Molecular Pathogenesis, Epidemiology, Risk Factors & Prognosis of Head and Neck Cancers in Relation to Human Papilloma Virus Infection

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Human papillomavirus (HPV) is well known to cause cervical cancer and has now been increasingly implicated as a risk factor for head and neck squamous cell carcinoma (HNSCC). More than 100 types of HPV have been identified but HPV type 16 genome is the one most commonly detected in head and neck cancers, followed by HPV type 18. Two viral oncoproteins E6 and E7 are implicated to play an important role in promoting tumorogenesis by degrading p53 and Rb gene products respectively and thus disrupting the cell cycle. The HPV positive HNSCC patients tend to be younger, non smokers and non drinkers. Oral sex is thought to be the probable mode of transmission for the HPV infection. Patients with HPV positive tumors have at least 50 percent reduced risk of death from HNSCC when compared with HPV-negative patients. Understanding the molecular biology of HPV-positive tumors could lead to the development of diagnostic, therapeutic, preventive and screening measures for HNSCC in the future.

Key Words: Head and neck squamous cell carcinoma (HNSCC), Human papillomavirus (HPV).

Human papillomavirus (HPV) is known to cause tumors in humans. Human papillomaviruses are DNA viruses that are members of the papovavirus family. More than 100 types of humanpapilloma virus (HPV) have been identified and the family is highly ubiquitous in the human population[1]. All the papillomaviruses regardless of the host specificity, are quite similar in physical structure and genome organization. The virion is composed of a double stranded, circular, 8000 base pair DNA genome encased in a naked icosahedral capsid about 55nm in diameter[2].

Different HPV types are strictly epitheliotropic and this tropism can be further subdivided into mucosal or cutaneous specificity which can also extend to the precise anatomical site susceptible to infection. A number of these papillomaviruses have been implicated in a variety of clinical conditions, ranging in severity from cutaneous warts of the hand (HPV type 1) to squamous cell carcinoma in renal transplant recipients (HPV type 5 and 8) and to anogenital and most importantly cervical cancer (HPV 16, 18, 31, 33 and 45).[3,4] HPV 16, 18, 31, and 45, account for nearly 80% of the cases of cervical squamous cell carcinoma[5] with HPV 16 alone accounting for about half the cases worldwide (6). HPV 18 is the most prevalent type in cervical adenocarcinomas (55%), followed by HPV 16 (32%) and HPV 45 (10%).[7]

Oral human papillomavirus (HPV) infection is now recognized to play a role in the pathogenesis of head and neck squamous cell carcinomas (HNSCC).[8-14] HPV genomic DNA has been detected in head and neck cancers[15-17] mostly of HPV type 16,[18,19] but its etiologic role in the development of HNSCC had remained unclear. Recent epidemiologic and molecular data suggest that human papillomavirus (HPV) infection of the upper airway may promote head and neck tumorigenesis.[20,21] Several studies have shown that tonsillar and oropharyngeal carcinomas contained HPV DNA more commonly than cancers at other head and neck sites.[8,10,15,22] This might be because HPV has facilitated access to the basal mucosal cells in the tonsillar crypts.[15,23]

A clonal association of HPV with cancer cells is strongly supported by a specific localization of HPV in tumor cells at all cancer stages (pre invasive, invasive and lymph node) and its probable integration into the genome of some tumors. In contrast to the HPV negative oropharyngeal cancers these cancers have distinct pathology (more frequently basloid), molecular biology (fewer p53 mutations), risk factors (less associated with alcohol consumption and perhaps smoking), and clinical prespective (good prognosis)[9].

The molecular pathogenesis of HPV in the causation of head and neck cancers that follows would help us have a better insight into the disease process.

Molecular Pathogenesis

Human papillomavirus genome consists of eight to nine open reading frames and non- coding upstream regulatory region containing the origin of replication and enhancer and promoter sequences. The unique factor binding sites likely explain the affinity for different tissues.[24] The viral genes expressed from several promoters via splicing of polycistronic mRNAs, are termed either early (E) or late (L) depending on when they are expressed during infection. Although the transforming activity of certain HPV types is clinically important, the normal viral life cycle is intended solely to replicate the virus; transformation occurs only as a mistake. During carcinogenic progression the viral genome frequently becomes integrated into the host genome but the integration is non specific with respect to host genome but is specific with respect to viral genome. After integration only

two viral genes E6 and E7 are consistently expressed in HPV associated cancers. Moreover viral transcription/ replication factor E2 expression is disrupted which functions as a transcriptional repressor of viral gene expression. As a result, the expression of E6 and E7, which is still driven by viral promoter/enhancer sequences, is dysregulated and is often higher in tumors.[reviewed in 25 & 26]

E6 Protein

E6 protein from HPV 16 and 18 are able to bind to the p53 product and this binding promotes the degradation of p53 by the ubiquitin pathway.[27,28] P53 links cell damage with DNA repair, cell cycle arrest, and apoptosis. Following DNA damage, there is rapid increase in p53 levels. At the same time, kinases such as DNA-dependent protein kinase and ATM (ataxia–telangectasia mutated) are activated and phosphorylate p53 which becomes an active transcription factor and increases the transcription of the Cyclin dependent kinase (CDK) inhibitor p21. p21 then haults the cell at G1/S check point giving the cell enough time to repair the DNA damage. This is done by GADD45 (growth arrest and DNA damage) the transcription of which is also increased by phosphorylated p53. If the DNA is repaired successfully p53 activates MDM2 by feedback mechanism which degrades p53 itself relieving the cell cycle block. If there is a failure of DNA repair p53 increases the transcription of pro-apoptotic gene BAX resulting in cell apoptosis.

E6 mediated degradation of p53 is dependent upon a cellular protein, E6 associated protein (E6-AP) also known as UBE3A.[29-31] E6-AP does not require E6 in order to function as a ubiquitin protein ligase since it has been shown that E6-AP alone is capable of ubiquitinating itself and a number of other cellular proteins[32] and E6-AP, in fact, belongs to the family of closely related ubiquitin ligase. E6 protein has been shown to stimulate the degradation of both c-Myc and Bak through the interaction with E6-AP[33,34] but in these cases it appears to be an enhancement of a normally occurring interaction. In the degradation of p53, E6 appears to have diverted E6-AP to an unnatural target. It has been assumed that degradation of p53 contributes to the oncogenic potential of high risk HPV. E6 protein from both high and low risk HPV were also shown to be capable of binding to p53 without inducing this degradation.[35,36] This interaction prevents the p53 mediated transcriptional repression of TATA containing promoters[37] and also represses p53's transactivation of promoters containing p53 response elements.[38] P53 degradation emerges as one of the prime causes for chromosomal instability of high risk human papillomavirus containing cells,[39-41] with resulting mutational consequences for HPV-positive cells. The presence of E6 also enhances the integration of foreign DNA into host cell genome.[42] Recent observation suggests that E6-AP-mediates ubiquitination and degradation of the src family tyrosine kinase Blk but it is conceivable that the presence of E6 partially blocks this degradation and thereby stabilizes the respective kinase and stimulate mitotic activity.[43] This could explain, in part, growth-stimulatory functions of the E6 protein of high risk HPVs.[44]

In addition, however E6 reveals a remarkable pleiotropism in binding further host-cell proteins:

It interacts with calcium-binding protein ERC 55 homologue, E6BP.[45] This appears to be a vitamin D receptor (VDR)-associated factor[46] and E6's interaction may perhaps prevent the mediation of vitamin D3's growth suppressive effect.

E6 also interacts with paxillin.[47,48] Paxillin is involved in signal transduction between the plasma membrane and focal adhesions and the cytoskeleton and it is activated in response to a number of mitogenic stimuli. It is possible that E6 induced disruption of the cytoskeleton and consequent interference with signal transduction can release the cell from certain cell cycle controls.[47]

E6 also interacts with mammalian homologue of the Drosophila discs large protein (DLG).[49] Since DLG has been implicated in the control of cell-cell contact and cell polarity[50,51] it is reasonable to speculate that this activity of E6 might be relevant for tumor progression.

E6 targeted protein 1 (E6TP1) is a negative regulator of Rap1 GTPase and could therefore be involved in the negative regulation of mitogenic signaling. E6 induced degradation of E6TP1 might restore this signaling and thence contribute to cellular immortalization.[52]

E6 also binds to the interferon regulatory factor3 and inhibits the induction of interferon beta messenger RNA (mRNA) following Sendai virus infection.[53] This inhibition is not mediated by ubiquitination or degradation.

E6 has also been shown to overcome p53 independent apoptosis, this is at least in part, the result of E6-induced ubiquitin-mediated degradation of c-Myc and Bak proteins, both of which are associated with induction of apoptosis.[33,34,54]

Human papillomavirus has been shown to specifically upregulate telomerase activity during immortalization.[55-58] Mutational analysis of E6 reveals that the up regulation of telomerase, rather than the degradation of p53 is important for its immortalizing activity.

HPV E6 protein has also been implicated in processes resulting in perturbation of chromosomal structure and the control of normal cellular DNA replication, HPV 18 E6 had been shown to induce the ubiquitin mediated degradation of Mcm7, via E6-AP.[59] The Mcm7 protein is a part of the replication licensing factor whose binding to cellular DNA replication origins (*oris*) ensures that each *ori* fires only once in each round of DNA replication.[60,61] From the above discussion it is clear that many of the effects seen in cells upon over expression of E6 are not related solely to the degradation of p53.

E7 Protein

The HPV E7 protein is able to bind and interact with Rb gene product[62] and retinoblastoma protein (Rb)-related pocket proteins[63]; however the binding affinity of the low-risk

E7 protein is much lower than the high-risk E7 protein.[64] Rb gene product is a nucleo-phosphoprotein that plays a key role in regulating cell cycle. Rb exists in active hypophosphorylated state in quiescent cells and inactive hyperphosphorylated state in the G1/S cell cycle transition.[65] Hypophosphorylated Rb binds to a protein complex that contains E2F and a subunit called DP1. The E2F/DP1/Rb complex binds to promoters of E2F-responsive genes. These E2F targets include prominent cell cycle regulatory genes, such as cdc2, thymidine kinase, myb, dihydrofolate reducetase, and the E2F1 gene itself.[66] Bound to the E2F/DP1/Rb complex, such genes are silent because Rb recruits histone deacetylase, an enzyme that causes compaction of chromatin and inhibition of transcription. When the quiescent cells are stimulated by growth factor the concentrations of cyclin D and E go up, resulting in the activation of cyclin D-CDK4 and cyclin E-CDK2 at the G1/S restriction point and causing phosphorylation of Rb. Hyperphosphorylated Rb dissociates from complex activating transcription of E2F target gene that is essential for progression through S phase.[67] The binding of E7 to Rb protein and Rb-related pocket proteins results in phosphorylation of these protein and their enhanced degradation by ubiquitination,[68] and the release of transcription factors of the E2F family, activating transcription of genes regulating cell proliferation.[69,70] E7 protein of high risk HPVs are found in cyclin E[71] and in cyclin A[72] complexes. These complexes exhibit kinase activity. The activation of cyclin E followed by activation of cyclin A is mediated by E7 sequences required for transformation.[73] The E7 induced S phase entry is not accompanied by cyclin D activation, probably because of the formation of cyclin D/p16^{INK4} complexes in high risk human papillomavirus infected cells.[74] p16/INK4 binds to CDK4 and promotes the inhibitory effect of Rb. E7 protein also causes the inactivation of the cyclin-dependent kinase inhibitor p21^{CIP-1} [75,76] and p27^{KIP-1}.[77] This interaction uncouples the CDK activity from CDK inhibitors and is a major factor in growth stimulation of HPV infected cells. Similar to E6, E7 protein also inhibits the interferon signaling pathways by binding to the interferon regulatory protein p48.[78] High risk human papillomavirus E7 expression enhances the integration of foreign DNA into the host cell DNA[79] results in increase mutagenesis[74,80] and enhances mutagenicity of chemical carcinogens.[81]

Although E6 and E7 proteins may immortalize various types of human cells independently, their cooperative interaction leads to substantially enhanced immortalization efficiency.

EPIDEMIOLOGY & HPV TYPES IN HEAD AND NECK SQUAMOUS CELL CARCINOMA (HNSCC):

Kreimer et al[82] did PCR-based assays for HPV DNA in greater than 5,000 HNSCC biopsy specimens from 60 studies and found that one-fifth to one-fourth of SCCs from the oral cavity and larynx were HPV positive, but the prevalence in oropharyngeal SCCs was significantly greater amounting to more than one-third of the cases. This is consistent with the findings of other studies showing that HPV-positive tumors arise largely from the lingual and palatine tonsils in the oropharynx compared with other anatomic sites of the head and neck.[8,9,15-17,83-85] Furthermore according to this study;[82] HPV16, the most common HPV type detected in biopsies from women with cervical SCC was also the most common type detected in biopsies from HNSCCs. In the oropharynx, HPV16 accounted for the overwhelming majority of HPV-positive cases (86.7%), whereas the predominance of HPV16 was less striking in other head and neck sites. HPV18 the second most common type detected in HPV-positive cancers in this study, was found much less frequently in HPV-positive was found much less frequently in HPV-positive oropharyngeal SCCs (2.9%) compared with HPV-positive oral SCCs (34.5%) or HPV-positive laryngeal SCCs (17.2%). It therefore seems that the type distribution of HPVs in HNSCCs may also vary by head and neck site. Aside from HPV16 and HPV18, other oncogenic HPV types commonly detected in invasive cervical cancer biopsies (e.g., HPV31, 33, 35, 45, 56, 58, and 59) were rarely or never detected in HNSCC biopsies. Conversely, HPV 6, which has been designated as ''low-risk'' or ''non-oncogenic'' to the cervix[86] and is the cause of benign tumors in the aerodigestive and genital tract,[18] was present in a greater number of HNSCCs than any of the oncogenic types other than HPV16 and HPV18. The fact that HPV16 and HPV18 account for almost all oncogenic HPV types detected in HNSCC biopsies suggests that newly developed prophylactic vaccines for cervical cancer[87] should also be relevant for HNSCCs.

HPV prevalence in oral SCCs from Asia was reported to be considerably higher compared with the other geographic locations. Similarly, HPV prevalence was significantly higher in oropharyngeal SCCs from North America and Asia compared with Europe.[82] However, in the IARC study of HPV and oral and oropharyngeal cancers HPV prevalence did not differ significantly among Europe, North and South America, Asia, and Africa.[88] Important gaps remain on the topic of HPV prevalence in HNSCC in many parts of the world. In particular, almost 84% of the information on oropharyngeal SCCs derived from studies conducted in Europe and North America.

Risk factors & Clinical Implications

The etiology of head and neck squamous cell cancer is multifactorial with alcohol and tobacco consumption considered to be the main risk factors.[89-92] Average and poor oral hygiene and inadequate dental status[93] marijuana use[94] and genetic factors[95] are also implicated in increasing the risk for head and neck squamous cell carcinoma. The association between high-risk human papillomavirus infection and the risk of head and neck squamous cancer development dates to 1985, when HPV 16 was detected in oral squamous cell tumours.[96] Since then, HPV DNA has repeatedly been found in a variable proportion of head and neck cancers[9,14,83,97] and

it has been proposed that HPV DNA positive tumors form an etiologically distinct group of oral cavity/oropharyngeal tumors. In this group of tumors, distinct clinical and epidemiological characteristics can be found.[98] The risk factors for HPV-positive head and neck squamous cell carcinoma are discussed briefly in the following paragraphs.

The HPV-positive patient appears to be distinct from the HPV-negative patient with regard to alcohol and tobacco exposure history. HPV-positive HNSCC is more likely than HPV-negative HNSCC to occur in the nonsmoker and nondrinker.[9,13,83,99,100] Non smokers are approximately 15-fold more likely to develop HPV-positive HNSCC than smokers.[100] An inverse association between HPV status and alcohol use has also been reported.[9,16,84,100,101] Although evidence suggests that HPV is associated with cancers in nonsmokers and nondrinkers,[102] the degree to which oral HPV infection may combine with tobacco and/or alcohol use to increase risk of cancer is currently unclear, with some studies suggesting a synergistic effect with tobacco[8] or alcohol,[103] whereas others have found no such synergy.[88,104,105]

How oral HPV infection occurs was not firmly established but sexual transmission was thought to be the likely mode since numerous case control studies of cervical cancer patients have indicated that HPV infection is predominantly sexually transmitted.[106] Recently oral HPV infection has also been associated with sexual behavior, in particular with number of oral sex partners.[93,107] Other factors associated with elevated risk of oral HPV infection include increasing age, male sex, history of sexually transmitted disease HIV infection and severity of immunosuppression.[107,108] In three case-control studies, patients with oral cancer had had more sexual partners than controls, although the number of patients and controls who ever had oral-genital contact were not significantly different.[8,109,110] One of these studies[8] found that the associations with a higher life-time number of sexual partners and with a total of more than four partners with whom the subject engaged in oral sex was stronger for patients with tumors positive for HPV-16 DNA than for those whose tumors did not contain HPV-16 DNA. Other studies also support the finding that the risk of HNSCC is elevated among women with a higher number of sexual partners.[111] Risk factors among men include young age at first intercourse, number of sexual partners and a history of genital warts.[8] However, no significant sex-related difference in the risk of cancer associated with the presence of anti-HPV antibodies has been found.[14,112] Patients with HPV-positive HNSCC tend to be younger by approximately 5 years, on average, when compared with HPV-negative HNSCC.[84,105,113-115] A possible explanation for this could be that high-risk sexual behaviors were found to be more prevalent among HNSCC patients younger than 55 years when compared with those older than 55 years.[110]

Histologically, HPV-positive tumors tend to have a poorly differentiated and frequently basaloid histology[9-11,116-121] but patients with HPV- positive tumors have improved prognosis when compared with patients with HPV-negative tumors in the majority of studies.[9,15,83,100,113,122-124] The reason for the improved survival is unclear; however, improved radiation responsiveness, immune surveillance to viral antigens, and the absence of field cancerization in these patients who tend to be nonsmokers, have been postulated.[100,113,125,126] In addition, E6-related degradation of p53 in HPV-positive cancers may be functionally inequivalent to HPV-negative p53 mutations,[127,128] and therefore, HPV-positive tumors may have an intact apoptotic response to radiation and chemotherapy.[125,129]

Molecular Detection OF HPV

HPV can be detected by many methods but each has its own merits and demerits. The problem of contamination by miniscule amounts of RNA or DNA underlies all of the sensitive molecular assays.

The detection of antibodies to E6 and E7 has been demonstrated in cervical cancer patients by several studies.[130-132] However, in a small study of HNSCC patients only 12% had antibodies to E6 or E7 and none of the patients with oral tumors demonstrated seropositivity.[133] It is not known if antibody development to any region of the HPV genome is significant, or if there are particular antibodies that herald a worse prognosis. Furthermore antibody presence is not necessarily indicative of active infection, latent integration, or oncoprotein production that might be a clinically significant contributor to carcinogenesis. In addition, seropositivity may be a confounding factor associated with other risk factors for oral cancer, including tobacco and ethanol exposure.[134]

HPV DNA and E6 mRNA assays have also been performed. van Houten et al.[12] demonstrated by PCR that not all the HPV DNA positive HNSCC samples were positive for E6 mRNA, indicating that the presence of DNA does not necessarily indicate active viral replication. Since it is not clear that integration must occur for HPV to play a role in carcinogenesis and HPV is thought to exist in an episomal form[135-137] in the oral cavity; it is possible that HPV can be a transient infection that may or may not participate in the foundation of malignancy.

In situ hybridization (ISH) involves the use of type-specific radioactively labeled DNA probes complementary to HPV sequences for detection. The sensitivity of this assay was found to be at least on the order of 20-50 copies per cell.[138] It is the only clinically useful test to confirm the diagnosis of HPV.[139] However, p16 immunohistochemistry may serve as a surrogate marker for high-risk HPV because strong correlations have been found between diffuse nuclear and cytoplasmic p16 staining, HPV DNA by ISH (140) and real-time PCR.[99]

PCR is known to be a very sensitive assay for the detection of HPV DNA in any given sample.[141] Universal primers to conserved DNA sequences in HPV have been designed to the L1 region [also known as MY09/MY11],[142] the E1 region [also known as CPI and CPII],[143,144] the E6 region[145]

and the E7 region.[146] Furthermore, there is a host of other primers utilized that can be type-specific. The majority of studies have settled on the use of MY09/11 primers for detection, which yields a product size of ~ 450 base pairs.

Southern blot has long been one of the gold standard assays for the detection of HPV DNA. It offers the ability to distinguish between episomal and integrated DNA, and it can detect up to 0.1 copy per cell.[147] While Southern blot may boast a theoretically higher specificity, it is clearly less sensitive than PCR.[148,149]

Quantitative PCR utilizes a fluorescent probe that is cleaved upon each round of amplification by the DNA polymerase, and the degree of fluorescence in the reaction mixture is then measured. Real-time PCR can be performed on microdissected tumor DNA normalized to a single-copy human gene to demonstrate one or more viral copies per tumor cell.[99,150,151] Capone et al.[152] reported the detection of HPV DNA in the sera of patiens with HPV-associated HNSCC and proposed that quantitative real-time PCR is a more sensitive and specific assay to monitor the serum cell free HPV DNA. Thus this technique combines the sensitivity of PCR reaction with the specificity of a southern blot.

Every method has its drawbacks so critical evaluation of data based on the types of detection methods used as well as determination of what the data means in a clinical context is necessary for appropriate analysis.

Conclusion

There is increasing evidence that human papillomavirus might be one of the causative factors in a unique subset of HNSCC. Molecular mechanisms that reveal the ability of the high risk (type 16 and 18) HPV to disrupt key events in the regulation of cell cycle and apoptosis have now been identified and oral sex is considered to be the most likely route of transmission. The current data is however insufficient to quantify exactly the magnitude of increased risk for HNSCC in individuals infected with HPV. Because of the lack of this information there are currently no screening programmes and the clinical relevance of HPV-positive tumors lie in their good prognostic value. Further research could lead to the development of diagnostic, therapeutic, preventive and screening implications for the HPV-positive HNSCC.

References

1. de Villiers E-M, Lavergne D, McLaren K, Benton EC. Prevailing papillomavirus types in nonmelanoma carcinomas of the skin in renal allograft recipients. Int J Cancer. 1997; 73: 356–361.
2. Scheurer ME, Tortolero-Luna G, Adler-Storthz K. Human papillomavirus infection: biology, epidemiology, and prevention. Int. J. Gynaecol Cancer 2005; 15: 727-746.
3. zur Hausen H and Schneider A. (1987). In: The Papovaviridae, Salzman N and Howley PM. (eds). Vol 2, Plenum Press: New York, pp. 245-263.
4. zur Hausen H. Human papillomavirus in the pathogenesis of anogenital cancers. Virology 1991; 184: 9-13.
5. Bosch FX, Lorincz A, Munoz N, Meijer CJ, Shah KV. The causal relation between human papillomavirus and cervical cancer. J Clin Pathol 2002; 55: 244–65.
6. Clifford GM, Smith JS, Aguado T, Franceschi S. Comparison of HPV type distribution in high-grade cervical lesions and cervical cancer: a meta-analysis. Br J Cancer 2003; 89: 101–5.
7. 1Zielinski GD, Snijders PJ, Rozendaal L et al. The presence of high-risk HPV combined with specific p53 and p16INK4a expression patterns points to high-risk HPV as the main causative agent for adenocarcinoma in situ and adenocarcinoma of the cervix. J Pathol 2003; 201: 535–43.
8. Schwartz SM, Daling JR, Doody DR, Wipf GC, Carter JJ, Madeleine MM, et al. Oral cancer risk in relation to sexual history and evidence of humanpapillomavirus infection. J Natl Cancer Inst 1998; 90: 1626–36.
9. Gillison ML, Koch WM, Capone RB, et al. Evidence for a causal association between human papillomavirus and a subset of head and neck cancers. J Natl Cancer Inst 2000; 92: 709-720.
10. Andl T, Kahn T, Pfuhl A, et al. Etiological involvement of oncogenic human papillomavirus in tonsillar squamous cell carcinomas lacking retinoblastoma cell cycle control. Cancer Res 1998; 58: 5-13.
11. Wiest T, Schwarz E, Enders C, et al. Involvement of intact HPV16 E6/E7 gene expression in head and neck cancers with unaltered p53 status and perturbed pRb cell cycle control. Oncogene 2002; 21: 1510-1517.
12. van Houten VM, Snijders PJ, van den Brekel MW, Kummer JA, Meijer CJ, van Leeuwen, et al. Biological evidence that human papillomaviruses are etiologically involved in a subgroup of head and neck squamous cell carcinomas. Int J Cancer 2001; 93: 232-235.
13. Hafkamp HC, Speel EJ, Haesevoets A, et al. A subset of head and neck squamous cell carcinomas exhibits integration of HPV 16/18 DNA and over expression of p16INK4A and p53 in the absence of mutations in p53 exons 5-8. Int J Cancer 2003; 107: 394-400.
14. Mork J, Lie AK, Glattre E, et al. Human papillomavirus infection as a risk factor for squamous-cell carcinoma of the head and neck. N Engl J Med 2001; 344: 1125-1131.
15. Paz IB, Cook N, Odom-Maryon T, Xie Y, Wilczynski SP. Human papillomavirus (HPV) in head and neck cancer. An association of HPV 16 with squamous cell carcinoma of Waldeyer's tonsillar ring. Cancer 1997; 79: 595–604.
16. Haraf DJ, Nodzenski E, Brachman D, Mick R, Montag A, Graves D, et al. Human papilloma virus and p53 in head and neck cancer: clinical correlates and survival. Clin Cancer Res 1996; 2: 755–62.
17. Fouret P, Monceaux G, Temam S, Lacourreye L, St Guily JL. Human papillomavirus in head and neck

squamous cell carcinomas in nonsmokers. Arch Otolaryngol Head Neck Surg 1997; 123: 513–6.

18. IARC monographs on the evaluation of carcinogenic risks to humans.Vol. 64. Human papillomaviruses. Lyon, France: International Agency for Research on Cancer, 1995.
19. McKaig RG, Baric RS, Olshan AF. Human papillomavirus and head and neck cancer: epidemiology and molecular biology. Head Neck 1998; 20: 250-65.
20. Gillison M, Koch WM, Shah KV. Human papillomavirus and head and neck squamous cell carcinoma: are some head and neck cancers sexually transmitted disease? Curr Opin Oncol 1999; 11: 191–9.
21. Franceschi S, Munoz N, Bosch XF, Snijders PJ, Walboomers JM. Human papillomavirus and cancers of the upper aerodigestive tract: a review of epidemiological and experimental evidence. Cancer Epidemiol Biomarkers Prev 1996; 5: 567–75.
22. Snijders PJ, Cromme FV, van der Brule AJ, et al. Prevalence and expression of human papillomavirus in tonsillar carcinomas, indicating a possible viral etiology. Int J Cancer 1992; 51: 845-50.
23. Frisch M, Biggar RJ. Aetiological parallel between tonsillar and anogenital squamous –cell carcinomas. Lancet 1999; 354: 1442-3.
24. Gravitt PE, Shah KV. The biology of human papillomavirus infections. In: Rohan TE, Shah KV, eds. Cervical cancer: from etiology to prevention. Boston, MA: Kluwer Academic Publishers, 2004: 81–99.
25. zur Hausen H. Papillomavirus infections-a major cause of human cancers. Biochim Biophys Acta 1996; 1288: F55-78.
26. Papillomavirinae: the viruses and their replication. In: Fields BN, Knipe DM, Howley PM, editors. Fundamental virology. Philadelphia (PA): Lipincott-Raven; 1996. p.947-78.
27. Scheffner M, Werness BA, Huibregtse JM, Levine AJ, Howley PM. The E6 oncoprotein encoded by human papillomavirus 16 or 18 promotes the degradation of p53. Cell 1990; 63: 1129-1136.
28. Werness BA, Levine AJ, Howley PM. Association of human papillomavirus types 16 and 18 E6 proteins with p53. Science 1990; 248: 76-79.
29. Huibregtse JM, Scheffner M, Howley PM. A cellular protein mediates association of p53 with the E6 oncoprotein of human papillomavirus types 16 or 18. EMBO J 1991 Dec; 10 (13): 4129–4135.
30. Huibregtse JM, Scheffner M and Howley PM. Cloning and expression of the cDNA for E6-AP, a protein that mediates the interaction of the human papillomavirus E6 oncoprotein with p53. Mol Cell Biol 1993 Feb; 13 (2): 775-784.
31. Scheffner M, Huibregtse JM, Vierstra RD, Howley PM. The HPV-16 E6 and E6-AP complex functions as a ubiquitin-protein ligase in the ubiquitination of p53. Cell 1993 Nov; 75 (3): 495–505.
32. Nuber U, Schwarz SE, Scheffner M. The ubiquitin-protein ligase E6-associated protein (E6-AP) serves as its own substrate. Eur J Biochem. 1998; 254: 643–649.
33. Gross-Mesilaty S, Reinstein E, Bercovich B, Tobias KE, Schwartz AL, Kahana C, Ciechanover A. Basal and human papillomavirus E6 oncoprotein-induced degradation of Myc proteins by the ubiquitin pathway. Proc Natl Acad Sci USA 1998; 95: 8058-8063.
34. Thomas M, Banks L. Inhibition of Bak-induced apoptosis by HPV-18 E6. Oncogene 1998; 10: 2943-2954.
35. Crook T, Tidy JA and Vousden KH. Degradation of p53 can be targeted by HPV E6 sequences distinct from those required for p53 binding and trans-activation. Cell 1991; 67: 547-556.
36. Lechner MS, Laimins LA. Inhibition of p53 DNA binding by human papillomavirus E6 proteins. J Virol 1994; 68: 4262-4273.
37. Lechner MS, Mack DH, Finicle AB, Crook T, Vousden KH, Laimins LA. Human papillomavirus E6 proteins bind p53 in vivo and abrogate p53-mediated repression of transcription. EMBO J 1992; 11: 3045-3052.
38. Pim D, Storey A, Thomas M, Massimi P, Banks L. Mutational analysis of HPV-18 E6 identifies domains required for p53 degradation in vitro, abolition of p53 transactivation in vivo and immortalisation of primary BMK cells. Oncogene 1994; 9 (7): 1869-1876.
39. White AE, Livanos EM, Tlsty TD. Differential disruption of genomic integrity and cell cycle regulation in normal human fibroblasts by HPV oncoproteins. Genes Dev 1994; 8: 666-77.
40. Reznikoff CA, Belair C, Savelieva E, Zhai Y, Pfeifer K, Yeager T, et al. Long-term genome stability and minimal genotypic and phenotypic alterations in HPV16 E7-, but not E6-immortalized human uroepithelial cells. Genes Dev 1994; 8: 2227-40.
41. Havre PA, Yuan J, Hedrick L, ChoKR. Glazer PM. p53 inactivation by HPV 16 E6 results in increased mutagenesis in human cells. Cancer Res 1995; 55: 4420-4.
42. Kessis TD, Conolly DC, Hedrick L, Cho KR. Expression of HPV16 E6 or E7 increases integration of foreign DNA. Oncogene 1996; 13: 427-31.
43. Oda H, Kumar S, Howley PM. Regulation of the Src family tyrosine kinase Blk through E6AP-mediated ubiquitination. Proc Natl Acad Sci USA 1999; 96: 9557-62.
44. zur Hausen H. Papillomavirus causing cancer: Evasion from host-cell control in early events in carcinogenesis. J Natl Cancer Inst 2000; 92: 690-8.
45. Chen JJ, Reid CE, Band V, Androphy EJ. Interaction of papillomavirus E6 oncoproteins with a putative calcium-binding protein. Science 1995; 269: 529-31.
46. Imai T, Matsuda K, Shimojima T, Hashimoto T, Matsuhiro Y, Kitamoto T et al. ERC-55, a Binding Protein for

the Papilloma Virus E6 Oncoprotein, Specifically Interacts with Vitamin D Receptor among Nuclear Receptors. Biochem Biophys Res Commun 1997; 233 (3): 765-769.
47. Tong X, Howley PM. The bovine papillomavirus E6 oncoprotein interacts with paxillin and disrupts the actin cytoskeleton. Proc Natl Acad Sci USA 1997; 94: 4412-7.
48. Vande Pol SB, Brown MC, Turner CE. Association of bovine papillomavirus type 1 E6 oncoprotein with the focal adhesion protein paxillin through a conserved protein interaction motif. Oncogene 1998; 16: 43-52.
49. Kiyono T, Hiraiwa A, Fujita M, Hayashi Y, Akiyama T, Ishibashi M. Binding of high-risk human papillomavirus E6 oncoproteins to the human homologue of the Drosophila discs large tumor suppressor pretein. Proc Natl Acad Sci USA 1997; 94: 11612-6.
50. Lue RA, Marfatia SM, Branton D, Chishti AH. Cloning and characterization of hdlg: The human homologue of the Drosophila discs large tumor suppressor binds to protein 4.1. Proc Natl Acad Sci USA 1994; 91: 9818–9822.
51. Woods DF, Hough C, Peel D, Callaini G, Bryant PJ. Dlg protein is required for junction structure, cell polarity, and proliferation control in Drosophila epithelia. J Cell Biol 1996; 134 (6): 1469-1482.
52. Gao Q, Srinivasan S, Boyer SN, Wazer DE, Band V. The E6 oncoproteins of high risk papillomaviruses bind to a novel putataive GAP protein, E6TP1, and target it for degradation. Mol Cell Biol 1999; 19: 733-44.
53. Ronco LV, Karpova AY, Vidal M, Howley PM. Human papillomavirus 16 E6 oncoprotein binds to interferon regulatory factor-3 and inhibits its transcriptional activity. Genes Dev 1998; 12: 2061-72.
54. Thomas M, Banks L. Human papillomavirus (HPV) E6 interactions with Bak are conserved amongst E6 proteins from high and low risk HPV types. J Gen Virol 1999; 80: 1513-7.
55. Klingelhutz AJ, Foster SA, McDougall JK. Telomerase activation by the E6 gene product of human papillomavirus type 16. Nature 1996; 380: 79-82.
56. Stoppler H, Hartmann DP, Sherman L, Schlegel R. The human papillomavirus type 16 E6 and E7 oncoproteins dissociate cellular telomerase activity from the maintenance of telomere length. J Biol Chem 1997; 272: 13332-7.
57 Kiyono T, Foster SA, Koop I, McDougall JK, Galloway DA, Klingelhutz AJ. Both Rb/p16INK4a inactivation and telomerase activity are required to immortalize human epithelial cells. Nature 1998; 396: 84-8.
58. Filatov L, Golubovskaya V, Hurt JC, Byrd LL, Phillips JM, Kaufmann WK. Chromosomal instability is correlated with telomere erosion and inactivation of G2 checkpoint function in human fibroblasts expressing human papillomavirus type 16 E6 oncoprotein. Oncogene 1998; 16 (14): 1825-1838.
59. Kühne C and Banks L. E3-Ubiquitin ligase/E6-AP links multicopy maintenance protein 7 to the ubiquitination pathway by a novel motif, the L2G box. J Biol Chem 1998; 273: 34302-34309.
60. Blow J.J. Preventing re-replication of DNA in a single cell cycle: evidence for a Replication Licensing Factor. J Cell Biol 1993; 122: 993-1002.
61. Thömmes P, Kubota Y, Takisawa H and Blow JJ. The RLF-M component of the replication licensing system forms complexes containing all six MCM/P1 polypeptides. EMBO J 1997; 16: 3312-3319.
62. Dyson N, Howley PM, Munger K, Harlow E. The human papilloma virus-16 E7 oncoprotein is able to bind to the retinoblastoma gene product. Science 1989; 243: 934-7.
63. Dyson N, Guida P, Munger K, Harlow E. Homologous sequences in adenovirus E1A and human papillomavirus E7 proteins mediate interaction with the same set of cellular proteins. J Virol 1992; 66: 6893-902.
64. Heck DV, Yee CL, Howley PM, Munger K. Efficiency of binding the retinoblastoma protein correlates with the transforming capacity of the E7 oncoproteins of the human papillomaviruses. Proc Natl Acad Sci USA 1992; 89: 4442–6.
65. DeCaprio JA, Ludlow JW, Lynch D, Furukawa Y, Griffin J, Piwnica-Worms H et al. The product of the retinoblastoma gene has properties of a cell cycle regulatory element. Cell 1989; 58: 1085-1095.
66. Weinberg RA. The retinoblastoma protein and cell cycle control. Cell 1995; 81: 323-330.
67. Sher CJ, McCormick F: The RB and p53 pathways in cancer. Cancer Cell 2002; 2: 103-12.
68. Boyer SN, Wazer DE, Band V. E7 protein of human papilloma virus-16 induces degradation of retinoblastoma protein through the ubiquitin-proteasome pathway. Cancer Res 1996; 56: 4620-4.
69. Bagachi S, Raychaudhri P, Nevins JR. Adenovirus E1A proteins can dissociate heteromeric complexes involving the E2F transcription factor: a novel mechanism for E1A trans-activation. Cell 1990; 62: 659-69.
70. Bandra LR, Adamczewski JP, Hunt T, La Thangue NB. Cyclin A and the retinoblastoma gene product complex with a common transcription factor. Nature 1991; 352: 249-51.
71. McIntyre MC, Ruesch MN, Laimins LA. Human papillomavirus E7 oncoproteins bind a single form of cyclin E in a complex with ckd2 and p107. Virology 1996; 215: 73-82.
72. Arroyo M, Bagchi S, Raychaudhuri P. Association of human papillomavirus type 16 E7 protein with S-phase-specific E2F-cyclin A complex. Mol Cell Biol 1993; 13: 6537-46.
73. Zerfass K, Schulze A, Spitkovsky D, Friedman V, Henglein B, Jansen-Durr P. Sequential activation of cyclin E and cyclin A gene expression by human papilloma-

virus type 16 E7 through sequences necessary for transformation. J virol 1995; 69: 6389-99.
74. Xiong Y, Kuppuswamy D, Li Y, Livanos EM, Hixon M, White A, et al. Alteration of cell cycle kinase complexes in human papillomavirus E6- and E7-expressing fibroblasts precedes neoplastic transformation. J Virol 1996; 70: 999-1008.
75. Funk JO, Waga S, Harry JB, Espling E, Stillman B, Galloway DA. Inhibition of CDK activity and PCNA-dependent DNA replication by p21 is blocked by interaction with the HPV 16 E7 oncoprotein. Genes Dev 1997; 11: 2090-2100.
76. Jones DL, Alani RM, Munger K. The human papillomavirus E7 oncoprotein can uncouple cellular differentiation and proliferation in human keratinocytes by abrogating p21 Cip1-mediated inhibition of cdk2. Genes Dev 1997; 11: 2101-11.
77. Zerfass-Thome K, Zwerschke W, Mannhardt B, Tindle R, Botz JW, Jansen-Durr P. Inactivation of the cdk inhibitor p27KIP1 by the human papillomavirus type 16 E7 oncoprotein. Oncogene 1996; 13: 2323-30.
78. Barnard P, McMillan NA. The human papillomavirus E7 oncoprotein abrogates signaling mediated by interferon-alpha. Virology 1999; 259: 305-13.
79. Kessis TD, Conolly DC, Hedrick L, Cho KR. Expression of HPV 16 E6 or E7 increases integration of foreign DNA. Oncogene 1996; 13: 427-31.
80. White AE, Livanos EM, Tlsty TD. Differential disruptt ion of genomic integrity and cell cycle regulation in normal human fibroblasts by the HPV oncoproteins. Genes Dev 1994; 8: 666-77.
81. Magal SS, Jackman A, Pei XF, Schlegel R, Sheramn L. Induction of apoptosis in human keratinocytes containing mutated p53 alleles and its inhibition by both the E6 and E7 oncoproteins. Int J Cancer 1998; 75: 96-104.
82. Kreimer AR, Clifford GM, Boyle P, Franceschil S. Human papillomavirus types in head and neck squamous cell carcinomas worldwide: a systematic review. Cancer Epidemiol Biomarkers Prev 2005; 14 (2): 467–75.
83. Richtie JM, Smith EM, Summersgill KF, et al. Human papillomavirus infection as a prognostic factor in carcinomas of the oral cavity and oropharynx. Int J Cancer 2003; 104: 336-344.
84. Ringstorm E, Peters E, Hasegawa M, et al. Human papillomavirus type 16 and squamous cell carcinoma of the head and neck. Clin Cancer Res 2002; 8: 3187-3192.
85. Brandsma JL, Abramson AL. Association of papillomavirus with cancers of the head and neck. Arch Otolaryngol Head Neck Surg 1989; 115: 621-625.
86. Walboomers JM, Jacobs MV, Manos MM, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol 1999; 189: 12–9.
87. Galloway DA. Papillomavirus vaccines in clinical trials. Lancet Infect Dis 2003; 3: 469–75.
88. Herrero R, Castellsague X, Pawlita M, et al. Human papillomavirus and oral cancer: the International Agency for Research on Cancer multicenter study. J Natl Cancer Inst 2003; 95: 1772–83.
89. Franceschi S, Talamini R, Barra S et al. Smoking and drinking in relation to cancers of the oral cavity, pharynx, larynx, and esophagus in northern Italy. Cancer Res 1990; 50: 6502-6507.
90. Castellsague X, Quintana ML, Martinez MC et al. The role of type of tobacco and type of alcoholic beverage in oral carcinogenesis. Int J Cancer 2004; 108: 741-749.
91. Tuyns AJ, Estève J, Raymond L, et al. Cancer of the larynx/hypopharynx, tobacco and alcohol: IARC international case-control study in Turin and Varese (Italy), Zaragoza and Navarra (Spain), Geneva (Switzerland) and Calvados (France). Int J Cancer 1988; 41: 483-491.
92. Rothman K, Keller A. The effect of joint exposure to alcohol and tobacco on risk of cancer of the mouth and pharynx. J Chronic Dis 1972; 25: 711-6.
93. Rosenquist K, Wennerberg J, Schildt E, Bladström A, Hansson BG, Andersson G. Oral status, oral infections and some lifestyle factors as risk factors for oral and oropharyngeal squamous cell carcinoma. A population-based case-control study in southern Sweden. Acta Otolaryngol 2005; 125 (12): 1327-1336.
94. Zhang ZF, Morgenstern H, Spitz MR, Tashkin DP, Yu GP, Marshall JR et al. Marijuana Use and Increased Risk of Squamous Cell Carcinoma of the Head and Neck. Cancer Epidemiol Biomarkers Prev 1999; 8: 1071-1078.
95. Foulkes WD**,** Brunet JS**,** Sieh W**,** Black MJ**,** Shenouda G**,** Narod SA**.** Familial risks of squamous cell carcinoma of the head and neck: retrospective case-control study. BMJ 1996; 313: 716-721.
96. Loning T, Ikenberg H, Becker J et al. Analysis of oral papillomas, leukoplakias, and invasive carcinomas for human papillomavirus type related DNA. J Invest Dermatol 1985; 84: 417-420.
97. Gillison ML, Shah KV. Human papillomavirus-associated head and neck squamous cell carcinoma: mounting evidence for an etiological role for human papillomavirus in a subset of head and neck cancers. Curr Opin Oncol 2001; 13: 183-188.
98 Smith EM, Ritchie JM, Summersgill KF et al. Age, sexual behaviour and human papillomavirus infection in oral cavity and oropharyngeal cancers. Int J Cancer 2004; 108: 766-772.
99. Klussmann JP, Gultekin E, Weissenborn SJ, et al. Expression of p16 protein identifies a distinct entity of tonsillar carcinomas associated with human papillomavirus. Am J Pathol 2003; 162: 747-753.
100. Lindel K, Beer KT, Laissue J, et al. Human papillomavirus positive squamous cell carcinoma of the oropharynx: A radiosensitive subgroup of head and neck carcinoma. Cancer 2001; 92: 805-813.
101. Portugal LG, Goldenberg JD, Wenig BL, et al. Human papillomavirus expression and p53 gene mutations in

squamous cell carcinoma. Arch Otolaryngol Head Neck Surg 1997; 123: 1230-1234.

102 Gillison ML, Lowy DR. A causal role for human papillomavirus in head and neck cancer. Lancet 2004; 363: 1488-1489.

103. Smith EM, Ritchie JM, Summersgill KF, et al. Human papillomavirus in oral exfoliated cells and risk of head and neck cancer. J Natl Cancer Inst 2004; 96: 449-455.

104. Snijders PJ, Scholes AG, Hart CA, et al. Prevalence of mucosotropic human papillomaviruses in squamous-cell carcinoma of the head and neck. Int J Cancer 1996; 66: 464-9.

105. Cruz IB, Snijders PJ, Steenbergen RD, et al. Age-dependence of human papillomavirus DNA presence in oral squamous cell carcinomas. Eur J Cancer B Oral Oncol 1996; 32B: 55-62.

106. Kjaer SK, Chackerian B, van den Brule AJ, et al. High-risk human papillomavirus is sexually transmitted: Evidence from a follow-up study of virgins starting sexual activity (intercourse). Cancer Epidemiol Biomarkers Prev 2001; 10: 101-106.

107. Kreimer AR, Alberg AJ, Daniel R, et al. Oral human papillomavirus infection in adults is associated with sexual behaviour and HIV serostatus. J Infect Dis 2004; 189: 686-698.

108. Coutlee F, Trottier AM, Ghattas G, et al. Risk factors for oral human papillomavirus in adults infected and not infected with human immunodeficiency virus. Sex Transm Dis 1997; 24: 23-31.

109. Smith EM, Hoffman HT, Summersgill KS, Kirchner HL, Turek LP, Haugen TH. Human papillomavirus and risk of oral cancer. Laryngoscope 1998; 108: 1098-103.

110. Maden C, Beckmann AM, Thomas DB, et al. Human papillomaviruses, herpes simplex viruses, and the risk of oral cancer in men. Am J Epidemiol 1992; 135: 1093-1102.

111. Rajkumar T, Sridhar H, Balaram P, et al. Oral cancer in Southern India: The influence of body size, diet, infections and sexual practices. Eur J Cancer Prev 2003; 12: 135-143.

112. Bjørge T, Dillner J, Anttila T, et al. Prospective seroepidemiological study of role of human papillomavirus in non-cervical anogenital cancers. BMJ 1997; 315: 646-9.

113. Mellin H, Friesland S, Lewensohn R, et al. Human papillomavirus (HPV) DNA in tonsillar cancer: clinical correlates, risk of relapse, and survival. Int J Cancer 2000; 89: 300-304.

114. Strome SE, Savva A, Brissett AE, et al. Squamous cell carcinoma of the tonsils: A molecular analysis of HPV associations. Clin Cancer Res 2002; 8: 1093-1100.

115. Sisk EA, Soltys SG, Zhu S, et al. Human papillomavirus and p53 mutational status as prognostic factors in head and neck carcinoma. Head Neck 2002; 24: 841-849.

116. van Houten VM, Snijders PJ, van den Brekel MW, et al. Biological evidence that human papillomaviruses are etiologically involved in a subgroup of head and neck squamous cell carcinomas. Int J Cancer 2001; 93: 232-235.

117. Wilczynski SP, Lin BT, Xie Y, et al. Detection of human papillomavirus DNA and oncoprotein overexpression are associated with distinct morphological patterns of tonsillar squamous cell carcinoma. Am J Pathol 1998; 152: 145-156.

118. Klussmann JP, Weissenborn SJ, Wieland U, et al. Human papillomavirus-positive tonsillar carcinomas: A different tumor entity? Med Microbiol Immunol (Berl) 2003; 192: 129-132.

119. Zoltan S, Szabolcs O, Miklos K. Indications for molecular detection of human papillomavirus (HPV) [in Hungarian]. Magy Onkol 2002; 46: 235-237.

120. El-Mofty SK, Lu DW. Prevalence of human papillomavirus type 16 DNA in squamous cell carcinoma of the palatine tonsil, and not the oral cavity, in young patients: A distinct clinicopathologic and molecular disease entity. Am J Surg Pathol 2003; 27: 1463-1470.

121. Poetsch M, Lorenz G, Bankau A, et al. Basaloid in contrast to nonbasaloid head and neck squamous cell carcinomas display aberrations especially in cell cycle control genes. Head Neck 2003; 25: 904-910.

122. Li W, Thompson CH, O'Brien CJ, et al. Human papillomavirus positivity predicts favourable outcome for squamous carcinoma of the tonsil. Int J Cancer 2003; 106: 553-558.

123. Schwartz SR, Yueh B, McDougall JK, et al. Human papillomavirus infection and survival in oral squamous cell cancer: A population-based study. Otolaryngol Head Neck Surg 2001; 125: 1-9.

124. Weinberger PM, Yu Z, Haffty BG, et al. Molecular classification identifies a subset of human papillomavirus–associated oropharyngeal cancers with favorable prognosis. J Clin Oncol 2006; 24: 736-747.

125. DeWeese TL, Walsh JC, Dillehay LE, et al. Human papillomavirus E6 and E7 oncoproteins alter cell cycle progression but not radiosensitivity of carcinoma cells treated with low-dose-rate radiation. Int J Radiat Oncol Biol Phys 1997; 37: 145-154.

126. Mellin Dahlstrand H, Lindquist D, Bjornestal L, et al. P16 (INK4a) correlates to human papillomavirus presence, response to radiotherapy and clinical outcome in tonsillar carcinoma. Anticancer Res 2005; 25: 4375-4383.

127. Butz K, Whitaker N, Denk C, et al. Induction of the p53-target gene GADD45 in HPV-positive cancer cells. Oncogene 1999; 18: 2381-2386.

128. Huang H, Li CY, Little JB. Abrogation of P53 function by transfection of HPV16 E6 gene does not en-

hance resistance of human tumour cells to ionizing radiation. Int J Radiat Biol 1996; 70: 151-160.
129. Ferris RL, Martinez I, Sirianni N, et al. Human papillomavirus-16 associated squamous cell carcinoma of the head and neck (SCCHN): A natural disease model provides insights into viral carcinogenesis. Eur J Cancer 2005;41: 807-815.
130. Jochmus-Kudielka I, Schneider A, Braun R, Kimmig R, Koldovsky U, Schneweis KE, et al. Antibodies against the human papillomavirus type 16 early proteins in human sera: correlation of anti-E7 reactivity with cervical cancer. J Natl Cancer Inst 1989; 81: 1698-1704.
131. Combita AL, Bravo MM, Touze A, Orozco O, Coursaget P. Serologic response to human oncogenic papillomavirus types 16, 18, 31, 33, 39, 58 and 59 virus-like particles in Colombian women with invasive cervical cancer. Int J Cancer 2002; 97: 796-803.
132. Zumbach K, Kisselijov F, Sacharova O et al. Antibodies against oncoproteins E6 and E7 of human papillomavirus types 16 and 18 in cervical carcinoma patients from Russia. Int J Cancer 2000; 85: 313-318.
133. Zumbach K, Hoffmann M, Kahn T, Bosch F, Gottschlich S, Gorogh T et al. Antibodies against oncoproteins E6 and E7 of human papillomavirus types 16 and 18 in patients with head-neck squamous-cell carcinoma. Int J Cancer 2000; 85: 815-818.
134. Ha PK, Califano JA. The role of human papillomavirus in oral carcinogenesis. Crit Rev Oral Bio Med 2004; 15 (4): 188-196.
135. Maitland NJ, Cox MF, Lynas C, Prime SS, Meanwell CA, Scully C. Detection of human papillomavirus DNA in biopsies of human oral tissue. Br J Cancer 1987; 56: 245-250.
136. Watts SL, Brewer EE, Fry TL. Human papillomavirus DNA types in squamous cell carcinomas of the head and neck. Oral Surg Oral Med Oral Pathol 1991; 71: 701-707.
137. Yeudall WA, Campo MS. Human papillomavirus DNA in biopsies of oral tissues. J Gen Virol 1991; 72: 173-176.
138. Syrjanen S, Partanen P, Mantyjarvi R, Syrjanen K. Sensitivity of in situ hybridization techniques using biotin- and 35S-labeled human papillomavirus (HPV) DNA probes. J Viro Methods 1988; 19: 225-238.
139. Fakhry C, Gillison ML. Clinical implications of human papillomavirus in head and neck cancers. J Clin Oncol 2006; 24: 2606-2611.
140. Begum S, Gillison ML, Ansari-Lari MA, et al. Detection of human papillomavirus in cervical lymph nodes: A highly efffective strategy for localizing site of tumor origin in patients with metastatic squamous cell carcinoma of the head and neck. Clin Cancer Res 2003; 9: 6469-6475.
141. Shibata DK, Arnheim N, Martin WJ. Detection of human papillomavirus in paraffin-embedded tissue using the polymerase chain reaction. J Exp Med 1988; 167: 225-230.
142. Snijders PJ, Meijer CJ, Walboomers JM. Degenerate primers based on highly conserved regions of amino acid sequence in papillomaviruses can be used in a generalized polymerase chain reaction to detect productive human papillomavirus infection. J Gen Virol 1991; 72: 2781-2786.
143. Gregoire L, Arella M, Campione-Piccardo J, Lancaster WD. Amplification of human papillomavirus DNA sequences by using conserved primers. J Clin Microbiol 1989; 27: 2660-2665.
144. Tieben LM, ter Schegget J, Minnaar RP, Bouwes Bavinck JN, Berkhout RJ, Vermeer BJ, et al. Detection of cutaneous and genital HPV types in clinical samples by PCR using consensus primers. J Virol Methods 1993; 42: 265-279.
145. Maitland NJ, Bromidge T, Cox MF, Crane IJ, Prime SS, Scully C. Detection of human papillomavirus genes in human oral tissue biopsies and cultures by polymerase chain reaction. Br J Cancer 1989; 59: 698-703.
146. Evander M, Wadell G. A general primer pair for amplification and detection of genital human papillomavirus types. J Virol Methods 1991; 31: 239-250.
147. Syrjanen SM. Basic concepts and practical applications of recombinant DNA techniques in detection of human papillomavirus (HPV) infection. APMIS 1990; 98: 95-110.
148. Schiffman MH. Validation of hybridization assays: correlation of filter in situ dot lot and PCR with Southern blot. IARC Sci Publ 1992; 119: 169-179.
149. Frazer IH, Leonard JH, Schonrock J, Wright RG, Kearsley JH. HPV DNA in oropharyngeal squamous cell cancers: comparison of results from four DNA detection methods. Pathology 1993; 25: 138-143.
150. Gravitt PE, Peyton C, Wheeler C, et al. Reproducibility of HPV 16 and HPV 18 viral load quantitation using TaqMan real-time PCR assays. J Virol Methods 2003; 112: 23-33.
151. Ha PK, Pai SI, Westra WH, et al. Real-time quantitative PCR demonstrates low prevalence of human papillomavirus type 16 in premalignant and malignant lesions of the oral cavity. Clin Cancer Res 2002; 8: 1203-1209.
152. Capone RB, Pai SI, Koch WM, Gillison ML, Danish HN, Westra WH et al. Detection and quantitation of human papillomavirus (HPV) DNA in the sera of patients with HPV-assosaited head and neck squamous cell carcinomas. Clin Cancer Res 2000; 6: 4171-4175.