MINI-REVIEW

Human Papillomavirus Type 16/18 Oncoproteins: Potential Therapeutic Targets in Non-smoking Associated Lung Cancer

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Abstract

   High-risk human papillomavirus (HPV) especially HPV-16 and HPV-18 types are speculated to be important risk factors in non-smoking associated lung cancer in Asia. Increasing evidence has demonstrated that HPV oncoproteins may contribute to lung tumorigenesis and cell transformation. Importantly, HPV 16/18 E6 and E7 oncoproteins can mediate expression of multiple target genes and proteins, such as p53/pRb, VEGF, HIF-1α, cIAP-2, and hTERT, and contribute to cell proliferation, angiogenesis and cell immortalization through different signaling pathways in lung cancer. This article provides an overview of experiment data on HPV-associated lung cancer, describes the main targets on which HPV E6/E7 oncoproteins act, and further discusses the potential signaling pathways in which HPV E6/E7 oncoproteins are involved. In addition, we also raise questions regarding existing problems with the study of HPV-associated lung cancer.

Keywords: Human papillomavirus - E6 - E7 - lung cancer - p53 - hypoxia inducible factor-1α

Introduction

   Human papillomaviruses (HPVs), double-stranded and non-enveloped DNA viruses, belong to the papillomaviridae family. Clinically, HPVs are commonly divided into two subtypes, the high-risk and low-risk type of HPV. Evidences from epidemiological, clinical, and basic studies have demonstrated that HPV infection is the necessary cause of cervix uterine cancer, especially high-risk type HPV16, which is the most predominant type indentified in cervical cancer accounting for about 50% of cases (zur Hausen, 2009). In addition, high-risk HPV 16, 18, 31, 33, 45, 52, and 61 have been found to be related with the cancers in vulva, vagina, penis, anus, oral cavity, and otopharynx, while low-risk HPV 6 and 11 may be associated with genital warts (Wang et al., 2007). During the past 20 years, variety of HPV in different subtypes has also been detected in lung cancer. These subtypes typically include HPV 6, 11, 16, 18, 31, 33 and 35, particularly HPV-16 and 18 (Park et al., 2007). With the progress of the study, HPV has been hypothesized to be a possible contributory agent for lung cancer. However, the hypothesis has not been widely accepted, and the role of HPV in lung carcinogenesis remains controversial.

   The main controversial point is that the prevalence of HPV in clinical specimens is widely divergent in different geographic regions and histological tissue types. The overall HPV prevalence ranged from 0.0 to 78.3%, with a higher prevalence in Asia, especially of Taiwanese and Chinese mainland patients, compared with the low or no HPV prevalence in European and America (Cheng et al., 2001; Srinivasan et al., 2009; Koshiol et al., 2011). This variation was once attributed to the different detection methods in library used (Guo et al., 2012).

   A recent meta-analysis suggested that the reported variability in HPV prevalence in lung cancer is better explained by geographical study origin and histological types of cancer than detection method itself (Syrjanen, 2012). But this is not a definitive answer to the question. Recently, Goto et. al (Goto et al., 2011) detected HPV infection in 176 lung squamous cell carcinoma and 128 lung adenocarcinoma from 8 areas in Asia using the same detection method and in the same library. But no significant difference of histological types was found (squamous cell carcinoma vs adenocarcinoma, 6.3% vs 7%) in the prevalence rate of HPV. Iwakawa et al. (2010) examined paraffin-embedded lung cancer tissues of Japanese patients, who are ethnically and geographical close to Taiwanese, for presence of HPV16/18/33 DNA and found no HPV DNA in the specimens. In addition, it is also difficult to interpret why HPV-associated lung cancers in Taiwanese women are more prevalent in nonsmokers, because smoking is an additional risk factor for the development of HPV-associated cervical cancer and should exert the same effect on HPV-mediated lung tumorigenesis (Cheng et al., 2001).

   A study showed that four covariates were also significantly associated with HPV detection rate. i) older age, ii) older age at smoking initiation, iii) fewer years of active smoking, iv) fewer total pack years (Syrjanen et

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al., 2012). In addition, gender and genetic polymorphism of cancer susceptibility genes should be considered when HPV was detected (Chiou et al., 2003; Cheng et al., 2004; Fei et al., 2006; Wang et al., 2006; Nunobiki et al., 2011). The relationship between HPV and lung cancer is really confusing, so more influencing factors should be considered in order to figure out the truth.

Malignant transformation caused by HPV is mainly attributed to three oncoproteins: E5, E6, and E7 (zur Hausen, 2002). It has been well established that E6 and E7 oncoproteins play a pivotal role in cervical cancer development. In recent years, a growing body of evidence has also implicated the potential role of E6 or E7 in lung carcinogenesis. Back in 1994, Australian scientists found human mesothelial and bronchial epithelial cells transfected with HPV16 E6 and E7 genes underwent at least 20 more population doublings than the normal cells (De Silva et al., 1994). In 2003, Kinjo et al. found that E2 and E6-E7 were expressed in one of the HPV transfected lung adenocarcinoma and colonic adenocarcinoma cell clones, and the cells transfected with plasmids containing HPV type 16 genes exhibited clear squamous metaplasia (Kinjo et al., 2003). E6 oncoprotein combines a conserved structural scaffold with highly variable surfaces, and E6 C-terminal was the main functional domain. E6 from high risk HPV has a four amino acid stretch ETQV/L at C-terminus, known as the PDZ-binding motif (Thomas et al., 2001). This binding site could gather PDZ domain proteins through hydrogen bonds, leading to the degradation of these proteins. Usually, PDZ domain proteins function as cellular organizing centers for signal transduction, transcriptional regulation, receptor assembly, and cell polarity (Jones et al., 2006; Zhang et al., 2007). The amino acid sequences of E7 could be divided into three conserved regions CR1, CR2, and CR3 (Phelps et al., 1988). CR2 mediates high-affinity binding to the retinoblastoma tumor suppressor protein family, which is required for E7 immortalizing and transforming activities. CR3 contains two CXXC motifs forming a zinc binding domain, which plays an important role in mediating protein dimerization and interacting with several E7-interaction proteins (Braspenning et al., 1998; Todorovic et al., 2011). More importantly, multiple oncogenic target genes, as described below in detail, may play important roles in the progression of lung tumorgenesis caused by HPV oncoproteins, especially by HPV16/18 E6 and E7 oncoproteins.

E6 Oncoprotein and p53

p53, a tumor suppressor gene, controls cell proliferation and apoptosis. It could keep integrity of the genome by inducing cell cycle arrest or apoptosis in response to DNA damage.

Now it has become clear that HPV E6 protein binds p53 and triggers its degradation through the ubiquitin pathway. E6 firstly binds to the cellular ubiquitination enzyme E6-AP, and then forms a complex with p53, leading to its proteolytic degradation. This could trigger cell cycle disruption, proliferation promotion, and apoptosis inhibition (Helt et al., 2002). The continuous degradation

of p53 by E6 protein is thought to be equivalent to p53 inactivation by genetic alterations. Although accumulating evidence has shown that HPV16/18 E6 oncoprotein was indeed expressed in lung tumors and involved in p53 inactivation (Cheng et al., 2007), the correlation between HPV infection and p53 mutations is still controversial, thus the relationship between p53 mutations and E6 expression is hard to clarify. For other HPV-associated tumors, p53 mutations in the context of E6 is incongruous, and most studies find that p53 mutation and HPV E6 expression is inversely correlated.

In lung cancer, previous studies have shown that HPV16/18 E6 contributes to p53 inactivation to down-regulate p21WAF1/CIP1and mdm2 transcription (Cheng et al., 2007). Wu et al. reported that DDX3 synergistically enhanced p53-activated p21 transcription (Wu et al., 2011). Sp1 is the bridge between p53 and p21. Moreover, DDX could enhance the interaction between p53 and Sp1 and the binding affinity of Sp1 onto the p21 promoter. But DDX transcription is positively regulated by p53, and p53 inactivated by E6 will lead to the down-regulation of p21 transcription. These reports suggest that E6 down-regulated p21 through p53-DDX pathway, which would enhance tumor progression in HPV-associated lung cancer (Wu et al., 2011). Further analysis showed that p53 binding ability was also modulated by p53 status besides E6 (Wu et al., 2011). Moreover, E6 can also suppress p53 by targeting p73, p300, and CREB-binding protein (Zimmermann et al., 1999; Park et al., 2001; Thomas et al., 2005). In addition, E7 has also been shown to be able to alter the levels as well as several functions of p53 (Jones et al., 1999; Eichten et al., 2002; Liu et al., 2007), but no related report has been found in lung cancer cells.

It was reported that E6 could down-regulate caveolin-1 via inactivation of p53 in lung cancer cells, and low level of caveolin-1 expression could partially revert HPV-mediated cell transformation (Razani et al., 2000). Carraresi L et al. found that the expression of caveolin-1 was reduced in the absence of both p53 and pRB in small lung carcinomas (Carraresi et al., 2006), indicating that the expression of caveolin-1 was also related with pRB status. Ikezoe T et al. found that non-small cell lung cancer (NSCLC) cells lost their sensitivity to oridonin-induced growth inhibition and apoptosis when p53 was suppressed by over-expression of HPV-16 E6 (Ikezoe et al., 2003).

E6 Oncoprotein and Human Telomerase Reverse Transcriptase

Human telomerase reverse transcriptase (hTERT), a key rate-limiting factor, controls the expression and activation of telomerase and plays an important role in tumorigenesis (Saini et al., 2009). The activation of telomerase at the pre-crisis stage promotes somatic cells to escape from crisis and becomes immortalized, and the dysregulation of telomerase leads to its activation in approximately 90% of human cancers (Kyo et al., 2008). Previous studies have shown that the activation of telomerase was closely related to the infection of HPV. Cheng et al. found that hTERT mRNA levels were obviously higher in HPV-16/18 E6-positive lung cancers

(Cheng et al., 2008). Sen et al. found that there was 71% correlation between telomerase activity and HPV-16/18 infection in lung cancer cells and cervical cancer cells, which may be related to the abnormal expression of hTERT (Sen et al., 2002). These studies have shown that the activation of telomerase was closely related to the infection of HPV.

c-Myc and Sp1 are two important transcription factors locating on the hTERT promoter. Sp1 contains three zinc finger motifs which bind to GC-rich sequence and can activate gene transcription (Dynan et al., 1983). c-Myc plays an important role in the induction of apoptosis. Previous studies have shown that E6 can activate hTERT by forming a complex with E6-AP or by interacting with c-Myc or other transcription factors locating on the hTERT promoter (Veldman et al., 2001; McMurray et al., 2003). For example, Cheng et al. found that Sp1 cooperated with c-Myc to activate hTERT transcription in HPV E6-positive lung cancer cells. Further results showed that c-Myc and Sp1 on the hTERT promoter were regulated by E6 in TL-1 lung cancer cells (Cheng et al., 2008). A soft-agar assay showed that the number of colonies of HPV E6-positive TL-1 cells formed in the soft agar was decreased significantly after transfection with E6-RNAi. However, the colonies did not form when the E6-positive TL-1 cells were transfected with hTERT-RNAi. These results suggest that the transcriptional activation of the hTERT gene regulated by E6 plays an important role in HPV-16/18-infected tumorigenesis in TL-1 lung cancer cells.

E6 Oncoprotein and Cellular Inhibitor of Apoptotic Protein 2

Cellular inhibitor of apoptotic protein 2 (cIAP-2), also known as MIHC and HIAP-1, inhibits the proteolytic activity of mature caspases. cIAP-2 was shown to be an important anti-apoptotic factor in cells expressing HPV-16 E6 and E7 oncoproteins (Yuan et al., 2005). E6 activated NF-κB and induced NF-κB elements binding at the cIAP-2 gene promoter. In lung cancer cells, Wu et al. (Wu et al., 2010) found that cIAP-2 could be up-regulated by E6 through the induction and activation of two NF-κB elements binding at −209 to −200 and −146 to −137 of the cIAP-2 gene promoter. E6 activated NF-κB not through the degradation of p53 or the activation of hTERT, but through the induction of nuclear binding activity of p52-containing NF-κB complexes in a PDZ binding motif-dependent manner (Wu et al., 2010). But cIAP-2 was still over-expressed in the TL cells which were transfected with E6△PDZ, lacking the PDZ binding motif (Wu et al., 2010). These findings suggest that cIAP-2 may also be up-regulated by other factors.

The presence of HPV DNA was significantly related to epidermal growth factor receptor (EGFR) mutations, and EGFR TK domain mutations that can lead to lung cancer pathogenesis were much more frequent in never smokers (Ohtsuka et al., 2006; Kato et al., 2012). cIAP-2 up-regulated by E6 was not only through NF-κB pathway but also through EGFR/PI3K/AKT pathway (Wu et al., 2010). Importantly, cIAP2 up-regulated by E6 via the

EGFR/PI3K/AKT pathway in lung cancer cells may be more effective than via the NF-κB pathway (Wu et al., 2010). The phosphorylation of CREB played a crucial role in the up-regulation of cIAP-2 by E6 in TL-1 and TL-4 cells via the EGFR/PI3K/AKT pathway. It has been shown that one CREB binding site in cIAP-2 promoter region was responsible for E6-induced cIAP-2 up-regulation in TL-1 cells.

E7 Oncoprotein and pRb

pRb, another well-known cellular tumor suppressor gene besides p53, involves in multiple processes such as cell cycle progression, DNA repair, apoptosis, differentiation, senescence, and chromatin remodeling. Usually, the impairment of pRb functions is closely associated with a higher incidence of lung cancer. But direct association between E7 and pRb is less reported, and most evidence benchmarks the study of other tumors. The E7 protein from high-risk HPV has a strong affinity to bind to unphosphorylated pRb. Binding of oncoprotein E7 to pRb can lead to the displacement of E2F transcription factor, consequently leading to the loss of the checkpoint control at G1/S transition and an uncontrolled cell proliferation (Hoenil et al., 2005). The CR3 zinc binding domain of the E7 protein contains two surface patches: one is required for E2F binding, whereas the other is required for pRb binding. E7 binding to one surface patch leads to the displacement of E2F from the other surface patch. Importantly, no homology has been detected between CR3 region of HPV E7 and other human proteins, providing a good target for designing anti-cancer drugs (Liu et al., 2006).

It is demonstrated that pRb degradation, not solely binding, is also important for the E7-induced inactivation of pRB (Gonzalez et al., 2001). Wang et al. (2008) found that there was an obvious relationship between the presence of high-risk type HPV and pRb depletion in lung cancer. pRb depletion is possibly associated with E7 induced pRb degradation. HPV-16 E7 also targets pRB-related proteins p107 and p130 degradation by a proteasome-dependent mechanism. The over-expression of p16, one of cyclin-dependent kinase inhibitor, is suggested to be a useful indicator of pRb degradation by HPV16 E7. p16 prevents the phosphorylation of pRb, which is over-expressed when pRb is inactivated.

E6/E7 Oncoprotein, Vascular Endothelial Growth Factor, Hypoxia-inducible Factor-1α

In tumor, a link between the functions of tumor suppressors and angiogenesis has long been established (Gomez et al., 2003). A less extent E6 was reported to increase the expression of vascular endothelial growth factor (VEGF), which may be p53-independent (Walker et al., 2011). Our previous studies showed that HPV-16 E6 could up-regulate hypoxia-inducible factor-1 α (HIF-1 α) and VEGF protein expression. Moreover, the increased expression of VEGF induced by HPV-16 E6 was HIF-1 α-dependent (Li et al., 2011). HIF-1α plays an important role in lung development and lung diseases such as

pulmonary hypertension, acute lung injury, and cancer (Shimoda et al., 2011). But the relationship between HIF-1α and p53 is not clear in HPV-induced lung cancer, and the role of E6 oncoprotein needs to be studied. A growing body of evidence has shown the relationship between HIF-1α and p53. For example, when HIF-1α is activated in hypoxic cells, MDM2 promotes p53 accumulation and the transcription of p53 target genes (Chen et al., 2003). Hypoxia induces transcriptional activity of p53, and p53 is stabilized through a physical association with HIF-1α (An et al., 1998). Further studies showed that the HIF-1α O2-dependent degradation domain and N-TAD domain could bind to p53 tetramers under physiological conditions (Sanchez et al., 2005). These results indicated that E6 up-regulated VEGF may be through HIF-1α-p53 pathway.

Walker et al. (2011) found that HPV E7 oncoprotein increased the levels of VEGF in a pRb-independent manner. Similarly, our previous studies showed that HPV-16 E7 oncoproteins could up-regulate the expression of VEGF in a HIF-1α-dependent manner in A549 cells, leading to tumor angiogenesis promotion in lung cancer cells (Li et al., 2011). What's more, HPV-16 E7 oncoprotein could enhance HIF-1α accumulation (Li et al., 2011). These results also suggest that there may be some intrinsically links between pRB and HIF-1α in lung cancer cells, and interaction between pRB and HIF-1α could lead to the up-regulation of VEGF. The relationship between pRB and HIF-1α has been confirmed in HEK 293 cells by Budde et al. (2005). They found that over-expression of pRB could stimulate transcriptional activation of HIF-1α. Furthermore, pRB could bind to HIF-1α and cause HIF-1α transcriptional activation, and the HIF-1α domain spanning amino acids 530-694 was mapped to be an interaction surface for pRB. Additionally, HIF-1α can reverse the function of transcription repressor pRB.

E6/E7 Oncoprotein and Interleukin, Mcl-1

Interleukins (ILs) stimulate growth-related activities of leukocytes as well as other cell types. ILs can enhance cell proliferation, differentiation, DNA synthesis, the secretion of other biologically active molecules, and the response to immune and inflammatory stimuli. Tartour et al. demonstrated that IL-17 could promote cell growth through the up-regulation of IL-6 expression in nude mice with cervical cancer (Numasaki et al., 2003). In addition, IL-17 was reported to enhance the angiogenesis and growth of NSCLC in the severe combined immunodeficiency mouse model (Numasaki et al., 2005). Chang et al. found that IL-17/Mcl-1 levels were elevated by HPV16 E6 (Chang et al., 2010). They also identified that there were significant correlations between IL-17 and IL-6 (P<0.001) or between IL-17 and Mcl-1 (P<0.001) (Cheng et al., 2008). Further, the up-regulated IL-17 levels lead to the increased Mcl-1

expression through the PI3K pathway and promote lung tumor cell progression through p53- and IL-6-independent mechanisms (Cheng et al., 2008). Similarly, Cheng et al. has shown that IL-6 and Mcl-1 protein levels were also elevated in HPV 16/18 E6- and E7- transfected A549 cells and HPV-16-infected TL-1 cells, mainly via PI3K/Akt pathway, while a positive IL-6 expression was the prerequisite of Mcl-1 expression in lung tumor (Chang et al., 2010). These findings indicate that E6 can up-regulate Mcl-1 through p53-PI3K/Akt-(IL-6)-(IL-17) pathway. Intriguingly, the IL-6 level in HPV18 E6-transfected cells was significantly higher than that in HPV16 E6 counterparts, but the IL-6 level in HPV 16 E7 was similar to that in HPV 18 E7 transfectants (Chang et al., 2010). Our previous studies found that over-expression of HPV-16 E6 and E7 oncoproteins led to HIF-1α-dependent increase of IL-8 in NSCLC cells (Li et al., 2011). The expression of IL-8, an important pro-angiogenic factor, was up-regulated by HPV-16 oncoproteins may contribute to the development and progression of NSCLC.

E6/E7 oncoprotein and Aryl Hydrocarbon Receptor

Aryl Hydrocarbon Receptor (AHR), a cytosolic ligand-activated transcription factor, mediates many toxic and carcinogenic effects in animals and humans AHR can trigger signaling pathways to adjust proliferation, differentiation, or apoptosis through ligand-dependent or completely ligand-independent mechanisms (Puga et al., 2002). It was reported that AHR haplotypes influenced risk of lung cancer (Kim et al., 2007; Chen et al., 2009). AHR expression in lung adenocarcinoma tumor cells was higher than that in adjacent normal bronchiolar (Lin et al., 2003). Buonomo et al. (Buonomo et al., 2011) analyzed the gene expression profiles of small cell lung cancer (SCLC) transgenic mouse by Ingenuity Pathways Analysis (IPA) and found that E6/E7 coexpression was associated with cellular development, cell cycle, cellular growth, and proliferation.

These findings indicated the top five canonical pathways affected by HPV E6/E7 expression, namely the AHR signaling, BRCA1 in DNA damage response, LPS/IL-1 mediated inhibition of RXR function, CHK proteins in cell cycle checkpoint control, and pyrimidine metabolism. The most significant signal modulated by E6/E7 is the AHR signaling in that 50 genes have a direct connection with AHR (Table 1) (Buonomo et al., 2011). In lung cancer cells, AHR is a potent transcriptional co-activator of E2F1-dependent transcription (Watabe et al., 2010). AHR expression was also shown to up-regulate the expression of IL-6, OPN, IL-8, and CYP1B1, while down-regulate proflammatory COX-2 and PG production, leading to lung tumorigenesis and cell proliferation

Table 1. Genes Regulated in AHR Signaling Pathway

Up-regualted	Aldh1a3	Aldh1b1	Hspb3	Gm9769	Gstm5	E2f1	Cdkn1b	Cdkn2a	Mcm7	
	Pola1	Ccna2	Tfdp1	Chek1	Ccna1	Cdkn1a	Rb1	Dhfr	Ncoa7	Gstm5
Down-regualted	Tgfb2	Fas I	Fas	Gstel	Aldh6a1	Mgst1	Gsta4	Gstt2	Il1b	Cyp1a1
	Mgst2	A1dh1a1	Gstm3	Gstm1	Gm10639	Gsta3	Nqo1	Tgm2	Gstk1	Gstt1
	Slc35a2	Nfe212	Ers2	Slc35a2	Nfkb1	Ckd6	Rarb	Med1	Rarg	Nfia
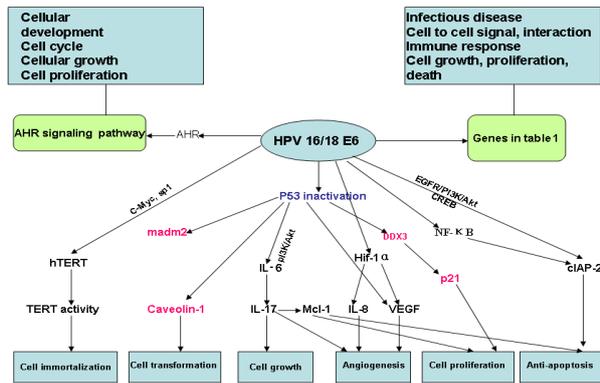
Figure 1. The Main Targets of HPV E6 Oncoprotein in Lung Cancer
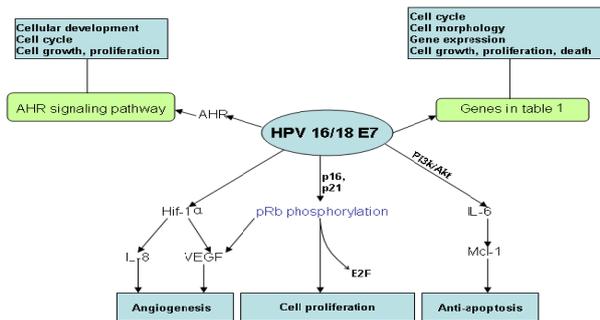
Figure 2. The Main Targets of HPV E7 Oncoprotein in Lung Cancer

promotion (Lin et al., 2003; Baglole et al., 2008; Podechard et al., 2008; Chen et al., 2012). Accumulating evidence has demonstrated the role of AHR in cell cycle control, but the underlying mechanisms are not completely clear. Even though several lines of evidence have pointed to the role of AHR in lung cancer, HPV E6 and E7 oncoproteins have not yet been demonstrated as etiological factors of lung cancer.

Therefore, more studies are needed to explore whether other signaling mediators related to AHR as shown in the gene network are also modulated by HPV E6/E7 oncoproteins and subsequently contribute to lung cancer development.

Oncoproteins Modulated by HPV 16 E6 and E7 Oncogenes

Ciotti et al. (2009) used two-dimensional gel electrophoresis gels to analyze the proteins which are regulated by the HPV16- E6 and E7 oncogenes in A549 cells. As a result, 17 different polypeptides were identified and biological associations among these proteins were modeled by IPA. These proteins regulated by the HPV16 E6 are functionally related to infectious disease, cell to cell signals and interactions, and immune responses, whereas the proteins regulated by the HPV16 E7 are related to the regulation of cell cycle, cell morphology, and cell death. However, the proteins up-regulated by both E6 and E7 are commonly involved in the regulation of cellular growth, proliferation, and death. Moreover, the proteins including Hsp27, annexin IV, Gp96, and tumor protein translationally-controlled 1 were some of the key genes involved in the gene network (Ciotti et al., 2009).

Conclusion and Future Vision

HPV infection is considered to be one cause of lung cancer, especially in Asia. Moreover, accumulating evidence has demonstrated that HPV16/18 E6 and E7 oncoproteins play an important role in lung carcinomas. HPV16/18 E6 and E7 have been shown to regulate the expression of various target genes and proteins including p53/pRb, VEGF, HIF-1α, cIAP-2, and hTERT, and contribute to cell proliferation, angiogenesis, cell immortalization etc. through different signaling pathways in lung cancer (Figures 1 and 2). Therefore, these findings suggest that HPV16/18 E6 and E7 oncoproteins and their target genes and proteins may be potential therapeutic targets in HPV-associated lung cancer.

However, some important questions are needed to be addressed before E6 and E7 oncoproteins could be considered as therapeutic targets of lung cancer. The first question is that what the route transmission of HPV to lung tissue is. The prevalence rate of HPV in the blood circulation of lung cancer and cervical cases was significantly higher compared with that of non-cancer controls (Chiou et al., 2003). Based on these findings, it was assumed that HPVs reach the lung tissue in a mucosal contact-blood circulation-lung tissue infection channel. It's worth mentioning that another possible route transmission is through oral cavity-larynx-lung tissue because of dangerous sexual contacts. Some reports also showed the low prevalence rate of HPV in normal lung tissue, so the second question is whether tumor tissue is more easily to be infected by HPV than normal tissue. If this hypothesis is right, it means that HPV does not have tumorigenicity for lung tissues or it is just a promoter of lung tumor development rather than it is the cause of lung carcinogenesis. On the contrary, if the infection chance is equal, we also need convincing experiment dates to prove the tumorigenicity of HPV according to the complexity of the unknowns, and three hypothesis are as follows. The first is that HPV could not make a contribution to tumorgenesis of lung tissues, where it could invade both normal lung and tumor tissues. The second is that HPV is the cause of lung cancer, and importantly we should make clear the incubation period of the HPV in normal lung tissues before our assumption is proved in case the misjudgment happens (Bishop et al., 2012). In 2012, Munoz et. al found the interaction between tobacco smoke and HPV16 E6/E7 oncoproteins for malignant transformation and tumorigenesis of lung epithelial cells (Munoz et al., 2012). And the third possibility is that HPV is too weak to cause lung tumorgenesis until appearance of other dangerous co-promoters. Our existing date, however, should not solve these problems. Resolution of these problems will play an important role in the prevention and prognosis of lung tumors.

Acknowledgment

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