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Review

The Biology and Life-Cycle of Human Papillomaviruses

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ABSTRACT

Human papillomaviruses (HPVs) comprise a diverse group, and have different epithelial tropisms and life-cycle strategies. Many HPVs are classified as low-risk, as they are only very rarely associated with neoplasia or cancer in the general population. These HPVs typically cause inapparent/inconspicuous infections, or benign papillomas, which can persist for months or years, but which are eventually resolved by the host's immune system. Low-risk HPVs are difficult to manage in immunosuppressed people and in individuals with genetic predispositions, and can give rise to papillomatosis, and in rare instances, to cancer. The high-risk HPV types are, by contrast, a cause of several important human cancers, including almost all cases of cervical cancer, a large proportion of other anogenital cancers and a growing number of head and neck tumours. The high-risk HPV types constitute a subset of the genus *Alphapapillomavirus* that are prevalent in the general population, and in most individuals cause only inconspicuous oral and genital lesions. Cancer progression is associated with persistent high-risk HPV infection and with deregulated viral gene expression, which leads to excessive cell proliferation, deficient DNA repair, and the accumulation of genetic damage in the infected cell. Although their life-cycle organisation is broadly similar to that of the low-risk HPV types, the two groups differ significantly in their capacity to drive cell cycle entry and cell proliferation in the basal/parabasal cell layers. This is thought to be linked, at least in part, to different abilities of the high- and low-risk E6 proteins to modulate the activity of p53 and PDZ-domain proteins, and the differential ability of the E7 proteins to target the several different members of the retinoblastoma protein family.

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1. The diversity of human papillomaviruses and the diseases that they cause

To date, more than 150 human papillomavirus (HPV) types have been completely sequenced (Fig. 1), along with over 60 animal papillomaviruses (PV) (see Papillomavirus Episteme (PaVE); http://pave.niaid.nih.gov/#home) and [1]). The presence of PVs in mammals, as well as in various diverse hosts, including birds, turtles and snakes, suggests that they may be ubiquitously present amongst present day amniotes (i.e., mammals, birds and reptiles) [2].

Papillomavirus types found in humans are divided into five genera based on DNA sequence analysis, with the different types having different life-cycle characteristics and disease associations [1,3–5] (Fig. 1). In recent years, it has become clear that many HPV types, including the majority of those contained within the Beta and Gamma genera, cause only asymptomatic infections in immunocompetent individuals and can be detected in skin swabs, and for some Gamma types, also in mucosal rinses [6–9]. Such viruses are well adapted to their host, and can in most instances complete their life-cycle and be maintained in the population without causing any apparent disease [5,10]. Such characteristics suggest that the PV-host interactions are very old, and that over time, this has lead to a balance between viral replication and immune tolerance [11]. Indeed, the evolutionary origins of PVs can be traced to the origin of the amniotes themselves (approximately 350 million years

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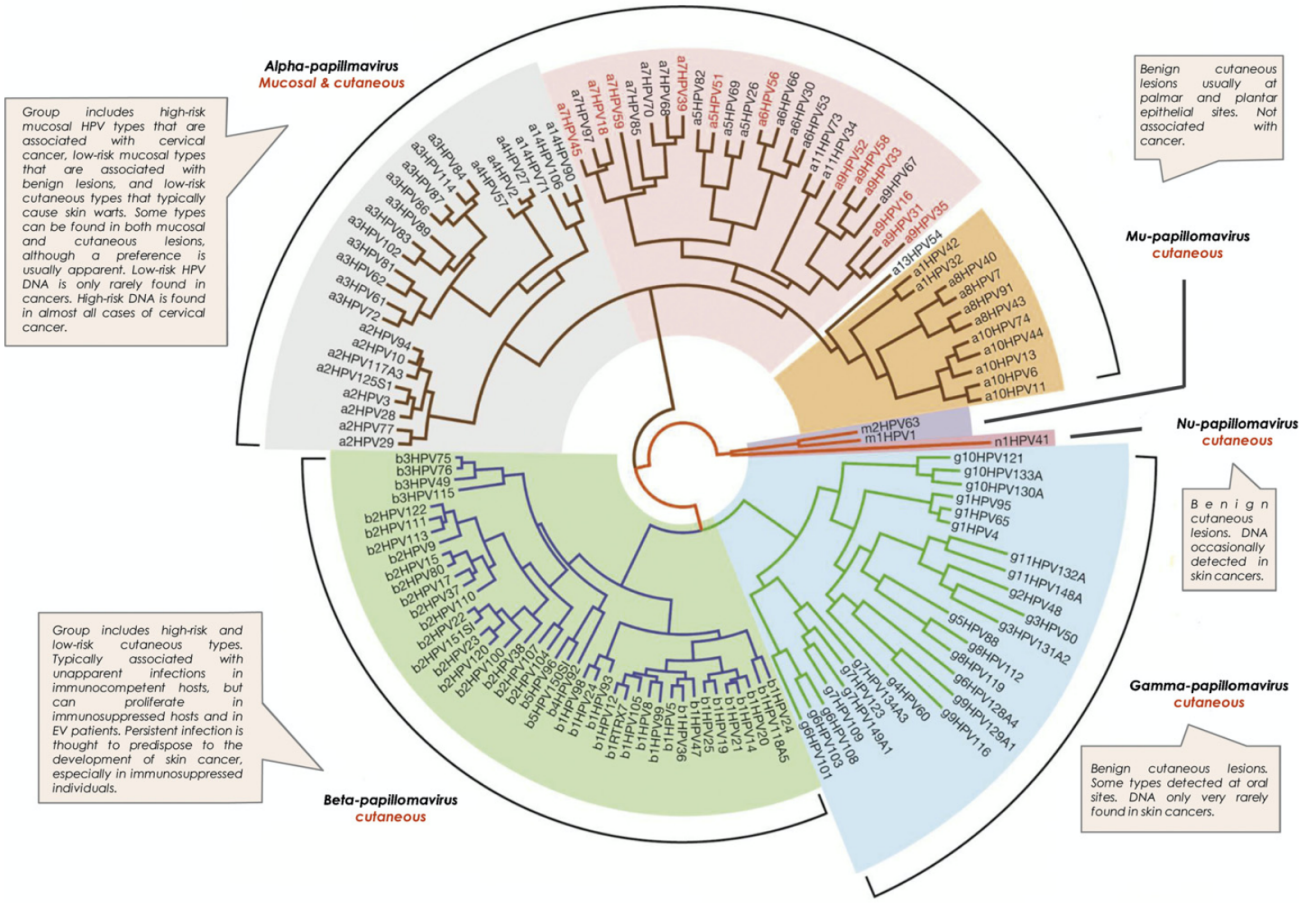

Figure 1. Evolutionary Relationship between Human Papillomaviruses.
Human Papillomaviruses comprise five evolutionary groups with different epithelial tropisms and disease associations. The Alpha papillomaviruses include the low-risk mucosal types (many of which are within the orange shaded branch) that cause genital warts, and the high-risk mucosal types (contained within the branch highlighted with pink shading) that can cause cervical neoplasias and cancer. Although the cutaneous HPV types (most of which are contained within the grey (Alpha), green (Beta) and blue (Gamma) shaded branches) are not generally associated with cancers, certain Beta types have been implicated in the development of non-melanoma skin cancers (NMSC) in immunosuppressed individuals and in epidermodysplasia verruciformis (EV) patients. Their possible role in cancer progression in the general population is currently unresolved. The image shows the best known maximum likelihood phylogenetic tree for the E1E2L2L1 genes of 132 HPVs. The sequences were aligned at the amino acid level with MUSCLE, filtered with GBLOCKS, and the corresponding codon sequences concatenated. Phylogenetic inference was performed with RAxML including three partitions per gene, one per codon position, using the GTR + G4 model. Branch lengths are proportional to substitutions per site.

ago [12–14]), with many evolutionary mechanisms contributing to their current diversity, including host/virus co-evolution, recombination, host-switching and the possible extinction of the PV lineage in some hosts [15].

In humans, the PV types that cause visible papillomas are generally of most concern for the individual, especially when they occur at oral or genital sites and are persistent. Approximately one-third of individuals who present for treatment with genital warts will still have their lesions 3 months later, with recurrence after treatment being a significant problem [16]. The low-risk Alpha types that cause these lesions (typically the Alpha 10 species [e.g., HPV6 and 11]; Fig. 1) are also implicated in the development of respiratory papillomatosis (RRP) [17]. Although rare, juvenile RRP (which affects around 4 per 100,000 children [18–20]) is a serious condition that can only be managed by repeated surgery, and can progress to cancer in a small percentage (approximately 5%) of persistently infected individuals where the infection spreads to the lung [20,21].

The various types of epithelial disease that HPVs cause (i.e., chronic asymptomatic infection or transient visible papillomas) appear linked to their different strategies of transmission and propagation within the epithelium, and probably also to their different interactions with the immune system [22]. During evolution, HPVs have adapted to specific epithelial niches, with different types having different disease associations and disease prevalence [13,14,23]. Amongst cutaneous HPVs, the diversity within the Alpha (species 2, 3, 4 and 14; see Fig. 1), Beta and Gamma genera contrasts sharply to what is seen in the apparently less successful Mu and Nu genera. The most well studied HPV types are, however, the mucosal Alpha types that cause cervical cancer (see Fig. 2A) [24], and for these the biology of disease is relatively well understood [3]. This is certainly the case for HPV16 (Fig. 2B) infections of the ectocervix and the cervical transformation zone where the majority of HPV16-associated cervical cancers develop (Fig. 3). The life-cycle organisation of HPV16 (and Alpha types in general) at other important epithelial sites, such as the anus, the endocervix, the penis [25,26] and the oropharynx [27] is, however, still poorly understood [28].

2. High- and low-risk types and their association with cancers

The Alpha PVs are divided into cutaneous and mucosal types, and the mucosal types are further subdivided into high-risk and

A

Genus + Species	Type	Invasive Cervical Cancer	IARC Category	Squamous Cell Carcinoma	Adeno Carcinoma	Tropism
Alpha 1	HPV32		3			mucosal
	HPV42					
Alpha 2	HPV3					cutaneous
	HPV10					
	HPV28		3			
	HPV29		3			
	HPV77		3			
	HPV94					
	HPV117					
	HPV125					
Alpha 3	HPV61	0.01	3			mucosal
	HPV62		3			
	HPV72		3			
	HPV81		3	0.4		
	HPV83		3	0.4		
	HPV84		3			
	HPV86		3			
	HPV87		3			
	HPV89		3			
	HPV102					
	HPV114					
Alpha 4	HPV2		3			cutaneous
	HPV27		3			
	HPV57		3			
Alpha 5	HPV26	0.37	2B	0.22		mucosal
	HPV51	1.25	1	0.75	0.54	
	HPV69	0.08	2B			
	HPV82	0.07	2B	0.26		
Alpha 6	HPV30	0.37	2B			
	HPV53	0.26	2B	0.04		
	HPV56	0.84	1	1.09		
	HPV66	0.08	2B	0.19		
Alpha 7	HPV18	10.28	1	11.27	37.3	
	HPV39	1.67	1	0.82	0.54	
	HPV45	5.68	1	5.21	5.95	
	HPV59	1.08	1	1.05	2.16	
	HPV68	1.04	2A	0.37		
	HPV70	0.11	2B			
	HPV85		2B			
	HPV97					
Alpha 8	HPV7		3		41.62	cutaneous (mucosal)
	HPV40		3		1.08	
	HPV43				0.54	
	HPV91	0.01	3		1.08	
Alpha 9	HPV16	61.35	1	54.38		mucosal
	HPV31	3.35	1	3.82	0.54	
	HPV33	3.83	1	2.06		
	HPV35	1.94	1	1.27		
	HPV52	2.71	1	2.25		
	HPV58	2.22	1	1.72		
	HPV67	0.31	2B			
Alpha 10	HPV6	0.11	3	0.07		mucosal
	HPV11	0.02	3	0.07		
	HPV13		3			
	HPV44	0.01	3			
	HPV74	0.01	3			
Alpha 11	HPV34	0.07				mucosal
	HPV73	0.52		0.49		
Alpha 12	HPV73					mucosal
Alpha 13	HPV54					
Alpha 14	HPV71					
	HPV90		3			
	HPV106		3			

B

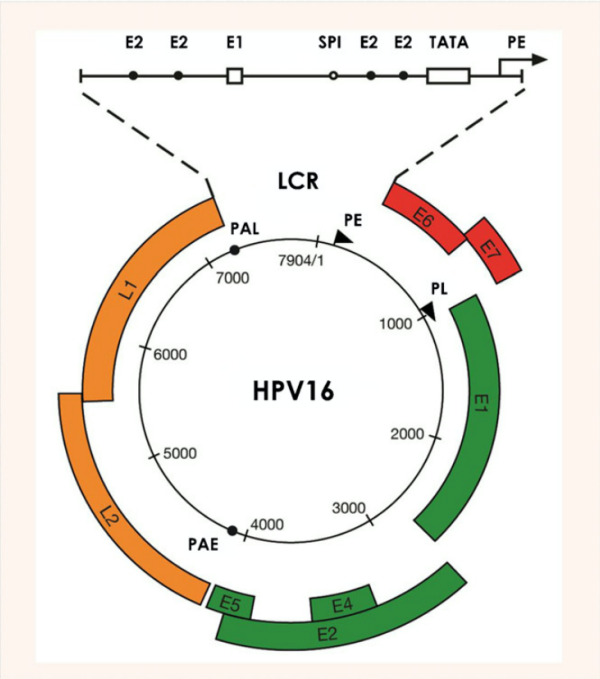

Figure 2. Alpha Papillomavirus Disease Association and Genome Organisation.
A. The high-risk Alpha types have been clearly linked with the development of squamous cell carcinoma (SCC) and adenocarcinoma (AC) of the cervix. IARC category 1 and 2A HPV types are classified (respectively) as carcinogenic and possibly-carcinogenic. Despite limited epidemiological data, the 2B classification is proposed for types that are probably carcinogenic because of their close phylogenetic relationship with the established carcinogenic types. HPV types in category 3 are considered non-carcinogenic. The remaining types have not yet been classified because of insufficient data. Types that are closely related evolutionarily (e.g., HPV16 and 31) can exhibit different degrees of cancer risk, which is thought to be related to different protein functions and patterns of gene expression. HPV16 is predominantly associated with SCC originating at the transformation zone and HPV18 with AC of the endocervix, but both can cause cancers at either type of tissue. Although cutaneous/mucosal classifications are not tight, the different Alpha species have tropism preferences which are indicated on the right. Data shown is based on information contained in [45,229,230].
B. The genome organisation of HPV16 is typical of the high-risk Alphapapillomaviruses (including HPV18), and comprises a long control region (LCR) and eight genes that are necessary for different stages of the virus life cycle. These genes encode a larger number of gene products as a result of mRNA splicing. The LCR contains binding sites for cellular transcription factors (e.g., SP1, AP1, Oct1), as well as for the viral E1 and E2 proteins that control viral replication and gene expression. HPV16 has two well-characterized promoter elements known as PE (early promoter; also referred to as p97) and PL (late promoter; also referred to as p670) that regulate the expression of differentially-spliced mRNAs during epithelial differentiation (position 97 and 670 in the HPV16 genome denote the 5′ cap site/RNA initiation site of viral transcripts). PAE and PAL indicate the positions of the early and late polyadenylation sites within the genome.

low-risk groups [1]. The cutaneous Alpha types are also 'low-risk', and include HPV2 and 57, which cause common warts, and HPV3 and 10, which cause flat warts [1,20].

The low-risk mucosal types (Fig. 2A), which despite their name can also cause cutaneous genital lesions, share a low-risk HPV life-cycle organisation and do not typically cause neoplasia [29] (Figs. 4B and 5). Cutaneous lesions caused by Alpha, Beta, Gamma and Mu types can become difficult to manage in patients with SCID (severe combined immunodeficiency) [30] and EV (epidermodysplasia verruciformis) and in organ transplant recipients and others who are pharmacologically immunosuppressed [31], with certain Beta types being associated with the appearance of neoplastic precursors (Bowen's disease, actinic keratosis) [32] and the development of non-melanoma skin cancer at sun-exposed sites in these individuals [6,31,33,34]. A predisposition to HPV-associated disease and cancer progression is also seen in WHIM syndrome (warts, hypogammaglobulinemia, infections, and myelokathexis) patients, which is associated with defective CXCR4 signalling [35]. The molecular defects that underlie these conditions are known [36], but it is not yet clear (in most cases) exactly how they predispose to disease and whether it is the infected keratinocyte [37,38] or the immune system that is primarily compromised [39,40]. Thus, the low-risk viruses are occasionally found to be associated with human cancers and can in some instances be associated with papillomatosis, especially in individuals with immune defects.

Carcinomas associated with the high-risk HPV types are, however, a far more significant burden [4,24]. Twelve HPVs (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59) are defined by the World Health Organisation (WHO) as being high-risk cancer-causing types (category 1 in Fig. 2A), with additional types (68, 73)[41–43] being recognised as 'possibly' cancer-causing (category 2 in Fig. 2A). Several other HPV types also belong to the high-risk clade based on evolutionary similarity to the known cancer-causing types [44,45] (shaded pink in Fig. 2A), although so far, the epidemiological data confirming this have not been obtained. Recent studies also suggest that variant lineages may differ in risk of persistence and association with high-grade disease. Together, these viruses cause approximately half a million cases of cervical cancer per year worldwide, with approximately half of these being fatal (530,000 cases per year with 275,000 deaths

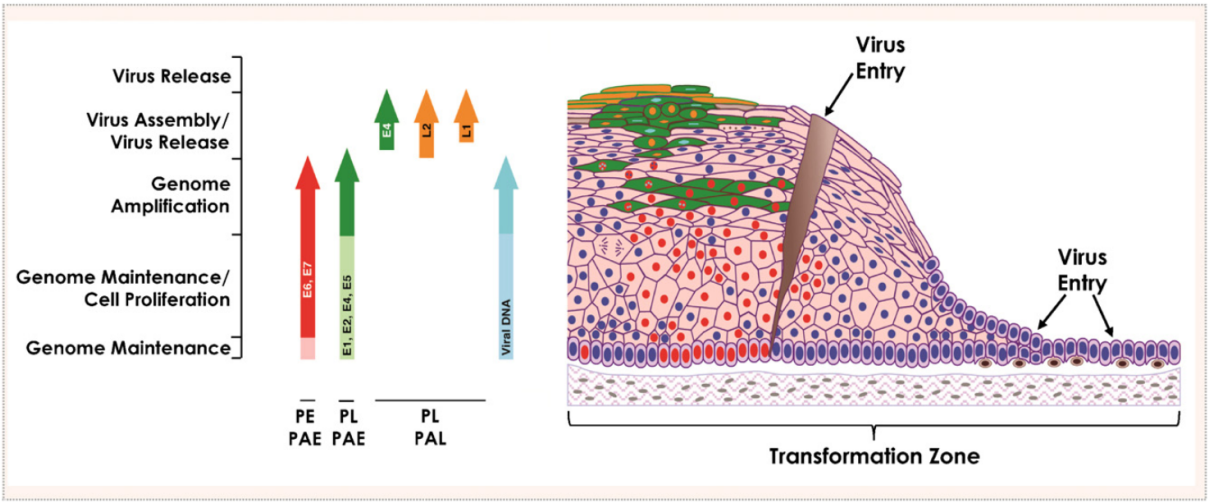

Figure 3. Life Cycle of High-Risk HPVs in Cervical Epithelium.
In multi-layered stratified epithelium, such as the ectocervix, infection is thought to require the presence of a microwound that allows the infectious virions to access the basal lamina. The infected basal cells form the reservoir of infection, and in these cells, the viral genome is maintained as a low copy number episome. As these cells divide, they produce daughter cells that are pushed outwards towards the epithelial surface. Different events in the virus life cycle are triggered at different stages during this migration. In lesions (such as CIN1) caused by high-risk HPV types (such as HPV16), cells in the lower layers express E6 and E7 and are driven through the cell cycle and are stimulated to divide (cycling cells marked with red nuclei). In the mid layers, proteins necessary for genome amplification become elevated in these cells, allowing genome amplification to occur. These cells express the viral E4 protein and are typically in the S or G2 phases of the cell cycle (E4 presence marked in green, with red nuclei indicating replication competence). In the upper epithelial layers, the cells leave the cell cycle, and in a subset of the E4-positive cells, the virus L2 and L1 proteins are made, allowing packaging of the amplified viral genomes. The site of expression of the different viral gene products is shown to the left of the image, with the key stages during productive infection listed alongside. At the cervical transformation zone and the endocervix, it is thought that HPV may also be able to infect columnar epithelial cells, the epithelial reserve cells, and cells at the squamo-columnar junction. Infection of these cell types may be associated with different patterns of disease progression and with the development of adenocarcinoma. IARC: International Agency for Research on Cancer; PAE: Position of the early polyadenylation site; PAL: Position of the late polyadenylation site; PE: Early promoter, also referred to as p97; PL: late promoter, also referred to as p670.

[WHO/ICO Information Centre on Human Papilloma Virus (HPV) and Cervical Cancer; http://www.who.int/hpvcentre/en/]). Importantly, these viruses are also associated with cancers at other sites, including the penis in men, the vagina and vulva in women and, in both genders, the anal transformation zone, the tonsils, oropharynx and base of tongue. It appears that deregulation of viral gene expression may occur to different extents at the different sites of high-risk HPV infection, and that squamo-columnar junctions, such as the cervical transformation zone, are particularly prone to neoplastic disease. Nevertheless, high-risk HPVs do not cause cancer in the vast majority of the individuals that they infect [3,24].

As with all HPV infections, the high-risk types are maintained in the general population because of productive infections rather than inadvertent cancers. Low-grade squamous intraepithelial lesions (LSIL), where infectious particles are produced, are generally flat and inconspicuous, and in most cases these will regress spontaneously within 18 months [4,46,47]. For reasons that we do not yet clearly understand, the high-risk HPV types have evolved the ability to persist, often for many years, and to drive cell proliferation in the basal and parabasal cell layers at some sites of infection [48,49]. This is not a prerequisite for virus production, and does not happen to any extent in lesions caused by low-risk types. High-grade lesions (high-grade squamous intraepithelial lesions; HSIL) are abortive infections in which normal patterns of early virus gene expression are perturbed [29]. In particular, it is thought that an elevation in the level of E6 and E7 is directly related to the increasing severity of neoplasia [50], and that the deregulated expression of these genes is directly responsible for the accumulation of genetic errors in the infected cell and the eventual integration of viral episomes into the host cell chromosome [51–53], which is seen in many cervical cancers [53–57]. Cancer progression is facilitated when integration preserves the integrity of the long control region (LCR) and the E6 and E7 genes and the 5′ portion of the E1 gene, but disrupts the ability of the integrated genome to express the DNA-binding protein that represses the viral early promoter, and the full-length E1 gene, which can regulate episomal copy number.

3. The normal productive life-cycle of high- and low-risk papillomaviruses

Whether a productive life-cycle is or is not completed depends on the nature of the epithelial site where infection occurs, as well as on the presence of external factors such as hormones [58] and cytokines [59]. Experimental models suggest that infection requires access of virus particles (composed of viral DNA and two capsid proteins, L1 and L2, which form icosahedral capsid [60,61]) to the basal lamina, and the interaction with heparin sulphate proteoglycans [62–64] and possibly also laminin [65]. Structural changes in the virion capsid, which includes furin cleavage of L2, facilitate transfer to a secondary receptor on the basal keratinocyte, which is necessary for virus internalization and subsequent transfer of the viral genome to the nucleus [22,66–69]. Although the Alpha 6 Integrin and growth factor receptors have (amongst others) been implicated in this process [70–75], the precise nature of the entry receptor remains somewhat controversial [67,75–78]. Once internalised, virions undergo endosomal transport, uncoating, and cellular sorting. The L2 protein-DNA complex ensures the correct nuclear entry of the viral genomes, while the L1 protein is retained in the endosome and ultimately subjected to lysosomal degradation [79,80].

In many cases, infection is thought to require epithelial wounding or micro-wounding to allow access of the virus to the basal lamina [67], and a role for the wound healing response in simulating the expansion of the infected cells has been suggested [3,67,81,82]. Indeed, active cell division, as would occur during wound healing, is thought to be necessary for entry of the virus genome into the nucleus, and it has been proposed that lesion formation requires

A

	High-Risk Alpha	Low-Risk Alpha
E6	encodes E6* products	no E6* products
	binding and degradation of… •p53 •specific PDZ-domain proteins (e.g. Dlg, MAGI-1, Scribble)	weaker binding (no degradation) of… •p53 •no binding of PDZ-domain proteins
	interact with the E6AP ubiquitin ligase inhibition of p53 transactivation and acetylation	
	inhibition of apoptosis	unknown
	bypass of growth arrest following DNA damage	normal growth arrest following DNA damage
	inhibition of keratinocyte differentiation	unknown
	inhibition of interferon response	weaker inhibition of interferon response
	activation of signaling pathways… •Akt •Wnt •Notch •mTORC1	unknown
	telomerase activation	no activation
	c-myc activation	no activation
E7	binding and degradation of… •pRb •p107 •p130 binding (no degradation) of… •E2F1 •Cullin2 •HDAC	weaker binding (no degradation) of… •pRb •p107 •E2F1 binding of… •p130
	binding of regulatory proteins including E2F6, p600, HAT, PP2A induction of cell cycle entry and DNA synthesis role in genome amplification	
	induction of genome instability	no stimulation of instability
	suppression of STAT-1 function	no suppression
	immortalization and transformation functions	no such functions
	activation of signaling pathways… •Akt	unknown

B

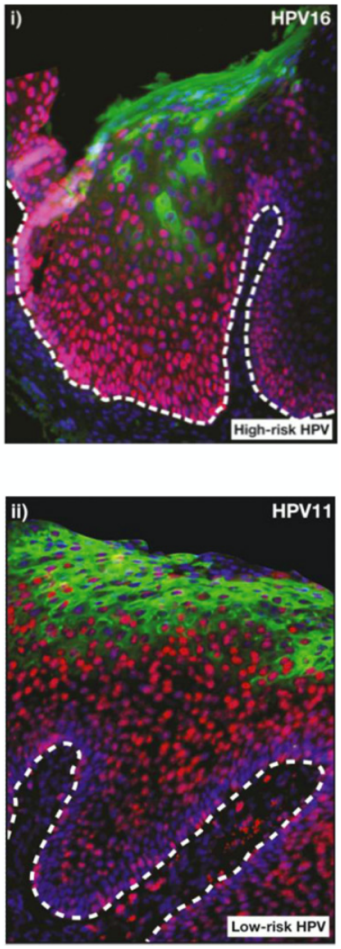

Figure 4. Protein Function and Patterns of Gene Expression in High and Low-Risk HPV Disease.
A. Key differences in E6/E7 protein function between the high- and-low-risk HPVs (based on fuller data presented in [105]). It is important to note that the high- and low-risk HPV types also have significant differences in promoter positioning and promoter regulation, as well as in patterns of mRNA splicing. These differences affect expression from the E6 and E7 genes [3,22]. Current thinking suggests that different patterns of viral gene expression (as well as different protein functions) play a major role in determining disease phenotype following infection.
B. Immunostaining of cervical lesions caused by high-risk (HPV16) and low-risk (HPV11) Alpha types reveals key differences in the life-cycle organisation of these viruses. In lesions caused by HPV16 (left), the stimulation of cell cycle entry (as visualized by staining for the cellular MCM protein (red)) is apparent in the basal layer and above, with some cells also being driven through mitosis. The blue stain (DAPI) highlights the condensed chromatin in these cells. The HPV16 E4 protein (green) appears as the red MCM signal begins to decline in the upper layers of the lesion. In lesions caused by HPV11 (right), the stimulation of cell cycle entry in the basal layers is much less obvious, and the red MCM signal indicating cell cycle entry (but not cell division) is apparent only in the upper epithelial layers. The E4 protein becomes abundant in the upper epithelial layers in cells that are strongly MCM positive and which are supporting viral genome amplification. The lower ability of low-risk HPV types to drive cell proliferation correlates with a lower incidence in neoplasia.

the initial infection of a mitotically active cell [83]. Given the diversity of HPV types and HPV-associated diseases, we should perhaps be cautious when making such broad generalisations regarding the route of infection, as multiple entry pathways have been invoked depending on the virus type under study [80,84–87].

The particular susceptibility of the transformation zone to cancer progression may also be linked to the increased accessibility and proliferation of the basal cell layers at this metaplastic epithelial site, particularly around the time of puberty and the onset of sexual activity [88]. In this case, we can hypothesize that the primary target cells for infection may be cells close to the squamo-columnar junction such as the epithelial reserve cells, which lie immediately underneath the columnar epithelium of the endocervix [89,90], and which eventually form the stratified epithelial layers of the transformation zone as the cervix matures. For some time now, the general hypothesis has been that lesion formation begins with the infection of a basal stem cell (rather than a basal transiently amplifying cell) and that the longevity of the stem cells is a key factor in the formation of a persistent lesion [3,50,91,92]. For the low-risk HPV types, which do not generally cause neoplasia and which do not massively stimulate basal cell proliferation, this is a plausible hypothesis, even though not yet formally proven. For the high-risk types, which can stimulate basal cell proliferation, it is less clear whether this is a necessity. The nature of the initially infected cell and how it relates to disease outcome is thus still a matter of speculation.

3.1. Genome maintenance and cell proliferation in the lower epithelial layers

Irrespective of the nature of the infected basal cell, it is generally thought that infection is followed by an initial phase of genome

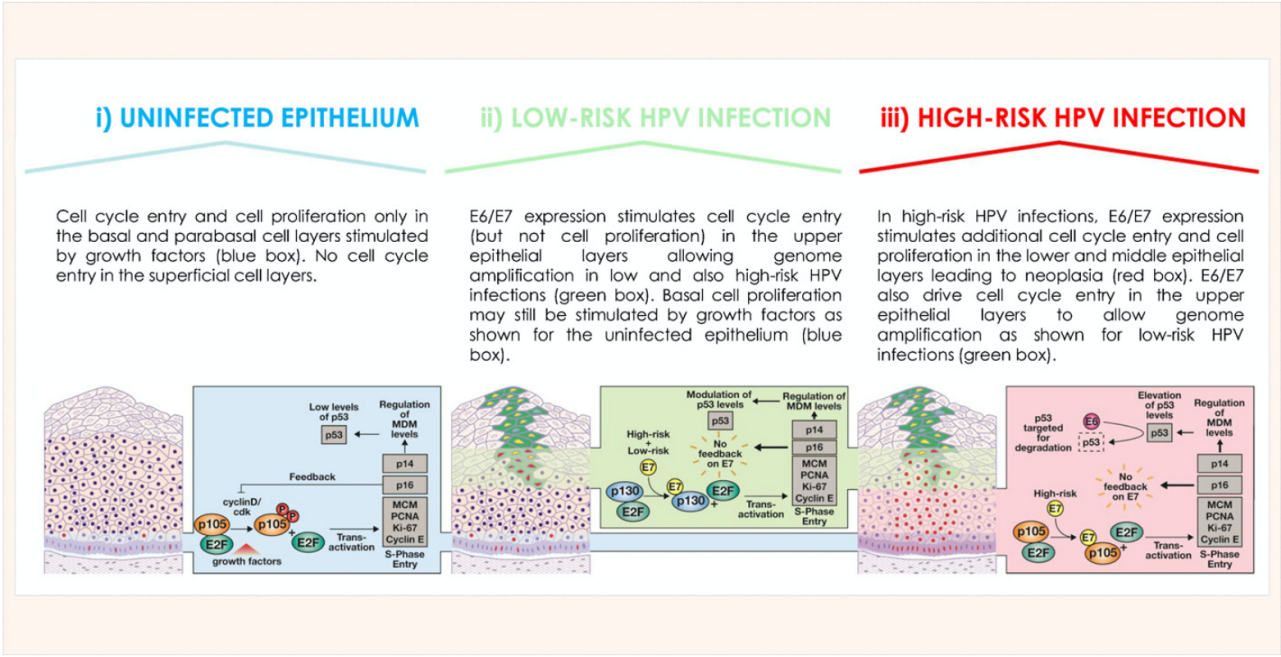

Figure 5. Regulation of Cell Cycle Entry and Proliferation in Infected and Uninfected Epithelium. The activities of the viral proteins underlie disease phenotype. This is apparent when the role of the high- and low-risk E6 and E7 proteins are considered in the context of disease as indicated below.
Uninfected epithelium. In uninfected epithelium (left), cell cycle entry (red nuclei) and cell division in the basal/parabasal cell layers is controlled by growth factors that stimulate the activity of G1 cyclins including CyclinD/Cdk. CyclinD/Cdk phosphorylates pRb and displaces it from E2F, which allows the transactivation of genes necessary for S-phase progression. As part of this regulated stimulation of cell cycle entry, p16ink4a forms a negative feedback loop that suppresses cyclinD/cdk activity, so preventing the over-expression of itself and other E2F-activated genes (MCM, Ki-67, PCNA). Because of this, p14arf levels remain low, which allows MDM to carry out its normal role of degrading p53. The molecular pathways and their regulation are shown to the right of the diagrammatic representation of the epithelium.
Low-risk HPV infection. In lesions caused by low-risk HPV types (centre), it is thought that basal cell proliferation is largely regulated by the presence of growth factors, as is seen in uninfected epithelium (left). The primary role of the HPV E6 and E7 proteins in these lesions is to drive cell cycle entry above the basal layer in order to facilitate HPV genome amplification (red nuclei in mid epithelial layers). This is thought to be dependent on the ability of E7 to bind the Rb family member p130, and to displace it and the associated E2F4 and five transcriptional repressors from target promoters required for S-phase gene expression (i.e., without the need for p130 phosphorylation). The transcriptional activators E2F1,2 and 3 can then occupy these vacant sites and stimulate expression of the host genes necessary for DNA replication and cell cycle progression (e.g., PCNA, MCM, CyclinA, CyclinE). Cells expressing the HPV E4 protein are shown in dark green, with L1 expression being shown in yellow. Cells in cycle are shown with red nuclei. The molecular pathways involved are shown to the right of the diagrammatic representation of the epithelium.
High-risk HPV infection. In high-risk HPV infections (right), an additional function of the high-risk E7 protein leads to the displacement of E2F 4 and 5 from Rb as well as p130 without the need for Rb phosphorylation. The absence of effective inhibition of cell cycle progression by p16ink4a can lead to its accumulation in the cell and to an elevation in MCM, Ki-67 and PCNA levels throughout the infected epithelial layers. The corresponding elevation in p14arf levels compromises the normal function of MDM in degrading p53, which subsequently leads to an increase in p53 abundance. P53-mediated cell cycle arrest is. however. countered in the proliferative cell layers by the high-risk E6 proteins, which associate with E6AP and mediate the ubiquination and proteosomal degradation of p53. Recent studies have suggested that certain biomarkers of high-risk HPV infection (such as p16ink4a) may be also be activated as a result of E7 mediated epigenetic programming [172] in addition to the mechanism described here. In the diagram shown, the locations of cells driven into cycle are marked by the red nuclei in the diagrammatic representation of the epithelium, with the yellow nuclei revealing the appearance of L1. The molecular pathways involved are shown to the right.

amplification, and then by maintenance of the viral episome at low copy number [83,93,94]. The copy number in the basal layer of lesions is often proposed as 200 or so copies per cell, based on the study of episomal cell lines derived from cervical lesions. In benign oral papillomas in animals, the basal copy number has been quantified using laser capture methods as 50 to 100 copies per cell [95], but it is likely that there will be variation from lesion to lesion and between different sites.

The viral replication proteins E1 and E2 are thought to be essential for this initial amplification phase, but may be dispensable for episomal maintenance-replication once the copy number has stabilised [96–98]. The precise role of E1 and E2 in the epithelial basal layer during natural infection needs further clarification however, given the proposed role of E2 in genome partitioning (see below). E2 also regulates viral transcription, and has multiple binding sites in the viral LCR (long control region or upstream regulatory region [URR]), and (during viral DNA replication) can recruit the viral E1 helicase to a specific E1 binding motif in the viral origin of replication. It has been speculated that the use of a viral DNA helicase (i.e., E1), which is distinct from the cellular replication helicases (MCM proteins), allows viral DNA replication to be disconnected from cellular DNA replication during genome establishment and amplification [3,99]. Although the role of viral and cellular helicases in genome maintenance still needs some clarification, several studies have proposed a role for E2 in the regulation of accurate genome partitioning during basal cell division [94]. In bovine PV, this involves the cellular Brd4 protein, but in HPVs, other E2 binding proteins appear to be involved in the tethering of viral episomes to the cellular chromatin during cell division [93,94,100–102].

The precise role of the HPV E6 and E7 proteins in infected basal cells is also uncertain, particularly for the low-risk HPV types (such as HPV6 or 11) that are not generally associated with neoplasia, and which are thought to produce lesions following the infection of a basal stem cell at the site of a wound or microwound. In these HPV types, the role of the wound healing response in driving the initial proliferation of the infected cell(s) may well be critical [103], with signalling from the local microenvironment influencing viral gene expression [104] and/or protein functions. In the case of the high-risk types that cause neoplasia, there is a clear role of the viral E6 and E7 proteins in driving cell proliferation in the basal and

parabasal cell layers, especially at cervical sites where neoplasia can occur [3]. It is also clear that there are many functional differences between the high and low-risk E6 and E7 proteins (see Fig. 4A and [105]), and that these contribute, along with differences in promoter activity and patterns of gene expression, to the different HPV-associated pathologies seen *in vivo*. Indeed, recent studies have suggested that the deregulation of E6/E7 expression, even in the absence of genome integration, is a critical event in determining neoplastic grade [106], which is classified according to the extent to which basal-like cells extend into suprabasal epithelial layers [107].

3.2. From genome maintenance to genome amplification in the upper epithelial layers

The E6/E7-mediated proliferation of the basal and parabasal cells following infection by the high-risk HPV types facilitates an expansion in lesion size, which is thought in part to be linked to specific functions of the high-risk E6 and E7 proteins (Fig. 4A). Functional differences between the high- and low-risk E7 proteins centre to a large extent on their differential ability to associate with members of the Retinoblastoma (Rb) protein (pRb) family, with the high-risk E7 proteins being able to bind and degrade both p105 and p107, which control cell cycle entry in the basal layer, as well as p130, which is involved in cell cycle re-entry in the upper epithelial layers ([48,108] and Figs. 4 and 5). The low-risk E7 proteins generally appear to have a lower affinity for p105 and p107 than the high-risk types, but can associate with and degrade p130 in order to create a replication-competent environment in the mid-epithelial layers that is suitable for genome amplification [105,109] (Fig. 5). An unfortunate characteristic of the high-risk E7 proteins however is their ability to stimulate host genome instability, particularly through deregulation of the centrosome cycle in the proliferating basal cells [110–115]. The PDZ–domain-binding motif, which is located at the C-terminus of all the high-risk E6 proteins, provides another key difference between high- and low-risk PVs. High-risk E6 proteins are able to interact with a several PDZ targets through this motif, many of which are involved in the regulation of cell polarity, cell proliferation and cell signalling [116,117]. A site for protein kinase A phosphorylation is found within the high-risk PDZ-domain binding motif and can negatively regulate the association of E6 with its PDZ domain-containing substrates [118]. Recent studies have further suggested that only particular PDZ pools or isoforms within the cell are susceptible to degradation [119,120], and that this function of E6 may be carefully regulated during the virus life-cycle [118]. Further studies are needed to precisely define the role of these interactions *in vivo*.

Other unique characteristics of the high-risk E6 proteins include their capacity to upregulate telomerase activity [121–123] and to maintain telomere integrity during repeated cell divisions, and their ability to mediate the degradation of p53 within the cell. Both high- and low-risk E6 proteins inactivate aspects of p53 function, which suggests an important life-cycle function, but only the high-risk types stimulate its ubiquitination and proteosome-dependent degradation [124–126]. In fact the high-risk types use degradatory pathways to target many of their substrates. For E7, this involves components of the CUL2 ubiquitin ligase complex, while for E6 it involves the cellular ubiquitin ligase E6AP [127]. With the use of more advanced proteomics technology, it is becoming clear that both E6 and E7 have a very large number of cellular substrates, and that the identity of these substrates differs between HPV types of the same high-risk clade, as well as between the high- and low-risk groupings themselves [128]. Indeed, there appears to be no single characteristic that can define high-risk types as cancer-causing. This is exemplified by studies showing very little concordance between cancer risk, and the capacity of the E6 oncoproteins from the high-risk types to degrade p53, degrade PDZ substrates and induce keratinocyte immortalisation. In the case of E6, recent structural studies are suggestive of a complex multimeric protein that has potential to associate with multiple protein partners at any given time [125,129]. While such functional differences undoubtedly contribute to the respective abilities of the high- and low-risk HPV types to cause neoplasia and cancer, it is important to remember that a key function of the E6 and E7 proteins in most HPV types is not to promote basal cell proliferation, but rather, to stimulate cell cycle re-entry in the mid-epithelial layers in order to allow genome amplification.

The expression of the E6 and E7 proteins in the upper epithelial layers allows the infected cell to re-enter S-phase, and for viral genome copy-number to rise. There is also a need for the viral replication proteins E1 and E2, which increase in abundance following the upregulation of the HPV 'late' or 'differentiation dependent' promoter [130]. In HPV16, this promoter (P670) resides within the E7 open reading frame near to nucleotide position 670. Thus, while the early promoter (P97 in HPV16) located in the LCR (long control region) can control the expression of transcripts with E6 and E7 as the first and second open reading frames, it appears that the differentiation-dependent promoter (P670) is positioned to upregulate the expression of E1 and E2 during differentiation to allow genome amplification. The epithelial cell that supports viral genome amplification, therefore, is subject to differentiation signals and can express well-defined markers of differentiation such as keratins 1 and 10 (cutaneous epithelia) or 4 and 13 (mucosa), while at the same time expressing markers of cell cycle entry, such as MCM, Ki-67, PCNA, CyclinE and CyclinA. Careful analysis suggests that, in the case of the low-risk HPV types, genome amplification begins as the infected cell undergoes cell cycle reactivation in the mid- to upper epithelial layers and enters an S phase-like state. For the high-risk types, this S phase-like state marks the upper proliferative layers within the neoplasia, rather than a region where cell cycle re-entry has occurred. HPV genome amplification persists as the 'differentiating' cell moves from an S-like to a G2-like phase, with viral genome amplification occurring primarily in G2 after cellular DNA replication has been completed [131,132]. Laser capture experiments in animal models have shown at least a 2-log increase in viral copy number per cell during the genome amplification phase [95].

In addition to E1 and E2, it is thought that the E4 and E5 proteins contribute indirectly to genome amplification success by modifying the cellular environment, with E5 also being involved in koilocyte formation [133]. E5 is a three-pass transmembrane protein with a cytoplasmic C-terminus [134]. It is believed to possess pore-forming capability and interferes with apoptosis [135] and the intracellular trafficking of endocytotic vesicles [136,137]. E5 is also thought to make an important contribution to genome amplification success through its ability to stabilize EGFR and to enhance EGF signalling and MAP Kinase activity [138–141] and to modulate both ERK 1/2 and p38 independently of EGFR [142,143].

The MAP Kinases ERK 1/2 are critical modulators of nuclear E1 accumulation through the phosphorylation and activation of the nuclear localisation signal within the E1 protein, and their activity is dependent on upstream MAPKs MEK 1/2 and p38. Through both the S and G2-like phases, the accumulation of Cyclins E and A and their associated cyclin-dependent kinase cdk2 further contributes by phosphorylation and inhibition of an E1 nuclear export sequence [144,145]. Recent work has suggested that other post-translational modifications in E1 (e.g., cleavage by caspases) also facilitate differentiation-dependent genome amplification, and that the accumulation of E1 in the nucleus may in itself enhance viral DNA replication at the expense of cellular replication through induction of a DNA damage response [146]. E4 is a viral protein that accumulates to

very high levels in cells that support virus synthesis [147,148], and it is likely that its primary function is in some aspect of virus release or transmission [149,150], with optimisation of genome amplification occurring indirectly [151–155]. For HPV16, the growth arrest functions of E4 contribute to amplification success.

3.3. The packaging of viral genomes and virus release

The completion of the HPV life cycle ultimately involves the expression of the minor coat protein (L2), the exit of the cell from the cell cycle, and the expression of the major coat protein L1 to allow genome packaging. This requires a change in splice site usage rather than promoter activation, leading to transcripts initiated at P670 (in HPV16) that terminate at the late polyadenylation site rather than the early site [3], an event that is aided by high levels of E2 expression [156,157]. Interestingly, this results in a switch from the production of an E1^E4, E5 message to an E1^E4, L1 message, as genome amplification gives way to genome packaging [22,157,158]. Genome encapsidation involves the recruitment of L2 to regions of replication via E2, prior to the expression of L1 and the assembly of the icosohedral capsid in the nucleus [159,160]. Virus maturation occurs in the most superficial, dying keratinocytes, which lose mitochondrial oxidative phosphorylation and convert from a reducing to an oxidizing environment just before virus release. This enables the progressive accumulation of disulphide bonds between the L1 proteins, leading to the production of extremely stable infectious virions [161,61]. Assembled particles contain 360 molecules of L1 arranged into 72 pentameric capsomeres, with a much smaller and variable number of L2 molecules, which can occupy capsomeres at the 5-fold axis of symmetry [60]. Although not precisely defined, the abundant E4 protein is thought to contribute to virion release and infectivity in the upper epithelial layers, as it assembles into amyloid fibres that disrupt keratin structure and compromise the normal assembly of the cornified envelope [148,150,162].

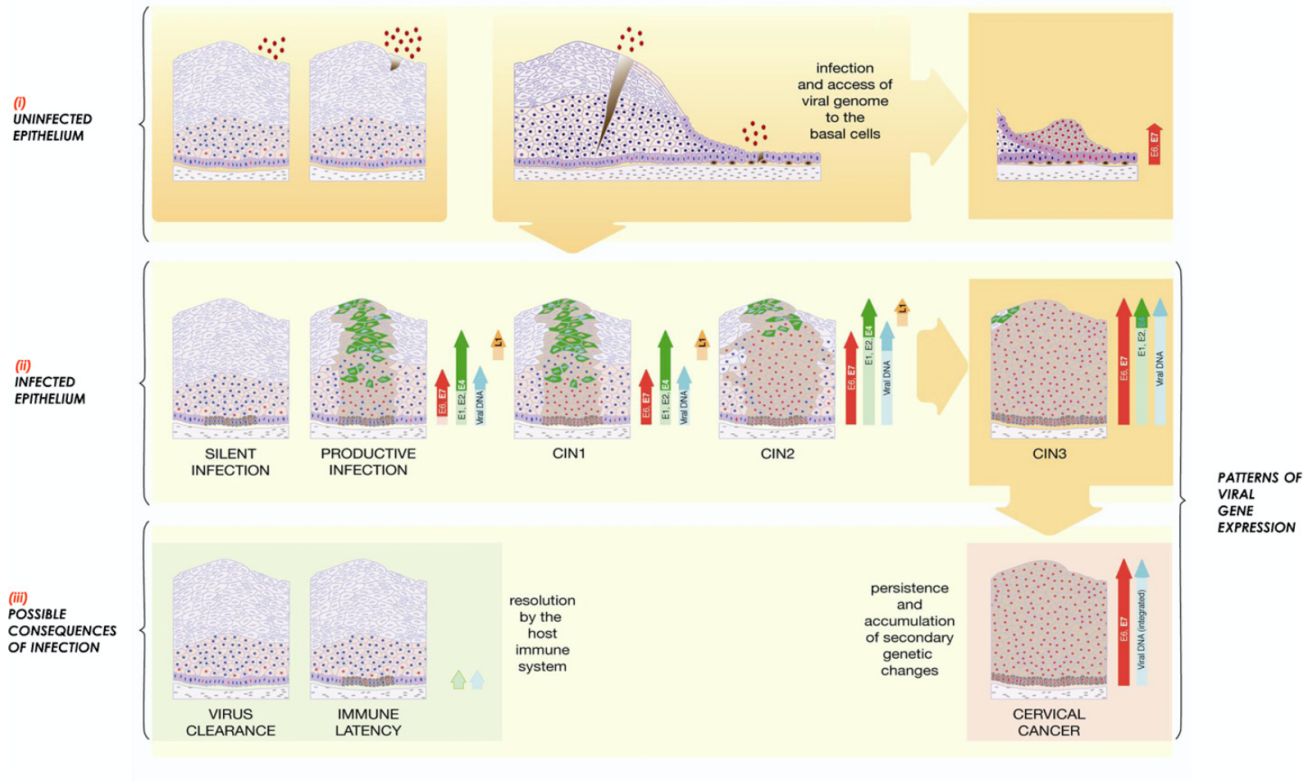

Figure 6. High-Risk HPV Infection and its Possible Consequences.
(i) The detection of HPV DNA in a tissue biopsy or in exfoliated cervical cells may indicate infection (productive (CIN1) or abortive (CIN3) as shown in (ii)), the presence of virus particles at the epithelial surface without infection (e.g. from recent transmission), or a latent or silent infection (as shown in (ii)). To resolve this ambiguity, markers of viral gene expression (such as mRNA or proteins) are useful in confirming the presence of active disease when HPV is detected using DNA-based tests. Infection requires the entry of HPV virions into the mitotically active epithelial cells of the basal layer, which in stratified epithelium is thought to require a microwound. In the columnar cell layers, infection is thought to be facilitated by the proximity of the target cell to the epithelial surface, which may allow the virus to access a cell type that is unable to support the full productive life cycle (right). The significance of infection of different cell types remains to be properly assessed.
(ii) Following infection (shown in (i)), expression from the viral genome can sometimes be suppressed (e.g., by genome methylation), leading to a 'silent' infection in which the viral genomes are retained in the basal layer without apparent disease. Infection may alternatively lead to an ordered pattern of viral gene expression leading to virus synthesis and release from the upper epithelial layers (productive infection or CIN1), or to deregulated viral gene expression and high-grade neoplasia (CIN2/CIN3). Persistent high-grade disease such as CIN2 and 3 is associated with a increasing risk of genome integration into the host cell chromosome and progression to cancer. Cells in cycle are indicated by the presence of red nuclei. Cells expressing E4 are shown in green, while those expressing L1 are shown in yellow. The brown shading on the diagrammatic representations of the epithelium identify all the cells (differentiated and un-differentiated) that contain viral genomes.
(iii) In most cases, HPV infections are resolved as a result of a cell-mediated immune response (left). This may lead to viral clearance or to viral latency and the persistence of viral episomes in the epithelial basal layer without life-cycle completion. Viral gene expression patterns during latency are not well characterised (E1, E2 expression postulated here as suggested from animal models [220]). Persistent deregulated gene expression, as occurs in CIN3 and following viral genome integration, can lead to the accumulation of secondary genetic changes in the infected host cell and development of cancer. This is facilitated by over-expression of the high-risk E6 and E7 proteins. Cells in cycle are shown by red nuclei. Brown shading in the immune latency state indicates cells harbouring viral episomes. In cervical cancer, the viral genome is often integrated with loss of expression of full-length E1, E2, E4 and E5, and the L1 and L2 capsid proteins, and with de-regulated expression of E6 and E7.

4. Life-cycle deregulation and cancer progression

The ordered expression of viral gene products that leads to virus particle production is disrupted in HPV-associated neoplasia (Figs. 6 and 7). In cervical disease, where most research has been done, it is generally thought that the levels of E6 and E7 expression increase from cervical intraepithelial neoplasia grade 1 to 3 (CIN1 to CIN3), and that these changes in gene expression directly underlie the neoplastic phenotype. In this scheme, CIN1 lesions typically retain the ability to complete the HPV life cycle and produce virus particles and can in fact resemble flat warts, which have a lower level of cell proliferation in the basal and parabasal layers [29]. The elevation of E6 and E7 expression in high-risk HPV infection that leads to the CIN2+ phenotype predisposes the cell to the accumulation of genetic changes, which increasingly contribute to cancer progression. According to this hypothesis, the relatively low levels of E6 and E7 present in CIN1 do not compromise the functions of their cellular targets sufficiently to facilitate cancer progression. The viral deregulation seen in CIN2/3+ is also thought to facilitate integration of the viral episome into the host cell chromosome, which can further deregulate the expression of E6 and E7; genes which are often referred to as viral oncogenes.

Although it is not clear exactly how gene expression from the viral episome can become deregulated in early CIN, data from the vaccine trials has indicated that CIN2+ can occur in young women soon after infection [163–166]. In these instances, deregulated gene expression may be driven by changes in cell signalling as can be brought about by hormonal changes [58], or epigenetic modifications such as viral DNA methylation, which may depend on the nature of the infected epithelial cell [167]. The HPV16 LCR contains hormone response elements that can be stimulated by estrogen, and there is ample evidence of cooperation between estrogen and HPV in the development of cervical cancer in both humans and in model systems [58,168–170]. In CIN, it has been reported that the LCR is differentially methylated according to disease severity, which suggests that epigenetic changes may also regulate gene expression [171] (and thus disease [106]). It is also thought that for HPV16 at least, the E7 protein can induce epigenetic changes that may contribute to changes in cellular gene expression [172–174].

Although common fragile sites (CFS) in the host cell genome are hot spots where integration is more likely to occur [53], integration

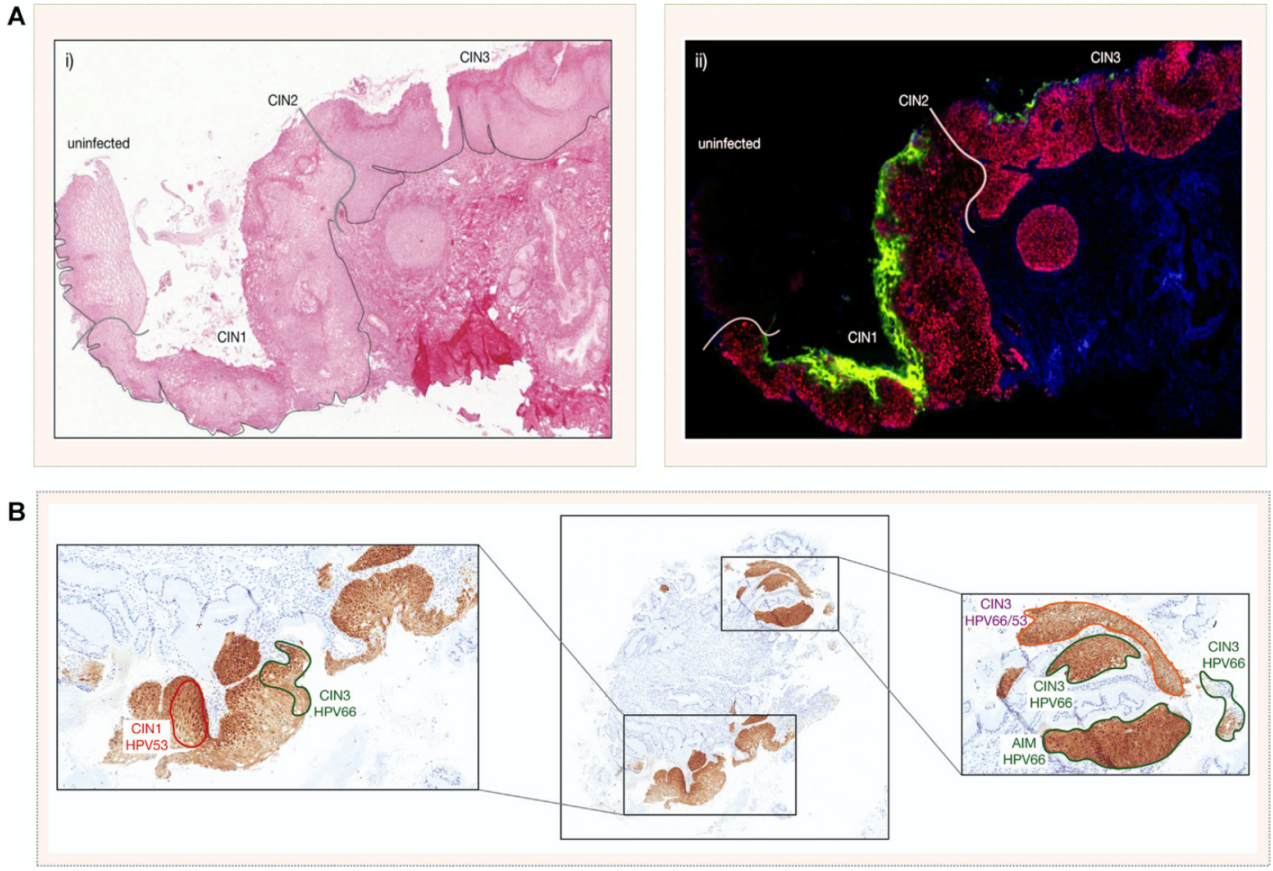

Figure 7. Biomarker Patterns and the Identification of Causal HPV Type in Disease of Different Severity.
A. Key life-cycle markers show distinct patterns of expression in cervical disease of different grades. A histology section is shown on the left of the figure to illustrate the typical pathology associated with either uninfected epithelium, CIN1, CIN2 or CIN3. The same piece of tissue stained with two biomarkers (MCM and HPV16 E4) is shown on the right. The cellular marker MCM (red) is expressed at low level in the basal and parabasal layers in uninfected tissue. As a surrogate marker of E7 expression, the MCM protein is elevated to different extents in neoplasia. In HPV-induced lesions (but not in normal metaplasia), the viral E4 protein (green) becomes abundant as MCM levels decline during differentiation. As an abundant viral protein, the detection of E4 using type-specific antibodies confirms HPV16 as the causative HPV type in this lesion.
B. Cervical biopsies often contain multiple HPVs, with different HPV types being associated with discrete areas of disease. A histology section of cervical tissue positive for HPV53 and HPV66, and which contained regions of CIN1, CIN3 and immature metaplasia is shown in the central panel. Some of the metaplastic areas show nuclear atypia (atypical immature metaplasia, AIM), with regions of p16 positivity (brown staining) in the CIN and AIM regions (shown in detail in the boxed enlargements). HPV66 was detected by laser capture microscopy and PCR (LCM-PCR) in all the CIN3 regions (suggesting causality). HPV56 was found in an area of CIN1, and was found together with HPV66 in one area of CIN3. In general, different HPV types are associated with discrete areas of disease except at junctional regions (where lesions abut or are in close proximity) where more than one HPV type may be found. CIN: Cervical intraepithelial neoplasia.

is, in general, a chance event that can sometimes result in the disruption of viral genes that regulate normal transcription from the LCR. Key amongst these is E2, which is a virally-encoded transcription factor that normally regulates E6/E7 abundance by binding to sites within the viral long control region (LCR). The majority of cervical cancers contain one or many copies of HPV, integrated more or less randomly into the host chromosome, with the viral integration site frequently lying within the regulatory E1 or E2 genes [55,175]. Integration and the loss of E6/E7 regulation can facilitate persistent high-level expression of these genes [176,177] and the accumulation of genetic errors that eventually lead to cancer [178]. In recent years, there has been much debate as to whether early integration events in CIN1 drive progression through CIN2 and CIN3 to cancer, or whether some degree of viral gene expression de-regulation underlies the early CIN2 and CIN3 phenotypes, and whether it is this initial deregulation that causes chromosome instability and thus facilitates integration (Figs. 6 and 7). In general, it is thought that integration occurs in high-grade lesions such as CIN2 and CIN3, and that once this occurs, the already deregulated expression of E6 and E7 can increase still further [50] or else be maintained at a constitutive level [179]. Cervical cancer can arise from cells containing exclusively episomes, and for HPV16, around 30% (26–76% depending on study) of cervical cancers develop in this way [54,180,181]. Around 70% of HPV16-associated cervical cancers contain integrated HPV16 sequences, while for HPV18, the viral genome is almost exclusively integrated [182–186]. In both cases, however, it is the long-term expression, and in particular, the over-expression of E6 and E7 and the accumulation of genetic errors, which are ultimately important in the progression from CIN3 to cervical cancer.

4.1. Other HPV types have different mechanisms of disease progression

Although most research on HPVs has focused on the high-risk types from the Alpha genus, it is apparent that the low-risk types can very occasionally be linked with cancer progression, such as in persistent RRP [187]. Several reports have suggested that duplications within the HPV genome or occasional integration may be important in these cases [188,189], but given the different functions of the low-risk E6 and E7 proteins, we would not expect the mechanisms of how these viruses predispose to cancer to be the same as for the high-risk types. Even so, it does appear that persistence is an important indicator of cancer risk in both cases, prompting the search for better methods of disease treatment for low-risk PV types.

Clearly, the genetic susceptibility of the host can play an important role in some cancers associated with low-risk HPV types, as evidenced from the study of WHIMS and EV [35,38], the latter of which is associated with Beta HPV types that are usually only associated with asymptomatic infection in the general population. In EV patients, Beta HPVs are clearly associated with the development of non-melanoma skin cancer (NMSC; the most common cancer in adult light-skinned populations [190]), but in the general population and in immunosuppressed individuals, this has been the subject of much debate [191–193]. These discussions have been stimulated, to a large extent, by the failure to detect Beta HPV DNA ubiquitously in skin cancers (in contrast to the situation seen for the high-risk Alpha PVs in cervical cancer), and the finding that HPVs from the Beta genus are prevalent in normal skin even in the absence of disease. It appears however that these viruses may stimulate cancer progression in a manner that is mechanistically different to HPVs from the high-risk Alpha group. Indeed, our current thinking suggests that the E6 and E7 proteins from these HPV types may exert their effects at an early stage in the carcinogenesis process by inhibiting normal DNA damage repair or apoptosis in response to sunlight [194–197]. According to this hypothesis, the accumulation of genetic errors in the infected cell leads eventually to changes in cellular phenotype and the eventual development of cancer, with loss of the Beta HPV genome from the cell as the rate of keratinocyte cell division increases ([198] and JD unpublished results). This model fits well with much of our data on the role of Beta HPV proteins and expression patterns, but still requires some confirmation, perhaps by the analysis of intermediate disease states during cancer progression.

Although there are many similarities in genome organisation of HPVs, there are many differences, both in protein function and expression patterns that underlie disease phenotype. The discovery of Gamma HPV types 101, 103 and 108 that lack an apparent E6 gene, and which are associated with cervical disease [199,200], emphasises the limitations of applying general principles across wider groupings. Such considerations should also be borne in mind when considering how HPV16 and 18 cause disease, and how even more closely related types, such as HPV16 and 31, function in infected epithelial tissue.

5. Lesion regression, latency and clearance

Although high-risk HPV infection is common, with over 80% of women becoming infected at some stage in their life, cervical cancer arises only rarely as a result of infection. Most infections are cleared as a result of a cell-mediated immune response, and do not persist long enough for deregulated gene expression and the accumulation of secondary genetic errors to occur. HPV16 has an average length of persistence that is longer than most other high-risk types, and this may contribute to its higher cancer risk [201,202]. Poorly understood differences in cell tropism and disease progression patterns associated with individual HPV types may underlie the higher association of HPV18 with adenocarcinoma (rather than squamous cell carcinoma) and its relative infrequence in CIN2. Indeed, our current thinking suggests that HPV16, 18 and 45, which are the primary cause of adenocarcinomas, may infect cells with potential for glandular differentiation [203], and that an abortive or semi-permissive infection in these cells is important for the development of adenocarcinoma. Recent studies have suggested that the infection of specific cells in the junctional region between the endo and ectocervix may in fact underlie the development of many cervical cancers [204].

In general however, genital tract infections by HPV are common in young sexually active individuals, with the majority (80–90%) clearing the infection without overt clinical disease. Most of those who develop benign lesions eventually mount an effective cell mediated immune response and the lesions regress. Regression of anogenital warts is accompanied histologically by a CD4+ T cell-dominated Th1 response, which is also seen in animal models of PV-associated disease [205–208]. Such models provide evidence that the response is modulated by antigen-specific CD4+ T cell dependent mechanisms. The failure to develop effective cell-mediated immunity to clear or control infection results in persistent infection, and in the case of the oncogenic HPVs, an increased probability of progression to high-grade intraepithelial neoplasia and invasive carcinoma.

Effective evasion of innate immune recognition seems to be the hallmark of HPV infections. The viral productive life cycle is exclusively intraepithelial, there is no viraemia, no viral-induced cytolysis or cell death, and viral replication and release is not associated with inflammation [209]. HPV globally down-regulates the innate immune signalling pathways in the infected keratinocyte, pro-inflammatory cytokines, particularly the Type I interferons, are not released, and the signals for Langerhans cell activation and migration and the recruitment of stromal dendritic cells (DCs)

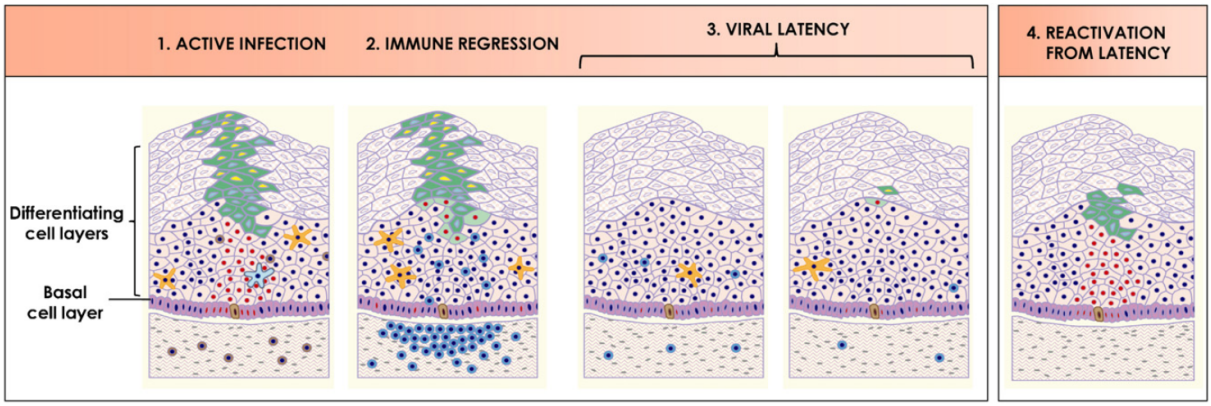

Figure 8. Immune clearance, latency and possible reactivation.
Several HPV proteins have roles in immune evasion as well as in cell cycle entry, which contributes to the ability of HPVs to persist in infected epithelium (see text). Immune regression does eventually occur in the majority of active infections however, presumably as a result of cross-priming of Langerhans cells (shown in orange in 1. Active infection (activated Langerhans cell shown in light blue)) and T-cell activation (resting T-cells shown in brown). The subsequent activation of a cell-mediated immune response leads to the accumulation of activated T-cells (blue circles) in the vicinity of the lesion (shown in 2. Immune regression), with some degree of lymphocyte infiltration. Our current thinking suggests that viral gene expression (marked by cells above the basal layer with red nuclei, green cytoplasm and yellow nuclei according to the different life-cycle stage (see Fig. 3)) is shut-off in the presence of the infiltrating lymphocytes (possibly as a result of cytokine signalling), but that the viral episomes are not effectively cleared from the basal cell layer, with occasional bursts of virus production (shown in 3. Viral latency) [95]. This model allows the possibility of reactivation, which may occur following a change in immune status (shown in 4. Reactivation from latency). It has been suggested that the viral episome may persist for an extended period of time in the slow-cycling epithelial stem cell in the absence of apparent disease.

and macrophages are either not present or inadequate [210]. Furthermore, the productively infected cells that express abundant viral proteins are shed from the epithelial surface, well away from circulating immune cells. For the high-risk Alpha types, many of the mechanisms of immune evasion have been established. The HPV16 E6 protein is known to interfere with Tyk2 function, and as a result is thought to affect STAT signalling [3,211,212]. Similarly, E7 can interfere with induction of Interferon response factor 1, and both E6 and E7 have been reported to reduce surface levels of E-Cadherin, which is thought to underlie the lower abundance of Langerhans cells (the epithelial DCs) in the vicinity of the lesion [213–216]. In addition, the high-risk E5 protein can interfere with the processing of classical MHC molecules to the cell surface, and compromises the display of viral peptides at the surface of the infected epithelial cell [217]. The low-level presentation of viral antigens (and active immune evasion strategies) in the absence of inflammation is thought to favour immune tolerance rather than an effector T cell response that can clear disease.

Although such tactics contribute to persistence, in most cases lesions are successfully resolved. Resolution of infection requires cross-priming of DCs followed by T-cell infiltration into the site of infection and shut-off of viral gene expression. As far as it is known, HPV gene expression is confined to keratinocytes and as a result of this, cross-presentation of HPV antigens by Langerhans cells (or other DCs) is considered essential for the induction of an effector T cell response to the nonstructural HPV proteins. Human Langerhans cells have been shown to prime and cross-prime naive CD8+ cells [218]; however, recent data in the mouse [219] suggests that in the skin (and probably other squamous surfaces) the important cross-presenting antigen-presenting cells are the Langerin+ve, CD103+ve DC, a subset most likely of dermal origin. Dermal DCs and macrophages recruited to HPV-infected epithelium may be key players in the recognition of HPV antigens and the induction of effector responses. However, the suboptimal codon usage by HPV that results in very low protein levels in infected cells could provide a further constraint on the effectiveness of cross-presentation by intra-epithelial DCs.

When lesion regression does occur, it is not associated with massive apoptosis or cell death, and it appears, from animal model studies, that the lesion is cleared by the replacement of actively infected cells with 'apparently normal cells' as the basal cells continue to divide. These 'apparently normal' cells may still contain viral genomes but without concomitant viral gene expression, and it has been suggested that the virus life cycle may become 're-activated' subsequently following immune suppression or changes in hormone levels (Fig. 8). Indeed, recent studies using laser capture approaches have demonstrated genome persistence in the epithelial basal layer for over a year following regression in experimental systems, and support a model in which the viral genome can persist in the epithelial stem cell [95,220]. Low-level viral gene expression and viral copy number have consistently been reported in studies of both asymptomatic infection and immune-mediated latency in humans and animal models [92,220–223]. Immunosuppression studies support the idea that reactivation can occur at the site of previous infection, and persistence following regression has also been suggested in humans, although the duration is not yet well defined [224]. It is clear that for cancer to develop, the virus has to evade immune detection over a prolonged period in order for genetic abnormalities to accumulate. Cervical cancer patients have been reported to have a reduced or non-existent T-cell response to antigens of the causal HPV type [59,225]. While this suggests that persistence may be linked to a failure of the immune response or an inability to recognise viral antigens, no clear link has yet been made with HLA type or other susceptibility indicators [226–228].

6. Conclusions

Human papillomaviruses have evolved over millions of years to survive in a wide range of animal species, including humans. As is typical of viruses that have co-evolved with their hosts, many PVs produce only chronic, inapparent infections, and produce virions from the surface of infected epithelium without apparent detriment to the host. This is the case for many Beta and Gamma HPV types. However, not all HPV types use the same strategy, and it appears that several of the Alpha PVs, in particular, have acquired immunoevasion strategies that allow them to cause persistent visible papillomas. As part of the PV life cycle in the epithelium, these viruses must activate the cell cycle in differentiating keratinocytes that would not normally be replication competent, so that they can

amplify their genomes and package them into infectious particles. To do this, they have evolved proteins (E6, E7 and E5) that interfere with normal cell cycle regulation, and can prevent apoptosis as a result of unscheduled DNA replication. In contrast to the low-risk HPV types, the high-risk Alpha PVs not only drive cell cycle entry in the upper epithelial layers, but (for reasons which are not yet clear) have E6 and E7 proteins that can stimulate the proliferation of infected basal cells and cause neoplasia. This additional characteristic reflects differences in the viral proteins but also differences in the way that the viral proteins are expressed in the basal layer and above. Indeed, it is generally accepted that deregulated expression of these cell cycle regulators underlies neoplasia and the eventual progression to cancer in individuals who cannot resolve their infection.

Although most work to date has focused on the study of high-risk HPV types, and in particular on HPV16 and 18, there will be a need in future to better understand the different risks associated with different high-risk types, and to more fully understand the molecular pathways that they subvert. Such approaches are expected to lead us eventually to the development of better strategies for disease treatment (i.e., targeted antivirals or immunotherapeutics), which are necessary to complement current methods of disease management (i.e., prophylactic vaccination, screening, surgical ablation or local immune modulation). It will also be important to consider high-risk HPV-associated diseases at sites other than the cervix, and to understand the mechanisms by which low-risk HPV types can give rise to papillomatosis and, rarely, cancer. Developing an understanding of the natural history of the Gamma and Beta HPV types both within disease and cancer, will also be an important part of this.

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References

[1] Bernard HU, Burk RD, Chen Z, van Doorslaer K, Hausen H, de Villiers EM. Classification of papillomaviruses (PVs) based on 189 PV types and proposal of taxonomic amendments. Virology 2010;401(1):70–9.
[2] Bravo IG, de Sanjosé S, Gottschling M. The clinical importance of understanding the evolution of papillomaviruses. Trends in microbiology 2010;18(10):432–8.
[3] Doorbar J. Molecular biology of human papillomavirus infection and cervical cancer. Clin Sci (Lond) 2006;110(5):525–41.
[4] Bosch FX, Burchell AN, Schiffman M, Giuliano AR, de Sanjose S, Bruni L, et al. Epidemiology and natural history of human papillomavirus infections and type-specific implications in cervical neoplasia. Vaccine 2008;26(Suppl 10):K1–16.
[5] Ekström J, Bzhalava D, Svenback D, Forslund O, Dillner J. High throughput sequencing reveals diversity of Human Papillomaviruses in cutaneous lesions. Int J Cancer 2011;129(11):2643–50.
[6] Nindl I, Gottschling M, Stockfleth E. Human papillomaviruses and non-melanoma skin cancer: basic virology and clinical manifestations. Dis Markers 2007;23(4):247–59.
[7] Gottschling M, Göker M, Köhler A, Lehmann MD, Stockfleth E, Nindl I. Cutaneotropic human beta-/gamma-papillomaviruses are rarely shared between family members. J Invest Dermatol 2009;129(10):2427–34.
[8] Bottalico D, Chen Z, Dunne A, Ostoloza J, McKinney S, Sun C, et al. The oral cavity contains abundant known and novel human papillomaviruses from the Betapapillomavirus and Gammapapillomavirus genera. J Infect Dis. 204(5):787-92.
[9] Ekstrom J, Forslund O, Dillner J. Three novel papillomaviruses (HPV109, HPV112 and HPV114) and their presence in cutaneous and mucosal samples. Virology 2010;397(2):331–6.
[10] Forslund O. Genetic diversity of cutaneous human papillomaviruses. J Gen Virol 2007;88(Pt 10):2662–9.
[11] Woolhouse M, Gaunt E. Ecological origins of novel human pathogens. Crit Rev Microbiol 2007;33(4):231–42.
[12] Garcia-Vallve S, Alonso A, Bravo IG. Papillomaviruses: different genes have different histories. Trends Microbiol 2005;13(11):514–21.
[13] Gottschling M, Stamatakis A, Nindl I, Stockfleth E, Alonso A, Bravo IG. Multiple evolutionary mechanisms drive papillomavirus diversification. Mol Biol Evol 2007;24(5):1242–58.
[14] Shah SD, Doorbar J, Goldstein RA. Analysis of host-parasite incongruence in papillomavirus evolution using importance sampling. Mol Biol Evol 2010;27(6):1301–14.
[15] Gottschling M, Göker M, Stamatakis A, Bininda-Emonds OR, Nindl I, Bravo IG. Quantifying the phylodynamic forces driving papillomavirus evolution. Mol Biol Evol 2011 Jul;28(7):2101–13.
[16] Lacey CJ, Lowndes CM, Shah KV. Chapter 4: Burden and management of non-cancerous HPV-related conditions: HPV-6/11 disease. Vaccine 2006;24(Suppl 3). S3/35-41.
[17] Major T, Szarka K, Sziklai I, Gergely L, Czeglédy J. The characteristics of human papillomavirus DNA in head and neck cancers and papillomas. J Clin Pathol 2005;58(1):51–5.
[18] Gerein V, Rastorguev E, Gerein J, Draf W, Schirren J. Incidence, age at onset, and potential reasons of malignant transformation in recurrent respiratory papillomatosis patients: 20 years experience. Otolaryngol Head Neck Surg 2005;132(3):392–4.
[19] Donne AJ, Clarke R. Recurrent respiratory papillomatosis: an uncommon but potentially devastating effect of human papillomavirus in children. Int J STD AIDS 2010;21(6):381–5.
[20] Hsueh PR. Human papillomavirus, genital warts, and vaccines. J Microbiol Immunol Infect 2009;42(2):101–6.
[21] Derkay CS. Task force on recurrent respiratory papillomas. A preliminary report. Arch Otolaryngol Head Neck Surg 1995;121(12):1386–91.
[22] Doorbar J. The papillomavirus life cycle. J Clin Virol 2005;32(Suppl):7–15.
[23] Chen Z, Schiffman M, Herrero R, Desalle R, Anastos K, Segondy M, et al. Evolution and taxonomic classification of human papillomavirus 16 (HPV16)-related variant genomes: HPV31, HPV33, HPV35, HPV52, HPV58 and HPV67. PLoS One 2011;6(5):e20183.
[24] zur Hausen H. Papillomaviruses in the causation of human cancers - a brief historical account. Virology 2009;384(2):260–5.
[25] Wikstrom A, Hedblad MA, Syrjanen S. Penile intraepithelial neoplasia: histopathological evaluation, HPV typing, clinical presentation and treatment. J Eur Acad Dermatol Venereol 2012 Mar;26(3):325–30.
[26] Silva RJ, Casseb J, Andreoli MA, Villa LL. Persistence and clearance of HPV from the penis of men infected and non-infected with HIV. J Med Virol 2011;83(1):127–31.
[27] Szentirmay Z, Pólus K, Tamás L, Szentkuti G, Kurcsics J, Csernák E, et al. Human papillomavirus in head and neck cancer: molecular biology and clinicopathological correlations. Cancer Metastasis Rev 2005;24(1):19–34.
[28] Syrjänen S, Lodi G, von Bültzingslöwen I, Aliko A, Arduino P, Campisi G, et al. Human papillomaviruses in oral carcinoma and oral potentially malignant disorders: a systematic review. Oral Dis 2011;17(Suppl 1): 58–72.
[29] Middleton K, Peh W, Southern S, Griffin H, Sotlar K, Nakahara T. Organization of human papillomavirus productive cycle during neoplastic progression provides a basis for selection of diagnostic markers. J Virol 2003;77(19):10186–201.

[30] Laffort C, Le Deist F, Favre M, Caillat-Zucman S, Radford-Weiss I, Debré M, et al. Severe cutaneous papillomavirus disease after haemopoietic stem-cell transplantation in patients with severe combined immune deficiency caused by common gammac cytokine receptor subunit or JAK-3 deficiency. Lancet 2004;363(9426):2051–4.
[31] Gewirtzman A, Bartlett B, Tyring S. Epidermodysplasia verruciformis and human papilloma virus. Curr Opin Infect Dis 2008;21(2):141–6.
[32] Lebwohl MG, Rosen T, Stockfleth E. The role of human papillomavirus in common skin conditions: current viewpoints and therapeutic options. Cutis 2010;86(5):suppl 1-11; quiz suppl 12.
[33] Dubina M, Goldenberg G. Viral-associated nonmelanoma skin cancers: a review. Am J Dermatopathol 2009;31(6):561–73.
[34] Weissenborn S, Neale RE, Waterboer T, Abeni D, Bavinck JN, Green AC, Harwood CA, et al. Beta-papillomavirus DNA loads in hair follicles of immunocompetent people and organ transplant recipients. Med Microbiol Immunol 2012 May;201(2):117–25.
[35] Kawai T, Malech HL. WHIM syndrome: congenital immune deficiency disease. Curr Opin Hematol 2009;16(1):20–6.
[36] Ramoz N, Rueda LA, Bouadjar B, Montoya LS, Orth G, Favre M. Mutations in two adjacent novel genes are associated with epidermodysplasia verruciformis. Nat Genet 2002;32(4):579–81.
[37] Chow KY, Brotin É, Ben Khalifa Y, Carthagena L, Teissier S, Danckaert A, et al. A pivotal role for CXCL12 signaling in HPV-mediated transformation of keratinocytes: clues to understanding HPV-pathogenesis in WHIM syndrome. Cell Host Microbe 2010;8(6):523–33.
[38] Lazarczyk M, Cassonnet P, Pons C, Jacob Y, Favre M. The EVER proteins as a natural barrier against papillomaviruses: a new insight into the pathogenesis of human papillomavirus infections. Microbiol Mol Biol Rev 2009;73(2):348–70.
[39] Gulino AV. WHIM syndrome: a genetic disorder of leukocyte trafficking. Curr Opin Allergy Clin Immunol 2003;3(6):443–50.
[40] Ortak T, Uysal AC, Alagoz MS, Orbay H, Sensoz O. Epidermodysplasia verruciformis: an unusual presentation. Dermatol Surg 2006;32(2):302–6.
[41] Muñoz N, Castellsagué X, de González AB, Gissmann L. Chapter 1: HPV in the etiology of human cancer. Vaccine 2006;24(Suppl 3). S3/1-10.
[42] Bouvard V, Baan R, Straif K, Grosse Y, Secretan B. El Ghissassi F, et al., A review of human carcinogens–Part B: biological agents. The lancet oncology 2009;10(4):321–2.
[43] Schiffman M, Clifford G, Buonaguro FM. Classification of weakly carcinogenic human papillomavirus types: addressing the limits of epidemiology at the borderline. Infect Agents Cancer 2009;4:8.
[44] Schiffman M, Herrero R, Desalle R, Hildesheim A, Wacholder S, Rodriguez AC, et al. The carcinogenicity of human papillomavirus types reflects viral evolution. Virology 2005;337(1):76–84.
[45] Muñoz N, Bosch FX, de Sanjosé S, Herrero R, Castellsagué X, Shah KV, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med 2003;348(6):518–27.
[46] Schiffman M, Castle PE, Jeronimo J, Rodriguez AC, Wacholder S. Human papillomavirus and cervical cancer. Lancet 2007;370(9590):890–907.
[47] Baseman JG, Koutsky LA. The epidemiology of human papillomavirus infections. J Clin Virol 2005;32(Suppl 1):S16–24.
[48] Barrow-Laing L, Chen W, Roman A. Low- and high-risk human papillomavirus E7 proteins regulate p130 differently. Virology 2010;400(2):233–9.
[49] Zhang B, Chen W, Roman A. The E7 proteins of low- and high-risk human papillomaviruses share the ability to target the pRB family member p130 for degradation. Proc Natl Acad Sci U S A 2006;103(2):437–42.
[50] Melsheimer P, Vinokurova S, Wentzensen N, Bastert G, von Knebel Doeberitz M. DNA aneuploidy and integration of human papillomavirus type 16 e6/e7 oncogenes in intraepithelial neoplasia and invasive squamous cell carcinoma of the cervix uteri. Clin Cancer Res 2004;10(9):3059–63.
[51] Van Tine BA, Kappes JC, Banerjee NS, Knops J, Lai L, Steenbergen RD, et al. Clonal selection for transcriptionally active viral oncogenes during progression to cancer. J Virol 2004;78(20):11172–86.
[52] Ziegert C, Wentzensen N, Vinokurova S, Kisseljov F, Einenkel J, Hoeckel M, et al. A comprehensive analysis of HPV integration loci in anogenital lesions combining transcript and genome-based amplification techniques. Oncogene 2003;22(25):3977–84.
[53] Thorland EC, Myers SL, Gostout BS, Smith DI. Common fragile sites are preferential targets for HPV16 integrations in cervical tumors. Oncogene 2003;22(8):1225–37.
[54] Pett M, Coleman N. Integration of high-risk human papillomavirus: a key event in cervical carcinogenesis? J Pathol 2007;212(4):356–67.
[55] Yu T, Ferber MJ, Cheung TH, Chung TK, Wong YF, Smith DI. The role of viral integration in the development of cervical cancer. Cancer Genet Cytogenet 2005;158(1):27–34.
[56] Dall KL, Scarpini CG, Roberts I, Winder DM, Stanley MA, Muralidhar B, et al. Characterization of naturally occurring HPV16 integration sites isolated from cervical keratinocytes under noncompetitive conditions. Cancer Res 2008;68(20):8249–59.
[57] Wilting SM, Steenbergen RD, Tijssen M, van Wieringen WN, Helmerhorst TJ, van Kemenade FJ, et al. Chromosomal signatures of a subset of high-grade premalignant cervical lesions closely resemble invasive carcinomas. Cancer Res 2009;69(2):647–55.
[58] Gariglio P, Gutiérrez J, Cortés E, Vázquez J. The role of retinoid deficiency and estrogens as cofactors in cervical cancer. Arch Med Res 2009;40(6):449–65.
[59] de Jong A, van Poelgeest MI, van der Hulst JM, Drijfhout JW, Fleuren GJ, Melief CJ, et al. Human papillomavirus type 16-positive cervical cancer is associated with impaired CD4+ T-cell immunity against early antigens E2 and E6. Cancer Res 2004;64(15):5449–55.
[60] Buck CB, Cheng N, Thompson CD, Lowy DR, Steven AC, Schiller JT, et al. Arrangement of L2 within the papillomavirus capsid. J Virol 2008;82(11):5190–7.
[61] Finnen RL, Erickson KD, Chen XS, Garcea RL. Interactions between papillomavirus L1 and L2 capsid proteins. J Virol 2003;77(8):4818–26.
[62] Johnson KM, Kines RC, Roberts JN, Lowy DR, Schiller JT, Day PM. Role of heparan sulfate in attachment to and infection of the murine female genital tract by human papillomavirus. J VIrol 2009;83(5):2067–74.
[63] Combita AL, Touzé A, Bousarghin L, Sizaret PY, Muñoz N, Coursaget P. Gene transfer using human papillomavirus pseudovirions varies according to virus genotype and requires cell surface heparan sulfate. FEMS Microbiol Lett 2001;204(1):183–8.
[64] Giroglou T, Florin L, Schäfer F, Streeck RE, Sapp M. Human papillomavirus infection requires cell surface heparan sulfate. J Virol 2001;75(3):1565–70.
[65] Culp TD, Budgeon LR, Marinkovich MP, Meneguzzi G, Christensen ND. Keratinocyte-secreted laminin 5 can function as a transient receptor for human papillomaviruses by binding virions and transferring them to adjacent cells. J Virol 2006;80(18):8940–50.
[66] Kines RC, Thompson CD, Lowy DR, Schiller JT, Day PM. The initial steps leading to papillomavirus infection occur on the basement membrane prior to cell surface binding. Proc Natl Acad Sci U S A 2009;106(48): 20458–63.
[67] Schiller JT, Day PM, Kines RC. Current understanding of the mechanism of HPV infection. Gynecol Oncol 2010;118(1 Suppl):S12–7.
[68] Bienkowska-Haba M, Patel HD, Sapp M. Target cell cyclophilins facilitate human papillomavirus type 16 infection. PLoS pathogens 2009;5(7):e1000524.
[69] Richards RM, Lowy DR, Schiller JT, Day PM. Cleavage of the papillomavirus minor capsid protein, L2, at a furin consensus site is necessary for infection. Proceedings of the National Academy of Sciences of the United States of America 2006;103(5):1522–7.
[70] Evander M, Frazer IH, Payne E, Qi YM, Hengst K, McMillan NA. Identification of the alpha 6 integrin as a candidate receptor for papillomaviruses. J Virol 1997;71(3):2449–56.
[71] McMillan NA, Payne E, Frazer IH, Evander M. Expression of the alpha6 integrin confers papillomavirus binding upon receptor-negative B-cells. Virology 1999;261:271–9.
[72] Shafti-Keramat S, Handisurya A, Kriehuber E, Meneguzzi G, Slupetzky K, Kirnbauer R. Different heparan sulfate proteoglycans serve as cellular receptors for human papillomaviruses. J Virol 2003;77(24):13125–35.
[73] Licitra L, Perrone F, Bossi P, Suardi S, Mariani L, Artusi R. High-risk human papillomavirus affects prognosis in patients with surgically treated oropharyngeal squamous cell carcinoma. J Clin Oncol 2006;24(36): 5630–6.
[74] Scheurer ME, Guillaud M, Tortolero-Luna G, McAulay C, Follen M, Adler-Storthz K. Human papillomavirus-related cellular changes measured by cytometric analysis of DNA ploidy and chromatin texture. Cytometry B Clinical Cytom 2007;72(5):324–31.
[75] Surviladze Z, Dziduszko A, Ozbun MA. Essential roles for soluble virion-associated heparan sulfonated proteoglycans and growth factors in human papillomavirus infections. PLoS Pathog 2012;8(2):e1002519.
[76] Patterson NA, Smith JL, Ozbun MA. Human papillomavirus type 31b infection of human keratinocytes does not require heparan sulfate. J Virol 2005;79(11):6838–47.
[77] Horvath CA, Boulet GA, Renoux VM, Delvenne PO, Bogers JP. Mechanisms of cell entry by human papillomaviruses: an overview. Virol J 2010;7:11.
[78] Sapp M, Bienkowska-Haba M. Viral entry mechanisms: human papillomavirus and a long journey from extracellular matrix to the nucleus. FEBS J 2009;276(24):7206–16.
[79] Bergant Marušič M, Ozbun MA, Campos SK, Myers MP, Banks L. Human Papillomavirus L2 facilitates viral escape from late endosomes via sorting nexin 17. Traffic 2012 Mar;13(3):455–67.
[80] Schelhaas M, Shah B, Holzer M, Blattmann P, Kühling L, Day PM, et al. Entry of human papillomavirus type 16 by actin-dependent, clathrin- and lipid raft-independent endocytosis. PLoS Pathog 2012;8(4):e1002657.
[81] Fuchs E, Nowak JA. Building epithelial tissues from skin stem cells. Cold Spring Harb Symp Quant Biol 2008;73:333–50.
[82] Ledwaba T, Dlamini Z, Naicker S, Bhoola K. Molecular genetics of human cervical cancer: role of papillomavirus and the apoptotic cascade. Biol Chem 2004;385(8):671–82.
[83] Pyeon D, Pearce SM, Lank SM, Ahlquist P, Lambert PF. Establishment of human papillomavirus infection requires cell cycle progression. PLoS Pathog 2009;5(2):e1000318.
[84] Smith JL, Campos SK, Wandinger-Ness A, Ozbun MA. Caveolin-1-dependent infectious entry of human papillomavirus type 31 in human keratinocytes proceeds to the endosomal pathway for pH-dependent uncoating. J VIrol 2008;82(19):9505–12.
[85] 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(6):3846–50.
[86] Day PM, Lowy DR, Schiller JT. Papillomaviruses infect cells via a clathrin-dependent pathway. Virology 2003;307(1):1–11.
[87] Hindmarsh PL, Laimins LA. Mechanisms regulating expression of the HPV 31 L1 and L2 capsid proteins and pseudovirion entry. Virol J 2007;4:19.

[88] Grayson W, Rhemtula HA, Taylor LF, Allard U, Tiltman AJ. Detection of human papillomavirus in large cell neuroendocrine carcinoma of the uterine cervix: a study of 12 cases. J Clin Pathol 2002;55(2):108–14.

[89] Gravitt PE, Lacey Jr JV, Brinton LA, Barnes WA, Kornegay JR, Greenberg MD, et al. Evaluation of self-collected cervicovaginal cell samples for human papillomavirus testing by polymerase chain reaction. Cancer Epidemiol Biomarkers Prev 2001 Feb;10(2):95–100.

[90] Bouvard V, Baan RA, Grosse Y, Lauby-Secretan B, El Ghissassi F, Benbrahim-Tallaa L, et al. Carcinogenicity of malaria and of some polyomaviruses. Lancet Oncol 2012;13(4):339–40.

[91] Egawa K. Do human papillomaviruses target epidermal stem cells? Dermatology 2003;207(3):251–4.

[92] Schmitt A, Rochat A, Zeltner R, Borenstein L, Barrandon Y, Wettstein FO, et al. The primary target cells of the high-risk cottontail rabbit papillomavirus colocalize with hair follicle stem cells. J Virol 1996;70(3):1912–22.

[93] Parish JL, Bean AM, Park RB, Androphy EJ. ChlR1 is required for loading papillomavirus E2 onto mitotic chromosomes and viral genome maintenance. Mol Cell 2006;24(6):867–76.

[94] McBride AA. Replication and partitioning of papillomavirus genomes. Adv Vir Res 2008;72:155–205.

[95] Maglennon GA, McIntosh P, Doorbar J. Persistence of viral DNA in the epithelial basal layer suggests a model for papillomavirus latency following immune regression. Virology 2011;414(2):153–63.

[96] Kim K, Lambert PF. E1 protein of bovine papillomavirus 1 is not required for the maintenance of viral plasmid DNA replication. Virology 2002;293(1):10–4.

[97] Angeletti PC, Kim K, Fernandes FJ, Lambert PF. Stable replication of papillomavirus genomes in Saccharomyces cerevisiae. J Virol 2002;76(7):3350–8.

[98] Egawa N, Nakahara T, Ohno S, Narisawa-Saito M, Yugawa T, Fujita M, et al. The E1 protein of human papillomavirus type 16 is dispensable for maintenance replication of the viral genome. Journal of Virology 2012;86(6): 3276–83.

[99] Blakaj DM, Fernandez-Fuentes N, Chen Z, Hegde R, Fiser A, Burk RD, et al. Evolutionary and biophysical relationships among the papillomavirus E2 proteins. Front Biosci 2009 Jan 1;14:900–17.

[100] McBride AA, Oliveira JG, McPhillips MG. Partitioning viral genomes in mitosis: same idea, different targets. Cell Cycle 2006;5(14):1499–502.

[101] Van Tine BA, Dao LD, Wu SY, Sonbuchner TM, Lin BY, Zou N, et al. Human papillomavirus (HPV) origin-binding protein associates with mitotic spindles to enable viral DNA partitioning. Proc Natl Acad Sci U S A 2004;101(12): 4030–5.

[102] Dao LD, Duffy A, Van Tine BA, Wu SY, Chiang CM, Broker TR, et al. Dynamic localization of the human papillomavirus type 11 origin binding protein E2 through mitosis while in association with the spindle apparatus. J Virol 2006;80(10):4792–800.

[103] Valencia C, Bonilla-Delgado J, Oktaba K, Ocádiz-Delgado R, Gariglio P, Covarrubias L. Human papillomavirus E6/E7 oncogenes promote mouse ear regeneration by increasing the rate of wound re-epithelization and epidermal growth. J Invest Dermatol 2008;128(12):2894–903.

[104] Rosenberger S, De-Castro Arce J, Langbein L, Steenbergen RD, Rösl F. Alternative splicing of human papillomavirus type-16 E6/E6* early mRNA is coupled to EGF signaling via Erk1/2 activation. Proc Natl Acad Sci U S A 2010;107(15):7006–11.

[105] Klingelhutz AJ, Roman A. Cellular transformation by human papillomaviruses: lessons learned by comparing high- and low-risk viruses. Virology 2012;424(2):77–98.

[106] Isaacson Wechsler E, Wang Q, Roberts I, Pagliarulo E, Jackson D, Untersperger C, et al. Reconstruction of human papillomavirus type 16-mediated early-stage neoplasia implicates e6/e7 deregulation and the loss of contact inhibition in neoplastic progression. J Virol 2012;86(11):6358–64.

[107] Jenkins D. Histopathology and cytopathology of cervical cancer. Dis Markers 2007;23(4):199–212.

[108] Roman A. The human papillomavirus E7 protein shines a spotlight on the pRB family member, p130. Cell Cycle 2006;5(6):567–8.

[109] Felsani A, Mileo AM, Paggi MG. Retinoblastoma family proteins as key targets of the small DNA virus oncoproteins. Oncogene 2006;25(38):5277–85.

[110] McLaughlin-Drubin ME, Munger K. The human papillomavirus E7 oncoprotein. Virology 2009;384(2):335–44.

[111] Duensing S, Munger K. The human papillomavirus type 16 E6 and E7 oncoproteins independently induce numerical and structural chromosome instability. Cancer Res 2002;62(23):7075–82.

[112] Duensing S, Munger K. Human papillomavirus type 16 E7 oncoprotein can induce abnormal centrosome duplication through a mechanism independent of inactivation of retinoblastoma protein family members. J Virol 2003;77(22):12331–5.

[113] Duensing S, Munger K. Mechanisms of genomic instability in human cancer: insights from studies with human papillomavirus oncoproteins. Int J Cancer 2004;109(2):157–62.

[114] Korzeniewski N, Spardy N, Duensing A, Duensing S. Genomic instability and cancer: lessons learned from human papillomaviruses. Cancer Lett 2011;305(2):113–22.

[115] Duensing A, Spardy N, Chatterjee P, Zheng L, Parry J, Cuevas R, et al. Centrosome overduplication, chromosomal instability, and human papillomavirus oncoproteins. Environ Mol Mutagen 2009;50(8):741–7.

[116] Javier RT. Cell polarity proteins: common targets for tumorigenic human viruses. Oncogene 2008;27(55):7031–46.

[117] Culp TD, Cladel NM, Balogh KK, Budgeon LR, Mejia AF, Christensen ND. Papillomavirus particles assembled in 293TT cells are infectious in vivo. J VIrol 2006;80(22):11381–4.

[118] Kühne C, Gardiol D, Guarnaccia C, Amenitsch H, Banks L. Differential regulation of human papillomavirus E6 by protein kinase A: conditional degradation of human discs large protein by oncogenic E6. Oncogene 2000;19(51):5884–91.

[119] Nicolaides L, Davy C, Raj K, Kranjec C, Banks L, Doorbar J. Stabilization of HPV16 E6 protein by PDZ proteins, and potential implications for genome maintenance. Virology 2011;414(2):137–45.

[120] Massimi P, Narayan N, Cuenda A, Banks L. Phosphorylation of the discs large tumour suppressor protein controls its membrane localisation and enhances its susceptibility to HPV E6-induced degradation. Oncogene 2006;25(31):4276–85.

[121] Galloway DA, Gewin LC, Myers H, Luo W, Grandori C, Katzenellenbogen RA, et al. Regulation of telomerase by human papillomaviruses. Cold Spring Harb Symp Quant Biol 2005;70:209–15.

[122] Gewin L, Galloway DA. E box-dependent activation of telomerase by human papillomavirus type 16 E6 does not require induction of c-myc. J VIrol 2001;75(15):7198–201.

[123] Klingelhutz AJ, Foster SA, McDougall JK. Telomerase activation by the E6 gene product of human papillomavirus type 16. Nature 1996;380:79–82.

[124] Fu L, Van Doorslaer K, Chen Z, Ristriani T, Masson M, Travé G, et al. Degradation of p53 by human Alphapapillomavirus E6 proteins shows a stronger correlation with phylogeny than oncogenicity. PLoS One 2010;5(9.).

[125] Zanier K, ould M'hamed ould Sidi A, Boulade-Ladame C, Rybin V, Chappelle A, Atkinson A, et al. Solution structure analysis of the HPV16 E6 oncoprotein reveals a self-association mechanism required for E6-mediated degradation of p53. Structure 2012;20(4):604–17.

[126] Pim D, Banks L. Interaction of viral oncoproteins with cellular target molecules: infection with high-risk vs low-risk human papillomaviruses. APMIS 2010;118(6-7):471–93.

[127] Tomaic V, Pim D, Banks L. The stability of the human papillomavirus E6 oncoprotein is E6AP dependent. Virology 2009;393(1):7–10.

[128] White EA, Sowa ME, Tan MJ, Jeudy S, Hayes SD, Santha S, 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(5):E260–7.

[129] Nominé Y, Masson M, Charbonnier S, Zanier K, Ristriani T, Deryckère F, et al. Structural and functional analysis of E6 oncoprotein: insights in the molecular pathways of human papillomavirus-mediated pathogenesis. Mol Cell 2006;21(5):665–78.

[130] Bodily J, Laimins LA. Persistence of human papillomavirus infection: keys to malignant progression. Trends Microbiol 2011;19(1):33–9.

[131] Banerjee NS, Wang HK, Broker TR, Chow LT. Human papillomavirus (HPV) E7 induces prolonged G2 following S phase reentry in differentiated human keratinocytes. J Biol Chem 2011;286(17):15473–82.

[132] 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(2):181–94.

[133] Krawczyk E, Suprynowicz FA, Liu X, Dai Y, Hartmann DP, Hanover J, et al. Koilocytosis: a cooperative interaction between the human papillomavirus E5 and E6 oncoproteins. Am J Pathol 2008;173(3):682–8.

[134] Krawczyk E, Suprynowicz FA, Sudarshan SR, Schlegel R. Membrane orientation of the human papillomavirus type 16 E5 oncoprotein. J Virol 2010;84(4):1696–703.

[135] Kabsch K, Mossadegh N, Kohl A, Komposch G, Schenkel J, Alonso A, et al. The HPV-16 E5 protein inhibits TRAIL- and FasL-mediated apoptosis in human keratinocyte raft cultures. Intervirology 2004;47(1): 48–56.

[136] Suprynowicz FA, Krawczyk E, Hebert JD, Sudarshan SR, Simic V, Kamonjoh CM, et al. The human papillomavirus type 16 E5 oncoprotein inhibits epidermal growth factor trafficking independently of endosome acidification. J Virol 2010;84(20):10619–29.

[137] Thomsen P, van Deurs B, Norrild B, Kayser L. The HPV16 E5 oncogene inhibits endocytic trafficking. Oncogene 2000;19(52):6023–32.

[138] Genther SM, Sterling S, Duensing S, Münger K, Sattler C, Lambert PF. Quantitative role of the human papillomavirus type 16 E5 gene during the productive stage of the viral life cycle. J Virol 2003;77(5):2832–42.

[139] Fehrmann F, Klumpp DJ, Laimins LA. Human papillomavirus type 31 E5 protein supports cell cycle progression and activates late viral functions upon epithelial differentiation. J Virol 2003;77(5):2819–31.

[140] Pim D, Collins M, Banks L. Human papillomavirus type 16 E5 gene stimulates the transforming activity of the epidermal growth factor receptor. Oncogene 1992;7(1):27–32.

[141] Straight SW, Hinkle PM, Jewers RJ, McCance DJ. The E5 oncoprotein of HPV16 transforms fibroblasts and effects the downregulation of the EGF receptor in keratinocytes. J Virol 1993;69:4521–32.

[142] Crusius K, Rodriguez I, Alonso A. The human papillomavirus type 16 E5 protein modulates ERK1/2 and p38 MAP kinase activation by an EGFR-independent process in stressed human keratinocytes. Virus Genes 2000;20(1): 65–9.

[143] Crusius K, Auvinen E, Steuer B, Gaissert H, Alonso A. The human papillomavirus type 16 E5-protein modulates ligand-dependent activation of the EGF receptor family in the human epithelial cell line HaCaT. Exp Cell Res 1998;241(1):76–83.

[144] Yu JH, Lin BY, Deng W, Broker TR, Chow LT. Mitogen-activated protein kinases activate the nuclear localization sequence of human papillomavirus type 11 E1 DNA helicase to promote efficient nuclear import. J Virol 2007;81(10):5066–78.

[145] Deng W, Lin BY, Jin G, Wheeler CG, Ma T, Harper JW, et al. Cyclin/CDK regulates the nucleocytoplasmic localization of the human papillomavirus E1 DNA helicase. J Virol 2004;78(24):13954–65.

[146] Moody CA, Fradet-Turcotte A, Archambault J, Laimins LA. Human papillomaviruses activate caspases upon epithelial differentiation to induce viral genome amplification. Proc Natl Acad Sci U S A 2007;104(49): 19541–6.

[147] Doorbar J, Foo C, Coleman N, Medcalf L, Hartley O, Prospero T, et al. Characterization of events during the late stages of HPV16 infection in vivo using high-affinity synthetic Fabs to E4. Virology 1997;238(1): 40–52.

[148] McIntosh PB, Martin SR, Jackson DJ, Khan J, Isaacson ER, Calder L, et al. Structural analysis reveals an amyloid form of the human papillomavirus type 16 E1–E4 protein and provides a molecular basis for its accumulation. J Virol 2008;82(16):8196–203.

[149] Doorbar J, Ely S, Sterling J, McLean C, Crawford L. Specific interaction between HPV-16 E1-E4 and cytokeratins results in collapse of the epithelial cell intermediate filament network. Nature 1991;352(6338): 824–7.

[150] Wang Q, Griffin H, Southern S, Jackson D, Martin A, McIntosh P, et al. Functional analysis of the human papillomavirus type 16 E1 = E4 protein provides a mechanism for in vivo and in vitro keratin filament reorganization. J Virol 2004;78(2):821–33.

[151] Gulliksen A, Keegan H, Martin C, O'Leary J, Solli LA, Falang IM, et al. Towards a Sample-In, Answer-Out Point-of-Care Platform for Nucleic Acid Extraction and Amplification: Using an HPV E6/E7 mRNA Model System. J Oncol 2012;2012:905024.

[152] Wilson R, Fehrmann F, Laimins LA. Role of the E1Ê4 protein in the differentiation-dependent life cycle of human papillomavirus type 31. J Virol 2005;79(11):6732–40.

[153] Wilson R, Ryan GB, Knight GL, Laimins LA, Roberts S. The full-length E1E4 protein of human papillomavirus type 18 modulates differentiation-dependent viral DNA amplification and late gene expression. Virology 2007;362(2):453–60.

[154] Wang Q, Kennedy A, Das P, McIntosh PB, Howell SA, Isaacson ER, et al. Phosphorylation of the human papillomavirus type 16 E1–E4 protein at T57 by ERK triggers a structural change that enhances keratin binding and protein stability. J Virol 2005;79(20):13150–65.

[155] Peh WL, Brandsma JL, Christensen ND, Cladel NM, Wu X, Doorbar J. The viral E4 protein is required for the completion of the cottontail rabbit papillomavirus productive cycle in vivo. J Virol 2004;78(4):2142–51.

[156] Ozbun MA, Meyers C. Human papillomavirus type 31b E1 and E2 transcript expression correlates with vegetative viral genome amplification. Virology 1998;248(2):218–30.

[157] Johansson C, Somberg M, Li X, Backström Winquist E, Fay J, Ryan F, et al. HPV-16 E2 contributes to induction of HPV-16 late gene expression by inhibiting early polyadenylation. EMBO J. 2012 May 22. doi: 10.1038/emboj.2012.147. [Epub ahead of print].

[158] Milligan SG, Veerapraditsin T, Ahamet B, Mole S, Graham SV. Analysis of novel human papillomavirus type 16 late mRNAs in differentiated W12 cervical epithelial cells. Virology 2007;360(1):172–81.

[159] Holmgren SC, Patterson NA, Ozbun MA, Lambert PF. The minor capsid protein L2 contributes to two steps in the human papillomavirus type 31 life cycle. J Virol 2005;79(7):3938–48.

[160] Day PM, Roden RB, Lowy DR, Schiller JT. The papillomavirus minor capsid protein, L2, induces localization of the major capsid protein, L1, and the viral transcription/replication protein, E2, to PML oncogenic domains. J Virol 1998;72(1):142–50.

[161] Buck CB, Thompson CD, Pang YY, Lowy DR, Schiller JT. Maturation of papillomavirus capsids. J VIrol 2005;79(5):2839–46.

[162] Brown DR, Kitchin D, Qadadri B, Neptune N, Batteiger T, Ermel A. The human papillomavirus type 11 E1–E4 protein is a transglutaminase 3 substrate and induces abnormalities of the cornified cell envelope. Virology 2006;345(1):290–8.

[163] Paavonen J, Jenkins D, Bosch FX, Naud P, Salmerón J, Wheeler CM, et al. Efficacy of a prophylactic adjuvanted bivalent L1 virus-like-particle vaccine against infection with human papillomavirus types 16 and 18 in young women: an interim analysis of a phase III double-blind, randomised controlled trial. Lancet 2007;369(9580):2161–70.

[164] Paavonen J, Naud P, Salmerón J, Wheeler CM, Chow SN, Apter D, Kitchener H. Efficacy of human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine against cervical infection and precancer caused by oncogenic HPV types (PATRICIA): final analysis of a double-blind, randomised study in young women. Lancet 2009;374(9686):301–14.

[165] Szarewski A, Poppe WA, Skinner SR, Wheeler CM, Paavonen J, Naud P, et al. Efficacy of the human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine in women aged 15-25 years with and without serological evidence of previous exposure to HPV-16/18. Int J Cancer 2012 Jul 1;131(1): 106–16.

[166] Quint W, Jenkins D, Molijn A, Struijk L, van de Sandt M, Doorbar J, et al. One virus one lesion - Individual components of CIN lesions contain a specific HPV type. J Pathol 2012 May;227(1):62–71.

[167] Ding DC, Chiang MH, Lai HC, Hsiung CA, Hsieh CY, Chu TY. Methylation of the long control region of HPV16 is related to the severity of cervical neoplasia. Eur J Obstet Gynecol Reprod Biol 2009;147(2):215–20.

[168] Pater MM, Hughes GA, Hyslop DE, Nakshatri H, Pater A. Glucocorticoid-dependent oncogenic transformation by type 16 but not type 11 human papilloma virus DNA. Nature 1988;335(6193):832–5.

[169] Piccini A, Storey A, Romanos M, Banks L. Regulation of human papillomavirus type 16 DNA replication by E2, glucocorticoid hormone and epidermal growth factor. J Gen Virol 1997;78(Pt 8):1963–70.

[170] Arbeit JM, Howley PM, Hanahan D. Chronic estrogen-induced cervical and vaginal squamous carcinogenesis in human papillomavirus type 16 transgenic mice. Proc Natl Acad Sci U S A 1996;93(7):2930–5.

[171] Vinokurova S, von Knebel Doeberitz M. Differential methylation of the HPV 16 upstream regulatory region during epithelial differentiation and neoplastic transformation. PLoS One 2011;6(9):e24451.

[172] McLaughlin-Drubin ME, Crum CP, Munger K. Human papillomavirus E7 oncoprotein induces KDM6A and KDM6B histone demethylase expression and causes epigenetic reprogramming. Proc Natl Acad Sci U S A 2011;108(5):2130–5.

[173] Laurson J, Khan S, Chung R, Cross K, Raj K. Epigenetic repression of E-cadherin by human papillomavirus 16 E7 protein. Carcinogenesis 2010;31(5):918–26.

[174] Hyland PL, McDade SS, McCloskey R, Dickson GJ, Arthur K, McCance DJ, et al. Evidence for alteration of EZH2, BMI1, and KDM6A and epigenetic reprogramming in human papillomavirus type 16 E6/E7-expressing keratinocytes. J Virol 2011;85(21):10999–1006.

[175] Wentzensen N, Ridder R, Klaes R, Vinokurova S, Schaefer U, Doeberitz MK. Characterization of viral-cellular fusion transcripts in a large series of HPV16 and 18 positive anogenital lesions. Oncogene 2002;21(3):419–26.

[176] Jeon S, Allen-Hoffmann BL, Lambert PF. Integration of human papillomavirus type 16 into the human genome correlates with a selective growth advantage of cells. J Virol 1995;69(5):2989–97.

[177] Jeon S, Lambert PF. Integration of human papillomavirus type 16 DNA into the human genome leads to increased stability of E6 and E7 mRNAs: implications for cervical carcinogenesis. Proc Natl Acad Sci U S A 1995;92(5):1654–8.

[178] Pett MR, Alazawi WO, Roberts I, Dowen S, Smith DI, Stanley MA, et al. Acquisition of high-level chromosomal instability is associated with integration of human papillomavirus type 16 in cervical keratinocytes. Cancer Res 2004;64(4):1359–68.

[179] Häfner N, Driesch C, Gajda M, Jansen L, Kirchmayr R, Runnebaum IB, et al. Integration of the HPV16 genome does not invariably result in high levels of viral oncogene transcripts. Oncogene 2008;27(11):1610–7.

[180] Vinokurova S, Wentzensen N, Kraus I, Klaes R, Driesch C, Melsheimer P, et al. Type-dependent integration frequency of human papillomavirus genomes in cervical lesions. Cancer Res 2008;68(1):307–13.

[181] Matsukura T, Koi S, Sugase M. Both episomal and integrated forms of human papillomavirus type 16 are involved in invasive cervical cancers. Virology 1989;172(1):63–72.

[182] Fehrmann F. Laimins LA.Human papillomaviruses: targeting differentiating epithelial cells for malignant transformation. Oncogene 2003;22(33): 5201–7.

[183] Badaracco G, Venuti A, Sedati A, Marcante ML. HPV16 and HPV18 in genital tumors: Significantly different levels of viral integration and correlation to tumor invasiveness. J Med Virol 2002;67(4):574–82.

[184] Woodman CB, Collins S, Rollason TP, Winter H, Bailey A, Yates M, et al. Human papillomavirus type 18 and rapidly progressing cervical intraepithelial neoplasia. Lancet 2003;361(9351):40–3.

[185] Cullen AP, Reid R, Campion M, Lörincz AT. Analysis of the physical state of different human papillomavirus DNAs in intraepithelial and invasive cervical neoplasms. J Virol 1991;65:606–12.

[186] Pirami L, Giache V, Becciolini A. Analysis of HPV16, 18, 31, and 35 DNA in pre-invasive and invasive lesions of the uterine cervix. J Clin Pathol 1997;50(7):600–4.

[187] Go C, Schwartz MR, Donovan DT. Molecular transformation of recurrent respiratory papillomatosis: viral typing and p53 overexpression. Ann Otol Rhinol Laryngol 2003;112(4):298–302.

[188] Rady PL, Schnadig VJ, Weiss RL, Hughes TK, Tyring SK. Malignant transformation of recurrent respiratory papillomatosis associated with integrated human papillomavirus type 11 DNA and mutation of p53. Laryngoscope 1998;108(5):735–40.

[189] DiLorenzo TP, Tamsen A, Abramson AL, Steinberg BM. Human papillomavirus type 6a DNA in the lung carcinoma of a patient with recurrent laryngeal papillomatosis is characterized by a partial duplication. J Gen Virol 1992;73(Pt 2):423–8.

[190] Pisani P, Bray F, Parkin DM. Estimates of the world-wide prevalence of cancer for 25 sites in the adult population. Int J Cancer 2002;97(1):72–81.

[191] Arnold AW, Hofbauer GF. Human papillomavirus and squamous cell cancer of the skin–epidermodysplasia verruciformis-associated human papillomavirus revisited. Curr Prob Dermatol 2012;43:49–56.

[192] Pfister H. Chapter 8: Human papillomavirus and skin cancer. J Natl Cancer Inst Monogr 2003;(31):52–6.

[193] Arron ST, Ruby JG, Dybbro E, Ganem D, Derisi JL. Transcriptome sequencing demonstrates that human papillomavirus is not active in cutaneous squamous cell carcinoma. J Invest Dermatol 2011;131(8):1745–53.

[194] Muschik D, Braspenning-Wesch I, Stockfleth E, Rösl F, Hofmann TG, Nindl I. Cutaneous HPV23 E6 prevents p53 phosphorylation through interaction with HIPK2. PLoS One 2011;6(11):e27655.

[195] Jackson S, Storey A. E6 proteins from diverse cutaneous HPV types inhibit apoptosis in response to UV damage. Oncogene 2000;19(4):592–8.
[196] Underbrink MP, Howie HL, Bedard KM, Koop JI, Galloway DA. E6 proteins from multiple human betapapillomavirus types degrade Bak and protect keratinocytes from apoptosis after UVB irradiation. J Virol 2008;82(21):10408–17.
[197] Viarisio D, Mueller-Decker K, Kloz U, Aengeneyndt B, Kopp-Schneider A, Gröne HJ, et al. E6 and E7 from beta HPV38 cooperate with ultraviolet light in the development of actinic keratosis-like lesions and squamous cell carcinoma in mice. PLoS Pathog 2011;7(7):e1002125.
[198] Borgogna C, Zavattaro E, De Andrea M, Griffin HM, Dell'Oste V, Azzimonti B, et al. Characterization of beta papillomavirus E4 expression in tumours from Epidermodysplasia Verruciformis patients and in experimental models. Virology 2012;423(2):195–204.
[199] Chen Z, Schiffman M, Herrero R, Desalle R, Burk RD. Human papillomavirus (HPV) types 101 and 103 isolated from cervicovaginal cells lack an E6 open reading frame (ORF) and are related to gamma-papillomaviruses. Virology 2007;360(2):447–53.
[200] Nobre RJ, Herráez-Hernández E, Fei JW, Langbein L, Kaden S, Gröne HJ, et al. E7 oncoprotein of novel human papillomavirus type 108 lacking the E6 gene induces dysplasia in organotypic keratinocyte cultures. J Virol 2009;83(7):2907–16.
[201] Schiffman M, Rodriguez AC, Chen Z, Wacholder S, Herrero R, Hildesheim A, et al. A population-based prospective study of carcinogenic human papillomavirus variant lineages, viral persistence, and cervical neoplasia. Cancer Res 2010;70(8):3159–69.
[202] Koshiol JE, Schroeder JC, Jamieson DJ, Marshall SW, Duerr A, Heilig CM, et al. Time to clearance of human papillomavirus infection by type and human immunodeficiency virus serostatus. Int J Cancer 2006;119(7):1623–9.
[203] de Sanjose S, Quint WG, Alemany L, Geraets DT, Klaustermeier JE, Lloveras B, et al. Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study. Lancet Oncol 2010;11(11):1048–56.
[204] Herfs M, Yamamoto Y, Laury A, Wang X, Nucci MR, McLaughlin-Drubin, et al. A discrete population of squamocolumnar junction cells implicated in the pathogenesis of cervical cancer. Proc Natl Acad Sci U S A 2012;109(26):10516–21.
[205] Nicholls PK, Klaunberg BA, Moore RA, Santos EB, Parry NR, Gough GW, et al. Naturally occurring, nonregressing canine oral papillomavirus infection: host immunity, virus characterization, and experimental infection. Virology 1999;265(2):365–74.
[206] Nicholls PK, Moore PF, Anderson DM, Moore RA, Parry NR, Gough GW, et al. Regression of canine oral papillomas is associated with infiltration of CD4+ and CD8+ lymphocytes. Virology 2001;283(1):31–9.
[207] Wilgenburg BJ, Budgeon LR, Lang CM, Griffith JW, Christensen ND. Characterization of immune responses during regression of rabbit oral papillomavirus infections. Comp Med 2005;55(5):431–9.
[208] Monnier-Benoit S, Mauny F, Riethmuller D, Guerrini JS, Căpîlna M, Félix S, et al. Immunohistochemical analysis of CD4+ and CD8+ T-cell subsets in high risk human papillomavirus-associated pre-malignant and malignant lesions of the uterine cervix. Gynecologic Oncol 2006;102(1):22–31.
[209] Stanley MA. Epithelial cell responses to infection with human papillomavirus. Clin Microbiol Rev 2012;25(2):215–22.
[210] Kanodia S, Fahey LM, Kast WM. Mechanisms used by human papillomaviruses to escape the host immune response. Curr Canc Drug Targ 2007;7(1):79–89.
[211] Li S, Labrecque S, Gauzzi MC, Cuddihy AR, Wong AH, Pellegrini S, et al. The human papilloma virus (HPV)-18 E6 oncoprotein physically associates with Tyk2 and impairs Jak-STAT activation by interferon-alpha. Oncogene 1999;18(42):5727–37.
[212] Nees M, Geoghegan JM, Hyman T, Frank S, Miller L, Woodworth CD. Papillomavirus type 16 oncogenes downregulate expression of interferon-responsive genes and upregulate proliferation-associated and NF-kappaB-responsive genes in cervical keratinocytes. J Virol 2001;75(9):4283–96.
[213] Perea SE, Massimi P, Banks L. Human papillomavirus type 16 E7 impairs the activation of the interferon regulatory factor-1. Int J Mol Med 2000;5(6):661–6.
[214] Um SJ, Rhyu JW, Kim EJ, Jeon KC, Hwang ES, Park JS. Abrogation of IRF-1 response by high-risk HPV E7 protein in vivo. Cancer Lett 2002;179(2):205–12.
[215] Caberg JH, Hubert PM, Begon DY, Herfs MF, Roncarati PJ, Boniver JJ, et al. Silencing of E7 oncogene restores functional E-cadherin expression in human papillomavirus 16-transformed keratinocytes. Carcinogenesis 2008;29(7):1441–7.
[216] Matthews K, Leong CM, Baxter L, Inglis E, Yun K, Bäckström BT, et al. Depletion of Langerhans cells in human papillomavirus type 16-infected skin is associated with E6-mediated down regulation of E-cadherin. J Virol 2003;77(15):8378–85.
[217] Ashrafi GH, Haghshenas M, Marchetti B, Campo MS. E5 protein of human papillomavirus 16 downregulates HLA class I and interacts with the heavy chain via its first hydrophobic domain. Int J Cancer 2006;119(9):2105–12.
[218] Merad M, Ginhoux F, Collin M. Origin, homeostasis and function of Langerhans cells and other langerin-expressing dendritic cells. Nature reviews Immunology 2008;8(12):935–47.
[219] Bedoui S, Whitney PG, Waithman J, Eidsmo L, Wakim L, Caminschi I. Cross-presentation of viral and self antigens by skin-derived CD103+ dendritic cells. Nature Immunol 2009;10(5):488–95.
[220] Zhang P, Nouri M, Brandsma JL, Iftner T, Steinberg BM. Induction of E6/E7 expression in cottontail rabbit papillomavirus latency following UV activation. Virology 1999;263(2):388–94.
[221] Maran A, Amella CA, Di Lorenzo TP, Auborn KJ, Taichman LB, Steinberg BM. Human papillomavirus type 11 transcripts are present at low abundance in latently infected respiratory tissues. Virology 1995;212(2):285–94.
[222] Abramson AL, Nouri M, Mullooly V, Fisch G, Steinberg BM. Latent Human Papillomavirus infection is comparable in the larynx and trachea. J Med Virol 2004;72(3):473–7.
[223] Kalantari M, Garcia-Carranca A, Morales-Vazquez CD, Zuna R, Montiel DP, Calleja-Macias IE, et al. Laser capture microdissection of cervical human papillomavirus infections: copy number of the virus in cancerous and normal tissue and heterogeneous DNA methylation. Virology 2009;390(2):261–7.
[224] Gravitt PE. The known unknowns of HPV natural history. J Clin Invest 2011;121(12):4593–9.
[225] Welters MJ, de Jong A, van den Eeden SJ, van der Hulst JM, Kwappenberg KM, Hassane S, et al. Frequent display of human papillomavirus type 16 E6-specific memory t-Helper cells in the healthy population as witness of previous viral encounter. Cancer Res 2003;63(3):636–41.
[226] Ades S, Koushik A, Duarte-Franco E, Mansour N, Arseneau J, Provencher D, et al. Selected class I and class II HLA alleles and haplotypes and risk of high-grade cervical intraepithelial neoplasia. Int J Cancer 2008;122(12):2820–6.
[227] Sheu BC, Chiou SH, Chang WC, Chow SN, Lin HH, Chen RJ, et al. Integration of high-risk human papillomavirus DNA correlates with HLA genotype aberration and reduced HLA class I molecule expression in human cervical carcinoma. Clin Immunol 2005;115(3):295–301.
[228] Zoodsma M, Nolte IM, Te Meerman GJ, De Vries EG, Van der Zee AG. HLA genes and other candidate genes involved in susceptibility for (pre)neoplastic cervical disease. Int J Oncol 2005;26(3):769–84.
[229] Muñoz N, Bosch FX, Castellsagué X, Díaz M, de Sanjose S, Hammouda D, et al. Against which human papillomavirus types shall we vaccinate and screen? The international perspective. Int J Cancer 2004;111(2):278–85.
[230] Geraets D, Alemany L, Guimera N, de Sanjose S, de Koning M, Molijn A, et al. Detection of rare and possibly carcinogenic human papillomavirus genotypes as single infections in invasive cervical cancer. Unpublished data.