Biology of Human Papillomavirus Infections in Head and Neck Carcinogenesis

Jaana Rautava · Stina Syrjänen

Received: 21 February 2012 / Accepted: 8 May 2012 / Published online: 3 July 2012
© Springer Science+Business Media, LLC 2012

Abstract The association between human papillomaviruses (HPV) and oral cancer was initially suggested nearly 30 years ago by us. Today, the research interest of head and neck squamous cell carcinoma (HNSCC) has substantially increased. HPV-associated HNSCC is considered a distinct clinical entity with better prognosis than the classical tobacco and alcohol associated cancers. HPV 16 seems to be the main genotype present in HNSCC and it most probably utilizes the same pathways in epithelial cell transformation as established for genital cancer. High-risk HPV E6 and E7 target the well characterized cellular proteins p53 and Rb, respectively. In addition, several other cellular targets of E6 and E7 have been identified. This review gives an overview on the biology of HPV which aids in dissecting the role of HPV in head and neck carcinogenesis. It also summarizes the possible pathways involved in creating new tools for diagnosis and therapy of HPV-associated HNSCC.

Keywords Mouth mucosa · Mouth neoplasms · Papillomavirus infections · HPV · Oncogenic proteins · Diagnosis · Transmission · Warts · Oral potentially malignant disorders · Cancer · Viral

Introduction

Human papillomaviruses (HPV) are small double-stranded, circular DNA viruses that can infect epithelial cells. Currently, nearly 150 different HPV types are recognized, of which 120 HPV types are fully sequenced. In this review, the basic concepts of viral molecular biology, control of HPV gene expression, the oncoproteins involved in HPV-induced malignant transformation, and deregulation of the normal cell cycle by HPV are covered briefly. Also, the salient features of the molecular biology specific to HPV and head and neck cancers are described.

Human Papillomaviruses (Structure and Classification)

HPVs have been traditionally referred to as “types”, with a type representing a cloned full-length HPV genome whose L1 nucleotide sequence is at least 10 % dissimilar from that of any other HPV type. The HPV types have also been grouped into mucosal or cutaneous types, based on their tropism for specific epithelial sites. Mucosal HPV types found preferentially in precancerous and cancerous lesions have been designated as ‘high-risk’ types and these include types 16, 18, 31, 33, 34, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, and 70. Mucosal HPVs found in benign genital warts and other non-malignant lesions are generally labeled as ‘low-risk’ types, the most important ones being HPV 6, 11, 42, 43, and 44 [1, 2].

In 2004, papillomaviruses (PV) were designated as a distinct family of viruses, the Papillomaviridae. This classification is based on phylogenetic analyses of the L1 gene, which is the most conserved gene in all known PVs [1]. The taxonomy levels include genera, species, genotypes, subtypes and variants. Most mucosal HPVs belong to the

J. Rautava · S. Syrjänen (✉)
Department of Oral Pathology, Faculty of Medicine,
Institute of Dentistry, University of Turku,
Lemminkäisenkatu 2, 20880 Turku, Finland
e-mail: stina.syrjanen@utu.fi

J. Rautava · S. Syrjänen
Department of Pathology,
Turku University Hospital, Turku, Finland

alpha genus (Fig. 1) while the skin types are included in the beta genus. The additional genera, gamma, mu and nu include also HPV types associated with skin papillomas and skin warts (verrucae) [1].

Fig. 1 The phylogenetic tree of genus alpha-papillomaviruses

Genomic Organization and Viral Gene Products

HPVs are non-enveloped, small DNA viruses with circular and double-stranded DNA genome of approximately 8,000 base pairs (bp). All putative protein coding sequences, called open reading frames (ORFs), are restricted to one strand. ORF is a segment of DNA that is large enough to encode for a protein. The coding sequences have been classified as early (E) containing the early genes E1, E2, E4, E5, E6 and E7, and late (L) containing the late genes encoding the major (L1) and minor (L2) capsid proteins. The structure and roles of the HPV proteins and their location in the host cell are summarized in Table 1. The genomic organization is presented in Fig. 2. The presence of E3 and E8 have been recently described only in a few HPV types but their function is unknown.

The PV virion is composed of 360 copies of the major capsid protein, L1, and up to 72 molecules of the minor capsid protein, L2. ORFs L1 and E6 are separated by 650–900 bp that do not encode proteins, i.e., a segment known as a non-coding region (NCR), long control region (LCR) or upstream regulatory region (URR). It contains

Table 1 Structure and role of HPV proteins and their location in the host cell

Protein	Molecular mass	Localization and amount in the cell	Function
<i>Early</i>			
E1	68–75 kDa 600–650 amino acids	Nuclear/+	Initiates replication of the viral genome. Activates helicase, keeps viral DNA episomal
E2	50 kDa 400 amino acids	Nuclear/+	Viral transcription and DNA replication. Segregation of viral genomes
E4	17 kDa: present mostly as a fusion protein, E1–E4. Two other forms are 16–10 kDa.	Cytoplasm/ +++	Facilitates packing of the viral genome. Maturation of viral particles. Destruction of cyokeratin filaments. Interaction with RNA helicase
E3			Not known
E5	8–10 kDa, hydrophobic	Cytoplasm/+	Interaction with EGF-receptor, activates PDGF-receptor. Oncoprotein, allows continuous proliferation of the host cell and delays differentiation
E6	16–18 kDa 150–160 amino acid	Nuclear/+	Blocks the normal regulation of host cell division. Degrades p53 in the presence of E6-AP. Interaction with several host proteins. Major oncoprotein, see Table 2
E7	11 kDa 100 amino acid	Nuclear/++	Blocks the normal regulation of host cell division. Binds to pRB-105, p107 ja p130. Interaction with several host proteins. Major oncoprotein, see Table 3
E8			Not known
<i>Late</i>			
L1	55–60 kDa	Nuclear/++++	Major capsid protein (n. 80 %)
L2	70 kDa	Nuclear/++	Minor capsid protein. Aids in viral localization into nucleus

promoter and enhancer DNA sequences critical to regulate viral replication and transcription by both viral and cellular genes. LCR with E2 binding sites and origin of replication (ori) is shown in Fig. 3. This region contains the highest degree of variation in the viral genome.

In cancer samples, HPV can be found in episomal (extra-chromosomal) form, integrated, or in mixed forms of both. Viral integration into the host-cell genome occurs

downstream of the early genes E6 and E7, often in the E1 or E2 region. This disruption results in a loss of negative-feedback control of oncogene expression by the viral regulatory E2 protein as shown in Fig. 2 [3]. Integrant-derived transcripts are more stable than those derived from episomal viral DNA, and HPV integration has been associated with a selective growth advantage for the affected cells [4].

The cellular abnormalities induced by HPV reflect complex interactions between viral- and cellular proteins. HPV can utilize three different strategies commonly used by higher organisms, including: (1) each piece of DNA can be translated theoretically into three different proteins, depending on the site where transcription begins; (2) different amino acid sequences from the same DNA sequence can be produced by RNA splicing. This indicates that mRNA is transcribed from DNA but is processed before the protein translation. Pieces of the mRNA sequence are deleted and the remaining pieces are spliced together to form a new strand of mRNA. The result is an mRNA sequence that differs from the original DNA sequence, and consequently a different protein will be encoded. As an example, low-risk types HPV-6 and HPV-11, have no introns within their E6 ORFs and express only a single E6 protein, in contrast to high-risk types HPV-16 and HPV-18 which can express a full length E6 protein and two truncated proteins (E*). Finally, (3) the third mode of function is a post-translational modification of the proteins.

For the control of RNA transcription, both promoters and enhancers are of importance. Promoters are consensus sequences known to be used for the same purposes in many different species. In HPV, consensus sequences are found where RNA polymerases bind to the DNA. In HPV, these sequences are typically TATAAA, located close to the starting point of RNA transcription. Two major promoters reside in the genomes of high-risk HPVs: an early promoter (p97 in HPV-16) located within the LCR and a late promoter (p670 in HPV-16) within E7. Transcripts initiated at p97 are polycistronic, with the potential to encode oncoproteins E6 and E7, as well as replication proteins E1 and E2. RNA polymerases need the presence of specific proteins, so called transcription factors, to recognize the promoter site and start the mRNA transcription. The efficiency of expression of particular HPV proteins depends on these

Fig. 2 Genomic organization of HPV type 16, presented together with the schematic presentation of HPV integration into host DNA

Fig. 3 Schematic presentation of the long control region (LCR) of HPV-16 showing the origin of replication and E2 binding sites

transcription proteins [3]. The protein expression is also affected by enhancers which are located even several thousands of nucleotides away from the gene to be transcribed. Enhancers can stimulate the effect of promoters. In HPVs, the enhancers might be even host-specific (e.g. keratinocyte specific), thus determining the host specificity of HPVs. Different genes can influence on the expression of each other by *cis* or *trans*-activation. In *cis* activation, a gene influencing the expression of another gene is in its close proximity. In *trans*-activation, the soluble gene product can migrate far from its original site of action. Thus, the position of the activating gene is not of importance.

HPV Life Cycle

In squamous epithelium, the productive cycle of HPV is intimately linked with differentiation factors expressed within various layers of the host epithelial cell [3, 5]. HPV cannot be cultured but only recently, an efficient virus propagation in vitro has been introduced [6]. HPVs infect undifferentiated basal cells of epithelia after trauma or erosion, and are maintained there at a low copy number. The productive phase of HPV infection is characterized by vegetative viral DNA replication. In this stage, the HPV genome is amplified up to more than 1,000 copies per cell. This will lead to expression of viral L genes, and mature viral particles are produced and released from the cells in the uppermost layers of differentiated epithelia. The schematic presentation of the expression of different HPV genes in HPV-infected epithelia with a productive infection is given in Fig. 4.

Fig. 4 Infectious cycle of HPV according to epithelial differentiation

Viral Entry in the Basal Cells

HPV can establish infection only after a successful binding with and entry into the host epithelial cell. Current concepts on cellular receptor binding and HPV entry into the host cell were recently reviewed by Letian and Tiarya in 2011 [7]. The first evidence of a putative HPV receptor was presented for $\alpha 6\beta 4$ integrins. [8]. It is highly expressed in laterally migrating epithelial cells, thus supporting HPV entry into wounded epithelia. Also cell surface heparin sulfate proteoglycans (HSPGs) have been claimed as HPV receptors. Of these HSPGs, syndecan-1 is the most likely receptor for HPV. Over-expression of syndecan-1 is also found in basal and parabasal cells following a trauma [9]. In their cellular entry, HPVs might also utilize a strategy of binding to several receptors, i.e., first to a primary receptor and then to be transferred to a secondary receptor. Thus, it seems likely that both $\alpha 6\beta 4$ integrin and HSPGs, functioning as the primary or secondary receptor, contribute individually or in combination to this process. It is also anticipated that other HPV receptors will be identified in the future.

After uptake, different HPV types require endosomal acidification for effective infection [10], probably first for viral uncoating. Different HPV types might enter the cells in distinct pathways, including clathrin- or caveolar-mediated endocytosis or clathrin- and caveolar-independent endocytosis [10, 11].

L2 is required for infectivity and required for egress of viral genomes from endosomes, while L1 does not appear to exit the endosomal compartment [12, 13]. Following endosomal escape, L2 accompanies the viral DNA to the nucleus. L2 interacts with microtubule network via dynein protein complex. The different steps of cell invasion by HPV are detailed in a recent review [14].

HPV Entry Into Tonsillar Crypt Epithelium

The presence of HPV has been shown in normal crypt epithelium [15]. The most prevalent HPV-associated cancer outside the genital region is tonsillar cancer which is thought to originate from the crypt epithelium [16]. Thus, the entry of HPV into crypt epithelium is of specific interest. The crypt epithelium of human palatine tonsils is a specialized squamous epithelium characterized by the presence of intraepithelial passages which are filled by non-epithelial cells. The passages are covered by the so-called M cells.

This epithelium contains patches of stratified squamous nonkeratinizing epithelium and patches of reticulated, sponge-like, epithelium [17]. The degree of reticulation of the epithelial cells and the infiltration of nonepithelial cells

varies. The major functions of the reticulated epithelium are: (1) to provide a favorable environment for the intimate contact between the effector cells of immune responses; (2) to facilitate direct transport of antigens; (3) to synthesize the secretory component continually; and (4) to contain a pool of immunoglobulins [17]. Thus, the reticulated epithelium lining of tonsillar crypts represents a specialized compartment, important in the first line immune defense. A breach in the continuity of the epithelium could explain the susceptibility of the tonsils to infection with viruses and bacteria. Also, M-cells could be the major port of HPV, as similarly shown for influenza A virus [18]. To summarize, HPV infection of crypt epithelium overlying the lymphoid tissue might be of more importance in evoking HPV-specific immunity than currently considered.

HPV Replication in Basal Cells

Only a portion of the virus reaching the nucleus seems to undergo replication and HPV replication remains confined to a small number of infected cells. Increasing inoculation leads to more viral DNA reaching the nucleus but not to a corresponding increase in viral replication. This could explain the patchy distribution of HPV DNA detected with in situ hybridization and the low amount of viral transcripts only detectable by highly-sensitive techniques. HPVs critically depend on the cellular machinery for the replication of their genome [3, 5]. During the course of infection in proliferating basal cells or in transformed cell systems, HPVs establish their genomes as low-copy-number, autonomously replicating episomes [3, 5, 19, 20]. HPV DNA replicates during the S phase, in synchrony with the host cell chromosome, and the mechanism of initiation of replication is fundamentally the same as that of eukaryotic chromosomes [19].

E2 is the initiating factor for HPV replication. E2 binds to LCR in HPV origin of transcription (ori) and facilitates the recruitment of E1, which is essential for viral replication [3, 20]. E2 protein is expressed in three forms, a full-length form that acts as a *trans*-activator, and two forms that lack the *trans*-activation domain. These truncated proteins suppress the transcriptional activity of the full-length E2. Target sequences for E2 are designated as E2 binding sites (E2BSs), four of which are located in highly-conserved positions in the regulatory region of a large group of HPVs, including all high-risk types, as shown in Fig. 3. E2 is also capable of binding to the mitotic chromatin by cellular protein Brd 4, aiding in segregation of viral DNA to the daughter cells during cell division [3, 19].

E1 ORF represents the most conserved structure among different PV types. E1 utilizes cellular molecules of the replication machinery, e.g. replication protein A,

topoisomerase I and polymerase alpha-primase. Unwinding of viral DNA is due to the helicase and ATPase activity of E1. E1 interacts with several cellular genes to synchronize the viral replication to host cell replication and is critical for G1/S transition and S phase. E1 also has an important role in retaining the episomal state of the viral molecule, i.e., keeping it separate from the host cell chromosome [3, 5, 19, 20].

Viral Genome Amplification

Viral amplification to over 1,000 copies is restricted to differentiated keratinocytes that are normally growth-arrested. Hence, the HPVs, especially viral proteins E5, E6 and E7, have developed strategies to subvert cellular growth regulatory pathways and are able to uncouple cellular proliferation and differentiation [3, 5, 19, 20]. E7 is the most important driver of the cell cycle deregulation (Fig. 5). The HPV E7 protein can induce cellular proliferation through several pathways by disrupting the activity of cyclin-dependent kinase (CDK) inhibitors p21 and p27, activation of CDKs and destabilization of the Rb tumor suppressor protein [for review see 20–24]. Rb family members (pRb, p107, p130) are instrumental in the control of cell cycle progression largely through regulation of the E2F family of transcription factors [23, 25]. Thus, binding of E7 to hypophosphorylated Rb results in the release of E2F factors that are necessary for transcription of genes involved in proliferation and cell cycle progression. The tight control of viral gene expression and function during infection is critical both for completion of the viral life cycle and for preventing malignant transformation. The activity of E7 can be controlled through a direct interaction with E2, resulting in an inhibition of transforming activity of E7 [20–25].

The best-characterized activity of E6 is its ability to bind and enhance the degradation of p53 through the ubiquitin–proteasome pathway as summarized in Fig. 6 [26, 27]. This results in a compromised ability of the host cell to engage cell cycle checkpoints and apoptotic responses. E6 proteins of both high-risk and low-risk HPVs have been shown to disrupt the transactivation potential of p53. Following the course of viral infection, the E2-induced E6 and E7 down-regulation releases p53 and p105Rb proteins, and the differentiation process can continue. The role of E6 and E7 in cell transformation is discussed later.

E5 is a membrane-associated protein and found within the cell as a homodimer [28]. E5 primarily plays a role in the productive phase of the viral life cycle. E5 also modulates EGFR-signaling pathways [39], as summarized in Fig. 7. E5 expression delays the internalization and degradation of EGFR, leading to increased levels of EGFR on

Fig. 5 Schematic presentation of progression toward malignancy caused by HPV oncoprotein E7. E7 targets pRb and deregulates the cell cycle restriction point (R). E2F transcription factors bind to DNA as homodimers or heterodimers in association with dimerization partner DP1 and regulate cell cycling by controlling the transcription of several cellular genes. pRb in hypo-phosphorylated form is associated with E2F molecules. pRb also effects on cellular gene expression by interaction of histone deacetylases (HDAC). Mitogenic signals activate Cyclins D1–D3, which promote progression through the G1–S phase of the cell cycle in a manner dependent on cyclin-

dependent kinases CDK4–6, and phosphorylate pRb causing the release of E2F/DP1. E7 binds to pRb and mimics its phosphorylation which leads the cell into the S phase. E7 also interacts with p21 leading to its degradation. p21(CDKN1A) plays a critical role in the cellular response to DNA damage, and its overexpression results in cell cycle arrest. The p16 protein [Cyclin-dependent kinase inhibitor-2A (CDKN2A) and also referred as p16(INK4)] binds to CDK4 and inhibits the ability of CDK4 to interact with cyclin D and stimulate passage through the G1 phase of the cell cycle

the surface of E5-expressing keratinocytes [29] through interaction with the 16 kD subunit of the vacuolar ATPase, which normally acts to acidify the endosomal environment [30].

HPV Particles and Their Release

After amplification of viral DNA, the next steps in the productive phase of the viral life cycle include: (1) the expression of E1–E4; (2) the onset of late genes L1 and L2; (3) viral DNA encapsidation; and (4) the release of newly-formed infectious virions. Activation of the late promoters leads to L1 and L2 gene expression. The onset of the L genes coding for L1 and the production of the virus are

strictly linked to the differentiation stage of the infected cells, and L1 proteins are detected primarily in terminally-differentiated cells in the upper layers of the epithelium [5]. L1 is the major capsid protein of HPV, forming 72 pentamers of five L1 molecules in addition to one L2 molecule. L1 has the capacity to self-assemble into virus-like particles (VLPs) when expressed as a eukaryotic recombinant protein [12, 31], which is used in the production of prophylactic HPV vaccines. L2 is needed for capsidation of HPV DNA, because L2 protein but not L1 protein, can bind HPV DNA.

E4 is involved in the release of viral particles from desquamating epithelial cells, but the mechanism is not fully understood as yet. E4 ORF represents a region of maximal divergence between different HPV types [5]. The

Fig. 6 Schematic presentation of progression toward malignancy caused by HPV oncoprotein E6. E6 protein binds to cellular ubiquitin ligase E6AP resulting in p53 degradation and blocking of p21.

HPV E6 also induces telomerase reverse transcriptase (hTERT) which prevents against telomerase erosion. hTERT is involved in cellular immortalization and progression of most cancers

Fig. 7 Schematic presentation of EGFR and the pathways activated after its phosphorylation. Oncoprotein E5 blocks ATPase and aids in recycling of EGFR and its expression on the cellular surface. E5 protein enhances cell proliferation also through the downregulation of tumor suppressor p21/27

most abundant viral message is formed by a single splice between the beginning of the E1 ORF and the E4 ORF and codes for the E1^{E4} protein. This protein can induce a

collapse of the cytokeratin (CK) network, whereas tubulin and actin networks remain unaffected by E1^{E4}, as does the nuclear lamina. The tight coupling of the E1^{E4} and L1

proteins at multiple time points suggests that the expression of both proteins is necessary to complete the virus life cycle. E4 has also been linked to apoptosis and modification of cornified cell envelopes.

Cell Transformation by HPVs

Figure 8 summarizes the critical steps in HPV-induced carcinogenesis as suggested by us in 2004 [32]. HPV can induce failures in cell cycle checkpoints, resulting in genetic instability which is the hallmark of human cancers. The major oncoproteins of HPV are E6 and E7, but also E5. They are also of importance in immune evasion, being

Fig. 8 Schematic presentation of the events involved in cancer development after HPV infection

involved in both innate and adaptive immunity as discussed shortly in the next paragraph below. As viral integration frequently leads to loss of E5 gene expression, it is critical in early stages of carcinogenesis, whereas E6 and E7 expression is needed for maintenance of the malignant phenotype [33].

The E6 protein is a major transforming protein of many PV types. However, E6 alone cannot immortalize human keratinocytes [for review see 19, 21–24, 33], but is capable of immortalizing mammary epithelial cells in vitro. E6-p53 complex formation requires an additional cellular protein, E6-AP, leading to complete degradation of p53. E6 binds directly to E6-AP protein, which functions as a specific ubiquitin-protein ligase targeting the E6-E6-AP complex to p53 [26]. E6 of the low-risk HPV types can also bind to E6-AP and complex to p53 but will not lead to degradation of p53. The low-risk HPVs have no introns within their E6 ORFs and they only express a single E6 protein. In contrast, high-risk HPV-16 and HPV-18 can express a full length E6 protein (E6), or truncated E proteins (E6*) termed E6*I and E6*II, by an alternative splicing within the E6 ORF. E6 protein of high-risk HPVs can bind to several cellular targets leading to interference with transcription, chromatin remodeling, cytokine signaling, protein degradation, cell polarity and apoptosis, leading to genomic instability [19, 21–24, 34, 35]. The cellular targets of E6 protein and their activities are summarized in Table 2.

The HPV E7 gene can immortalize human keratinocytes alone when expressed at high levels [for review see 24, 34, 35]. E7 protein of the low-risk HPV types also binds to pRb, although with lower affinity, but thus indicating its significance in transformation. However, it has to be noted that E7 of the low-risk HPV-1 can bind to pRb with the same affinity as E7 of HPV-16, indicating that mechanisms other than pRb binding are of importance for E7-associated malignant transformation [for review see 19, 21, 22, 24, 34, 35]. E7 subverts the normal activities of the cellular regulatory complexes by targeting several cellular proteins, which leads to interference of transcription, chromatin remodeling, DNA synthesis, cytokine signaling, genomic instability, apoptosis and cellular metabolism [19, 21–24, 34, 35]. The cellular target proteins of E7 and their activities are summarized in Table 3.

E5 induces cell transformation apparently via modulation of the tyrosine kinase growth factor receptor signaling pathways. The oncogenic activity of E5 is mediated principally by the growth factor receptors (Fig. 7). Although different domains of the PDGFR and EGFR are required for E5 activation, both receptors are activated directly by formation of an E5-containing complex. The role of E5 in HPV-induced carcinogenesis was reviewed by Maufort and coworkers in 2010 [36].

Table 2 Host cell proteins interacting with HPV E6 and their activities

Host protein	The role of host protein binding to E6
AMF-1/Gps2	Enhances p300 transcription
Bak	Proapoptotic protein, member of the Bcl-2 family
CBP/p300	p53 co-activator, signal regulator (activates cell cycle regulation, cell differentiation, immune defense), involved in chromatin remodeling
p/CAF	Chromatin remodeling
hTert	Extended proliferation
Mcm7	Extended proliferation
c-myc	Transcriptional factor, induces apoptosis
E6AP	Protein degradation, regulates signaling of cell proliferation by degrading Blk, a member of Src-family, involved in degradation of p53
p53	Tumor suppressor gene, regulates cellular response to mitotic stimuli
TBP	Transcriptional factor
E6TPI	GAP-protein, which activates GTPase and is a negative regulator of Rab-gene
ERC55 (E6BP)	Calcium binding protein which has a role in epithelial differentiation and blocking of apoptosis
hDlg/Sap97	Growth suppressor gene, which is human equivalent to Drosophila DLG. Important in epithelial cell polarization and cell adhesion
hScrib	Present in epithelial junctions, human equivalent to Drosophila growth suppressor gene Scrib (regulates cell adhesion and blocks epithelial growth)
Interferon regulating factor 3 (IRF3)	Cytokine signaling, positive transcriptional regulator of the IFN beta promoter
IFN-beta	Escape from immune surveillance, down regulates
IFN-alpha	Escape from immune surveillance, down regulates
STAT-1	Escape from immune surveillance, down regulates
IL-8 promoter	Escape from immune surveillance, down regulates
MAGI-1/2/3	Tight junction proteins; make complexes with β -catenin, regulator of PTEN-growth suppressor gene
Mcm7	Facilitates DNA-replication and starts it
Mupp1	Multiform PDZ-protein, signaling protein
Paxillin	Cell polarity and local adhesion protein, and the regulator of cytoskeleton protein actin
XRCC1	DNA repair protein
NHERF-1	Cellular signaling and transformation
PATJ	Tight junction protein

Table 2 continued

Host protein	The role of host protein binding to E6
PBM	Promote epithelial to mesenchymal transition-like changes, anchorage independent growth
TIP-1	Gain of function
PTPN3/PTPH1	Tyrosine phosphorylation of growth factor receptors
PTPN13	
Notch 1	Keratinocyte differentiation
E-cadherin	Adhesion between antigen presenting Langerhans cells and epithelial cells

Immune Escape and HPV

HPV infection can appear as acute, chronic or latent but the exact virological definitions for these HPV infections are still lacking, unlike for many other viruses (e.g. herpes simplex virus or hepatitis B). Persistent HPV infection (whether latent or chronic) is a key event for HPV-induced cellular transformation. Of importance in the establishment of persistent infection is the immune escape of HPV mainly orchestrated by E5, E6, and E7 as reviewed by Kanodia et al. 2007 [37] and Venuti et al. 2011 [38]. HPV-associated tumors exhibit loss of MHC Class I. Immunogenic peptides from both E7 and E6 proteins are not efficiently processed and presented by HPV+ tumor cells and this correlates with reduced expression of human leukocyte antigen (HLA) Class I, proteasome subunits, low-molecular-mass proteins (LMP), and the transporters of antigenic peptides (TAP) [37, 38]. HPV has also evolved mechanisms to avoid the effects of type I interferon (IFN) which is produced by infected cells and has anti-viral, antiproliferative, anti-angiogenic and immune-stimulatory activities [37]. E7 inhibits induction of IFN-alpha and genes stimulated by it via loss of ISG factor-3 (ISGF-3) transcription complex. The E6 protein of HPV also appears to target the IFN pathway. E6 binds to IRF-3, a factor that is produced constitutively in most cell types and is present in the cytoplasm in an inactive form [37]. HPV-16 E6 binding to IRF-3 inhibits its trans-activation function, thereby preventing transcription of IFN-beta mRNA. E6 also down-regulates STAT-1 and reduces the binding of STAT-1 to IREs. E5 plays an important role in escape of both innate and cell-mediated immunity [38]. E5 protein also down-regulates MHC/HLA Class I but selectively only the surface expression of HLA-A and HLA-B which present viral peptides to MHC Class I-restricted cytotoxic T lymphocytes (CTLs), but not the natural killer (NK) cell inhibitory ligands HLA-C and HLA-E [38]. The bridging of innate and adaptive immune responses can be affected

Table 3 Host cell proteins interacting HPV E7 and their activities

Host protein	The role of host protein binding to E7
Rb1	Transcription factor and control of cell division
p107	Transcription factor and control of cell division
RB2 (p130)	Transcription factor and control of cell division
E2F 1–5	Transcription factors, DNA synthesis
UBR4	
KCMF1	
AFT	Transcription factor
AP1	Transcription factor
TBP	Transcription factor
TAF110	Transcription factor
MPP2	Transcription factor
p27 (CKI)	Inhibitor of cyclin dependent kinase
p21 (CKI)	Inhibitor of cyclin dependent kinase
Cyclin A	
26S proteasome subunit S4	Protein degradation
TBP	Transcription initiation, TATA box binding protein
TAF110	Transcription initiation, TATA box binding protein, regulator of transcription
AP-1	Transcription factor
Mi2 (HDAC)	Histone deacetylase, chromatin remodelling
Mi1beta (HDAC)	Chromatin remodeling
BRAG1 (HDAC)	Chromatin remodeling
p300	Chromatin remodelling
CBP	Chromatin remodelling
pCAF	Chromatin remodelling
p53	Apoptosis
NF-kBh	Apoptosis
hTid	Apoptosis
p600	Apoptosis
IGFBP-3	Target of p53 transcription, regulates insulin like growth factor
M2 pyruvate kinase	m2 regulator via glycolysis
Glucosidase	Regulates glycolysis
hTid-1	Growth suppressor, human equivalent to drosophila tid56 dnaj, regulator of apoptosis
p48 (ISGF3)	Interferon regulating protein
TGF beta	Cytokine signaling
TNF alfa	Cytokine signaling
IFN	Cytokine signaling
IGF	Cytokine signaling
IRF-1	Regulator of IFN- β , inhibits activation of the IFN-beta promoter
IFN-alpha inducible genes	Induction inhibited via loss of ISGF-3 complex

Table 3 continued

Host protein	The role of host protein binding to E7
TAP	Alteration of MHC class I expression
MHC class I heavy chain promoter	Escape from immune surveillance
IL-8 promoter down regulated	Escape from immune surveillance
M2 pyruvate kinase	Cellular metabolism
Alpha-glucosidase	Cellular metabolism
Kinase B1	Cell cycle regulator in G2-phase
Smad 2, 3, 4	Regulates TGF- β signaling
P/CAF	Activator of IL-8 promoter in co-operation with CBP/p300
UBR4	Cellular transformation and anchorage-independent growth
CUL2	Proteasomal degradation of RB1
ZER1	Destabilization of RB1
CUL3	Protein degradation
NHE-1	Cytoplasmic alkalisation
TAP-1	Antigen processing pathway

by E5 via the inhibition of CD1d-mediated cytokine production [38]. In the future, potential new therapies of HPV infection could include not only the prevention of HPV entry into the cell but also interventions against HPV immune escape.

HPV and Head and Neck Cancer (HNSCC)

In 1977, zur Hausen suggested that HPV is involved in cervical carcinogenesis [2]. Seven years later, in 1983, we published the first evidence suggesting that a subgroup (some 20 %) of oral cancers is associated with HPV, based on detection of HPV structural proteins in these lesions using an antibody prepared against pooled HPV types [39]. We subsequently identified HPV types 11, 16 and 18 in these samples [40–49]. This concept has now been well accepted, and a growing body of evidence is supporting that approximately 20 % of oral cancers and 60–80 % of oropharyngeal cancers are caused by HPV [41–44]. In 2011, the International Agency of Research of Cancer (IARC), classifying both chemical and biological carcinogens to humans, declared that there is sufficient evidence that HPV-16 is causally associated with oral cancer [45]. Importantly, HPV-positive HNSCCs differ remarkably from HPV-negative HNSCCs in their clinical response and molecular properties. HPV-positive HNSCCs have a better overall-survival than HPV-negative HNSCCs [46, 47]. HPV-positive HNSCCs harbor the wild-type p53, while the classical smoking- and alcohol-induced cancers have mutated p53. However, it has been recently shown that also

among HPV-positive HNSCCs, the worst outcome is related to smoking [42, 47, 48]. Thus, these carcinogens might potentiate the transformation effect of HPV. This is supported by a bi-transgenic mouse model of HPV-associated HNSCC, developed by Lambert and coworkers. In this model, *K14E6* and *K14E7* mice were crossed but E6 and E7 could only induce a suprabasal DNA synthesis in the oral cavity [49, 50]. On the contrary, when the mice were treated with the oral carcinogen 4-nitroquinoline-n-oxide (4-NQO) in their drinking water as a co-carcinogen, the animals were dramatically more susceptible to carcinogenesis and developed tumors almost fully penetrant as compared to the low tumor incidence in the like-treated non-transgenic control group [51]. Subsequently, the same group showed that E7 is the dominant HPV oncoprotein in HNSCC, and they also reported that pRb/p107-deficient mice developed HNSCC as frequently as did HPV-16 E7 transgenic mice. Thus, inactivation of these two pocket proteins by E7 primarily drives E7 oncogenic properties in HPV-positive HNSCC [49].

The HPV copy numbers found in HNSCCs are often lower than detected in cervical cancers. Among HNSCC, the highest copy numbers are detected in palatine tonsillar cancers and the best survival figures are reported to those tonsillar cancers harboring the highest copy numbers and episomal form of HPV [52]. As discussed earlier, not all HPV genomes are replicating in the cells. Characteristic of chronic infections, in general, is the periodic occurrence (every now and then) of viral replication. If this is true for HPV, that could also partly explain the wide variation in HPV detection rates in HNSCCs; consequently, only highly-sensitive HPV testing methods and optimal sampling will result in HPV-positive results, as recently discussed [53].

It has been suggested that HPV infection is an early event in HPV-associated malignant transformation in HNSCCs. Also, the “hit-and-run” mechanism of HPV-mediated carcinogenesis in HNSCC has been discussed, as first suggested by us in the early 1990s. According to this concept, HPV infection is an early and possibly an initiating oncogenic event, but perhaps not needed in the later steps of malignant progression. This is supported by the fact that HPV is usually lost at early passages of cultured cells derived from the HNSCCs. Further evidence on the stepwise carcinogenesis is provided by our studies of the HPV-33-positive vaginal UT-DEC-1 cell line. It is known that not all vaginal cancers (approximately 40 %) are associated with HPV. In UT-DEC-1, HPV-33 was first episomal and became fully integrated at passage 20. The cells with integrated HPV always had a growth advantage over the cells with episomal HPV-33 [4]. In later passages, E6 expression increased in parallel with *hTERT*. However, we could identify an increase of *hTERT* activation but a decline of viral *E6/E7* mRNA expression [32]. We called

this “the point-of-no-return” in the progression toward malignancy (a potential “run” stage after the “hit” with HPV) [54]. We also selected five genes potentially important for this stepwise malignant progression and found that mRNA overexpression of *hTERT*, *DKC1*, *Bcl-2*, *S100A8*, and *S100A9* genes matched with the following events: (1) viral integration into the cell genome and episome loss; (2) the selection of cells with an acquired growth advantage and ability to maintain telomerase activity; and (3) the final stage of malignancy with permanently up-regulated telomerase [54]. Thus, integration of high-risk HPV is a key event in HPV-induced carcinogenesis. In early carcinogenesis, the concomitant presence of both circular HPV genomes and integrated form in the same cells might be the crucial event for additional chromosomal changes needed to acquire a growth advantage as supported by our in vitro model [4]. Thus, in early HPV infection, if both episomal and integrated forms of HPV exist in the same cell, the replication of integrated HPV leads to rearrangements within the integrated locus being thus the key event in the “hit-and-run” mechanisms [55]. The HPV biology of head and neck cancer has also recently been discussed in two excellent reviews [56, 57].

Because most of the HPV-positive HNSCCs have a wild type p53 (wtp53), and p53 is mutated in 60–80 % of HNSCC samples, it was expected that the rest of these tumors could be HPV-infected cancers with wtp53. However, this could not be confirmed. It has been recently shown that a better correlation between HPV-positivity and wtp53 in HNSCC can be obtained when E6 and E7 mRNA expression is used as the criteria for HPV-positivity [56]. Until now, less than half of the HPV infections detected in HNSCC have been shown to express viral E6 and E7 transcripts. As was discussed earlier, not all viral genomes will replicate and in chronic infection replication can be activated every now and then. It has been suggested that the detection of E6 and E7 mRNAs would thus be the method of choice in HNSCC, together with p16 expression which is a surrogate marker for HPV [58]. However, one has to remember that p16 is also up-regulated in cellular senescence. When p16 expression is used as a marker for HPV infection, approximately 10 % of these lesions may be false positives.

The role of HPV in the development of HNSCC includes a coordinated targeting of multiple pathways by HPV oncoproteins E5, E6 and E7 [21, 23, 36, 57, 59]. Modeling the molecular circuitry of cancer has been presented by Hahn and Weinberg in 2002 [60]. Among the important pathways involved in HPV-induced cancers, are (1) the p53 and pRb pathways (cell cycling), (2) the EGFR pathway (growth factor signaling), (3) the TGF β pathway (growth factor signaling), (4) PI3K–PTEN–AKT (evading apoptosis, which may also be one of the important

pathways providing better survival for HPV-positive HNSCC after irradiation) and, finally, (5) angiogenesis including hypoxia-inducible factor (HIF). Activation of Wnt signaling pathways by E6 and E7 in head and neck cancer has been presented by Rampias and coworkers in 2011 [61]. The consequence of the HPV interference in these pathways is the accumulation of mutations in cellular genes and genomic instability [56]. This finally will lead to full transformation. Wilting and coworkers in 2009 showed that loss at 13q and gain at 20q were frequent in HPV-positive carcinomas of both cervical cancers (CxSCC) and HNSCCs, but uncommon in HR-HPV-negative HNSCCs, indicating that these alterations are associated with HR-HPV-mediated carcinogenesis [62]. Within the group of HR-HPV-positive carcinomas, HNSCCs more frequently showed gains of multiple regions at 8q whereas CxSCCs more often showed loss at 17p. The understanding of the molecular events and pathways of HPV-associated cancers in general and especially of anatomy-related HPV cancers will provide opportunities to create new molecular markers for HPV diagnostics and new therapeutic approaches.

In conclusion, during initial infection HPV is episomal but can become integrated at an early stage of infection leading to the rearrangements within the integrated host gene locus. HPV-16 is the most potent HPV to cause cancer at several sites of the human body. The major HPV oncoproteins are HPV E6 and E7, which target multiple cellular pathways. Immune evasion is important in establishment of persistent HPV infection, which provides the greatest risk for the development of HPV-related cancer. E5, E6 and E7 also play an important role in evasion of both innate and adaptive immunity. HPV is causally associated with a subgroup of HNSCC and this evidence is strongest for oropharyngeal cancer. HPV-positive HNSCCs differ remarkably from HPV-negative HNSCCs in their clinical response, risk factors and molecular properties. Importantly, HPV-positive HNSCCs have better overall-survival than HPV-negative HNSCCs. Thus, further studies are needed to dissect the molecular mechanisms involved in HPV-associated HNSCC in order to create optimal prevention and treatment modalities.

Acknowledgments The other author (SS) has received consultancy fees from Sanofi Pasteur MSD.

References

- de Villiers EM, Fauquet C, Broker TR, et al. Classification of papillomaviruses. *Virology*. 2004;324:17–27.
- Zur Hausen H. Papillomaviruses and cancer: from basic studies to clinical application. *Nat Rev Cancer*. 2002;2:342–50.
- Thierry F. Transcriptional regulation of the papillomavirus oncogenes by cellular and viral transcription factors in cervical carcinoma. *Virology*. 2009;384:375–9.
- Ruutu M, Peitsaro P, Johansson B, Syrjänen S. Transcriptional profiling of a human papillomavirus 33-positive squamous epithelial cell line which acquired a selective growth advantage after viral integration. *Int J Cancer*. 2002;100:318–26.
- Doorbar J. The papillomavirus life cycle. *J Clin Virol*. 2005;32(Suppl 1):S7–15. Review.
- Wang HK, Duffy AA, Broker TR, Chow LT. Robust production and passaging of infectious HPV in squamous epithelium of primary human keratinocytes. *Genes Dev*. 2009;23:181–94.
- Letian T, Tianyu Z. Cellular receptor binding and entry of human papillomavirus. Cellular receptor binding and entry of human papillomavirus. *Virol J*. 2010;7:2. Review.
- Kajiji S, Tamura RN, Quaranta V. A novel integrin (alpha 6 beta 4) from human epithelial cells suggests a fourth family of integrin adhesion receptors. *EMBO J*. 1989;8:673–80.
- Shafit-Keramat S, Handisurya A, Kriehuber E, et al. Different heparan sulfate proteoglycans serve as cellular receptors for human papillomaviruses. *J Virol*. 2003;77:13125–35.
- Day PM, Lowy DR, Schiller JT. Papillomaviruses infect cells via a clathrin-dependent pathway. *Virology*. 2003;307:1–11.
- Bousarghin L, Touzé A, Sizaret PY, Coursaget P. Human papillomavirus types 16, 31, and 58 use different endocytosis pathways to enter cells. *J Virol*. 2003;77:3846–50.
- Zhou J, Sun XY, Stenzel DJ, Frazer IH. Expression of vaccinia recombinant HPV 16 L1 and L2 ORF proteins in epithelial cells is sufficient for assembly of HPV virion-like particles. *Virology*. 1991;185:251–7.
- Kämper N, Day PM, Nowak T, et al. A membrane-destabilizing peptide in capsid protein L2 is required for egress of papillomavirus genomes from endosomes. *J Virol*. 2006;80:759–68.
- Sapp M, Bienkowska-Haba M. Viral entry mechanisms: human papillomavirus and a long journey from extracellular matrix to the nucleus. *FEBS J*. 2009;276:7206–16. Review.
- Syrjänen S. HPV infections and tonsillar carcinoma. *J Clin Pathol*. 2004;57:449–55. Review.
- Kim SH, Koo BS, Kang S, Park K, Kim H, Lee KR, Lee MJ, Kim JM, Choi EC, Cho NH. HPV integration begins in the tonsillar crypt and leads to the alteration of p16, EGFR and c-myc during tumor formation. *Int J Cancer*. 2007;120:1418–25.
- Surján L. Immunohistochemical markers of tonsillar crypt epithelium. *Acta Otolaryngol*. 1988;105:60–3.
- Fujimura Y, Takeda M, Ikai H, Haruma K, Akisada T, Harada T, Sakai T, Ohuchi M. The role of M cells of human nasopharyngeal lymphoid tissue in influenza virus sampling. *Virchows Arch*. 2004;444:36–42.
- Ghittoni R, Accardi R, Hasan U, et al. The biological properties of E6 and E7 oncoproteins from human papillomaviruses. *Virus Genes*. 2010;40:1–13.
- Hamid NA, Brown C, Gaston K. The regulation of cell proliferation by the papillomavirus early proteins. *Cell Mol Life Sci*. 2009;66:1700–17.
- Moody CA, Laimins LA. Human papillomavirus oncoproteins: pathways to transformation. *Nat Rev Cancer*. 2010;10:550–60. Review.
- Finzer P, Aguilar-Lemarroy A, Rösl F. The role of human papillomavirus oncoproteins E6 and E7 in apoptosis. *Cancer Lett*. 2002;188:15–24. Review.
- Münger K, Baldwin A, Edwards KM, et al. Mechanisms of human papillomavirus-induced oncogenesis. *J Virol*. 2004;78:11451–60.
- Javier RT, Rice AP. Emerging theme: cellular PDZ proteins as common targets of pathogenic viruses. *J Virol*. 2011;85:11544–56. Review.
- van den Heuvel S, Dyson NJ. Conserved functions of the pRB and E2F families. *Nat Rev Mol Cell Biol*. 2008;9:713–24. Review.

26. Scheffner M, Werness BA, Huibregtse JM, et al. The E6 oncoprotein encoded by human papillomavirus types 16 and 18 promotes the degradation of p53. *Cell*. 1990;63:1129–36.
27. Werness BA, Levine AJ, Howley PM. Association of human papillomavirus types 16 and 18 E6 proteins with p53. *Science*. 1990;248:76–9.
28. Schlegel R, Wade-Glass M, Rabson MS, Yang YC. The E5 transforming gene of bovine papillomavirus encodes a small, hydrophobic polypeptide. *Science*. 1986;233:464–7.
29. Straight SW, Hinkle PM, Jewers RJ, McCance DJ. The E5 oncoprotein of human papillomavirus type 16 transforms fibroblasts and effects the downregulation of the epidermal growth factor receptor in keratinocytes. *J Virol*. 1993;67:4521–32.
30. Disbrow GL, Hanover JA, Schlegel R. Endoplasmic reticulum-localized human papillomavirus type 16 E5 protein alters endosomal pH but not trans-Golgi pH. *J Virol*. 2005;79:5839–46.
31. Kirnbauer R, Taub J, Greenstone H, et al. Efficient self-assembly of human papillomavirus type 16 L1 and L1-L2 into virus-like particles. *J Virol*. 1993;67:6929–36.
32. Peitsaro P, Ruutu M, Syrjänen S, Johansson B. Divergent expression changes of telomerase and E6/E7 mRNA, following integration of human papillomavirus type 33 in cultured epithelial cells. *Scand J Infect Dis*. 2004;36:302–4.
33. Duensing S, Münger K. Mechanisms of genomic instability in human cancer: insights from studies with human papillomavirus oncoproteins. *Int J Cancer*. 2004;109:157–62.
34. McLaughlin-Drubin ME, Münger K. The human papillomavirus E7 oncoprotein. *Virology*. 2009;384:335–44. Review.
35. White EA, Sowa ME, Tan MJ, et al. Systematic identification of interactions between host cell proteins and E7 oncoproteins from diverse human papillomaviruses. *Proc Natl Acad Sci U S A*. 2012;109:E260–7.
36. Maufort JP, Shai A, Pitot HC, Lambert PF. A role for HPV16 E5 in cervical carcinogenesis. *Cancer Res*. 2010;70:2924–31.
37. Kanodia S, Fahey LM, Kast M. Mechanisms used by human papillomaviruses to escape the host immune response. *Curr Cancer Drug Targets*. 2007;7:79–89.
38. Venuti A, Paolini F, Nasir L, Corteggio A, Roperto S, Campo MS, Borzacchiello G. Papillomavirus E5: the smallest oncoprotein with many functions. *Mol Cancer*. 2011;10:140. Published online 2011 November 11. doi:10.1186/1476-4598-10-140.
39. Syrjänen K, Syrjänen S, Lamberg M, et al. Morphological and immunohistochemical evidence suggesting human papillomavirus (HPV) involvement in oral squamous cell carcinogenesis. *Int J Oral Surg*. 1983;12:418–24.
40. Chang F, Syrjänen S, Nuutinen J, et al. Detection of human papillomavirus (HPV) DNA in oral squamous cell carcinomas by in situ hybridization and polymerase chain reaction. *Arch Dermatol Res*. 1990;282:493–7.
41. Hansson BG, Rosenquist K, Antonsson A, et al. Strong association between infection with human papillomavirus and oral and oropharyngeal squamous cell carcinoma: a population-based case–control study in southern Sweden. *Acta Otolaryngol*. 2005;125:1337–44.
42. Ang KK, Harris J, Wheeler R, et al. Human papillomavirus. *N Engl J Med*. 2010;363:24–53.
43. Mehanna H, Beech T, Nicholson T, et al. Prevalence of human papillomavirus in oropharyngeal and nonoropharyngeal head and neck cancer-systematic review and meta-analysis of trends by time and region. *Head Neck*. 2012. doi:10.1002/hed.22015. [Epub ahead of print].
44. Syrjänen S, Lodi G, von Bültzingslöwen I, et al. Human papillomaviruses in oral carcinoma and oral potentially malignant disorders: a systematic review. *Oral Dis*. 2011;Suppl 1:58–72. Review.
45. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Part B: biological agents LYON, France 2011, Volume 100B pp. 278–80.
46. Dayyani F, Etzel CJ, Liu M, et al. Meta-analysis of the impact of human papillomavirus (HPV) on cancer risk and overall survival in head and neck squamous cell carcinomas (HNSCC). *Head Neck Oncol*. 2010;2:15. Published online 2010 June 29. doi:10.1186/1758-3284-2-15.
47. Fakhry C, Westra WH, Li S, et al. Improved survival of patients with human papillomavirus-positive head and neck squamous cell carcinoma in a prospective clinical trial. *J Natl Cancer Inst*. 2008;100:261–9.
48. Sinha P, Logan HL, Mendenhall WM. Human papillomavirus, smoking, and head and neck cancer. *Am J Otolaryngol*. 2012;33:130–6.
49. Jabbar S, Strati K, Shin MK, Pitot HC, Lambert PF. Human papillomavirus type 16 E6 and E7 oncoproteins act synergistically to cause head and neck cancer in mice. *Virology*. 2010;407:60–7.
50. Song S, Liem A, Miller JA, Lambert PF. Human papillomavirus types 16 E6 and E7 contribute differently to carcinogenesis. *Virology*. 2000;267:141–50.
51. Strati K, Pitot HC, Lambert PF. Identification of biomarkers that distinguish human papillomavirus (HPV)-positive versus HPV-negative head and neck cancers in a mouse model. *Proc Natl Acad Sci U S A*. 2006;103:14152–7.
52. Mellin H, Dahlgren L, Munck-Wikland E, Lindholm J, Rabbani H, Kalantari M, Dalianis T. Human papillomavirus type 16 is episomal and a high viral load may be correlated to better prognosis in tonsillar cancer. *Int J Cancer*. 2002;102:152–8.
53. Rautava J, Syrjänen S. Human papillomavirus infections in the oral mucosa. *J Am Dent Assoc*. 2011;142:905–14. Review.
54. Koskimaa HM, Kurvinen K, Costa S, et al. Molecular markers implicating early malignant events in cervical carcinogenesis. *Cancer Epidemiol Biomarkers Prev*. 2010;19:2003–12.
55. Kadaja M, Isok-Paas H, Laos T, Ustav E, Ustav M. Mechanism of genomic instability in cells infected with the high-risk human papillomaviruses. *PLoS Pathog*. 2009;5(4):e1000397.
56. Leemans CR, Braakhuis BJ, Brakenhoff RH. The molecular biology of head and neck cancer. *Nat Rev Cancer*. 2011;11:9–22. Review.
57. Strati K, Lambert PF. Human papillomavirus association with head and neck cancers: understanding virus biology and using it in the development of cancer diagnostics. *Expert Opin Med Diagn*. 2008;2:11–20.
58. Lewis JS Jr, Thorstad WL, Chernock RD, et al. p16 positive oropharyngeal squamous cell carcinoma: an entity with a favorable prognosis regardless of tumor HPV status. *Am J Surg Pathol*. 2010;34:1088–96.
59. Saha A, Kaul R, Murakami M, Robertson ES. Tumor viruses and cancer biology: modulating signaling pathways for therapeutic intervention. *Cancer Biol Ther*. 2010;10:961–78. Review.
60. Hahn WC, Weinberg RA. Modelling the molecular circuitry of cancer. *Nat Rev Cancer*. 2002;2(331–341):61.
61. Rampias T, Boutati E, Pectasides E, et al. Activation of Wnt signaling pathway by human papillomavirus E6 and E7 onco-genes in HPV16-positive oropharyngeal squamous carcinoma cells. *Mol Cancer Res*. 2010;8:433–43.
62. Wilting SM, Smeets SJ, Snijders PJ, et al. Genomic profiling identifies common HPV-associated chromosomal alterations in squamous cell carcinomas of cervix and head and neck. *BMC Med Genomics*. 2009;2:32. doi:10.1186/1755-8794-2-32.