so, the exploitation for viral etiology for selected solid cancers should not cease. We are now entering a more mature phase of research with the realization that a considerable proportion of cancers are indeed caused by viruses. With the advent of new sequencing technologies, it is highly probable that this proportion will increase. The discovery of cancers with an infectious origin is critical to develop antilient viral drugs and immunological therapies. The recognition of the importance of viral cancers has already resulted in vaccines against HBV and high-risk HPV and targeted therapies against HCV and HIV, and will create more opportunities in cancer control.

Conclusion

This article highlights several important findings on lung cancer-associated oncogenic viruses. First, HPV is detected in a substantial fraction of human lung cancer tissues worldwide, with widely variable infection rates in lung cancer tissues depending on the geographical origin of the study. The association seems to be stronger in squamous cell carcinoma than in other lung cancer subtypes. Multiple oncogenic mechanisms have been proposed to play a part in HPV-infected NSCLC carcinogenesis, such as transcription of oncogenes that contribute to lung cancer cell transformation, induction of EGFR mutation, and clonal integration into the cellular genome. Furthermore, HPV might act as a cofactor of smoking exposures to facilitate lung cancer carcinogenesis. MCPyV has been put under the spotlight of searching oncogenic virus associated with lung cancer since the discovery of MCPyV being as a causative agent of MCC. Limited evidence indicates a role of MCPyV in lung cancer, especially NSCLC. Like HPV, MCPyV might also induce EGFR mutation in NSCLC, but through unknown mechanism. The association between MCPyV and lung cancer warrants further investigation. The association of EBV and lung cancer presents significant differences according to tumor histotype and geographical site. EBV is often detected in pulmonary LELC occurring in patients from east and southeast Asian and circulating EBV DNA in the plasma of patients with pulmonary LELC predicts disease recurrence. EBV may be related to pulmonary LELC, especially in east and southeast Asia, while a more general role of EBV in lung carcinogenesis seems unlikely. Although JSRV has been known to induce ovine lung adenocarcinoma through the viral envelope protein, no conclusive evidence exists related to the possible link between

JSRV and the development of lung adenocarcinomas in humans so far. Compared with the above four viruses, JCV are less-studied. Obviously, more research in the future is needed to get more insight into their role in lung carcinogenesis.

Abbreviations

NSCLC, non-small-cell lung cancer; SCLC, small-cell lung cancer; EBV, Epstein–Barr virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HTLV-1, human T-lymphotropic virus-1; HPV, human papillomavirus; KSHV, Kaposi's sarcoma herpesvirus; MCPyV, Merkel cell polyomavirus; JSRV, Jaagsiekte sheep retrovirus; HSPG, heparan sulfate proteoglycans; *FHIT*, fragile histidine triad; LOH, loss of heterozygosity; MA, microsatellite alterations; EMT, epithelial-mesenchymal transition; ECM, extracellular matrix; TIMP, tissue inhibitor of metalloproteinase; Mcl-1, myeloid cell leukemia-1; hTERT, human telomerase reverse transcriptase; IAP, antiapoptosis proteins; CREB, cAMP response element-binding protein; 8-OH-dG, 8-hydroxy-2'-deoxyguanosine; CSC, cigarette smoke components; LCR, long control region; B[a]P, benzo[a]pyrene; FOXM1, foxhead box M1; MTA-1, metastasis-associated protein-1; CDK, cyclin-dependent kinase; DDX3, dead-box RNA helicase; pRb, retinoblastoma suppressor protein; HDAC, histone deacetylase; DNMT3, DNA methyltransferase; MCC, Merkel cell carcinoma; SCC, squamous cell carcinoma; AC, adenocarcinoma; LELC, lymphoepithelioma-like carcinoma; DFS, disease-free survival; OPA, ovine pulmonary adenocarcinoma; Hyal2, hyaluronidase-2; Env, envelope; JCV: John Cunningham virus.

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Disclosure

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