

Review

The papillomavirus life cycle

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Abstract

Papillomaviruses infect epithelial cells, and depend on epithelial differentiation for completion of their life cycle. The expression of viral gene products is closely regulated as the infected basal cell migrates towards the epithelial surface. Expression of E6 and E7 in the lower epithelial layers drives cells into S-phase, which creates an environment that is conducive for viral genome replication and cell proliferation. Genome amplification, which is necessary for the production of infectious virions, is prevented until the levels of viral replication proteins rise, and depends on the co-expression of several viral proteins. Virus capsid proteins are expressed in cells that also express E4 as the infected cell enters the upper epithelial layers. The timing of these events varies depending on the infecting papillomavirus, and in the case of the high-risk human papillomaviruses (HPVs), on the severity of neoplasia. Viruses that are evolutionarily related, such as HPV1 and canine oral papillomavirus (COPV), generally organize their productive cycle in a similar way, despite infecting different hosts and epithelial sites. In some instances, such as following HPV16 infection of the cervix or cottontail rabbit papillomavirus (CRPV) infection of domestic rabbits, papillomaviruses can undergo abortive infections in which the productive cycle of the virus is not completed. As with other DNA tumour viruses, such abortive infections can predispose to cancer.

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Abbreviations: HPV, human papillomavirus; BPV, bovine papillomavirus; COPV, canine oral papillomavirus; CRPV, cottontail rabbit papillomavirus; PML, promyelocytic leukaemia; NMSC, non-melanoma skin cancer

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1. Diversity amongst human papillomaviruses

Papillomaviruses are a diverse group of viruses that have been found in more than 20 different mammalian species, as well as in birds and reptiles. Because of their medical importance, the human papillomaviruses (HPV) have been most extensively studied, and more than 100 different types have now been identified (Bernard, 2005). Although papillomavirus classification is based on nucleotide sequence homology, the differences between evolutionary groups are reflected to some extent, in the differences that exist in the biology of the different viruses. Genitally transmitted HPV types are contained within supergroup A (also known as Alpha papillomaviruses) (de Villiers et al., 2004; Myers et al., 1994) and viruses from this group, such as HPV6 and 11, are major sexually transmitted pathogens that are thought to affect around 1% of the sexually active population (Brentjens et al., 2002). These viruses can also infect oral sites where they are generally associated with benign papillomas. By contrast, the high-risk viruses from supergroup A, such as HPV16 and 18, cause mucosal lesions that can progress in some individuals to high-grade neoplasia and cancer (Bosch et al., 2002; Walboomers et al., 1999). Although viruses from supergroup A also include members whose primary tropism is for cutaneous sites, such as HPV2 or HPV10, these viruses share life cycle features that do not extend to papillomaviruses from other evolutionary groups (Middleton et al., 2003; Peh et al., 2002). HPV2 and closely related supergroup A papillomaviruses are the primary cause of common warts.

The second major group of human papillomaviruses are contained within supergroup B. Viruses from the B1 subgroup such as HPV5 (also known as Beta papillomaviruses) (de Villiers et al., 2004; Myers et al., 1994) cause inapparent or latent infections in the general population, but can become a problem in immuno-suppressed individuals and in individuals who have an inherited defect (Ramoz et al., 2002) which renders them susceptible to infection by papillomaviruses from the B1 supergroup. Such patients can develop skin cancers at the site of HPV infection, and it is thought that B1 viruses may be involved in the development of non-melanoma skin cancer (NMSC) in the general population (Harwood et al., 2004). By contrast, viruses from the B2 subgroup such as HPV4 (also known as Gamma papillomaviruses; de Villiers et al., 2004), cause cutaneous warts in the general population that can superficially resemble those caused by supergroup A papillomaviruses such as HPV2.

The remaining group of HPVs are contained within supergroup E (Myers et al., 1994) (also classified as Mu and Nu-papillomaviruses (de Villiers et al., 2004)). Only three human members from this group are known, and all cause cutaneous papillomas in the general population. HPV1 is the most well studied member of this group, and like HPV2 in supergroup A, causes verrucas and palmar warts.

2. Problems in developing a general model of HPV-associated disease

It is apparent from the above overview that different HPVs have evolved to fill different biological niches, and that in some instances, viruses from different evolutionary groups may be able to target the same epithelial site. Despite this apparent heterogeneity amongst HPVs, they all share certain features that allow them to produce infectious virions following infection. All known HPVs are exclusively epitheliotropic, and unlike certain animal papillomavirus types such as bovine papillomavirus type 1 (BPV1) or BPV2, they do not infect or express their gene products in the underlying dermis. Similarly, all produce infectious particles in the upper epithelial layers, although there appear to be differences in the extent of virus synthesis depending on whether transmission is through direct contact (e.g., genital warts), or whether it occurs indirectly (e.g., verrucas) (Middleton et al., 2003; Peh et al., 2002). While we have a basic understanding of how papillomaviruses cause disease, it is becoming apparent that the evolutionary backgrounds of the different viruses, their site of infection, and their mode of transmission must all be considered if the general model is to be applied to particular HPV types. Differences in regulatory sequences and coding potential within the viral genome are likely to underlie the significant differences that are apparent in the biology of different papillomavirus types.

3. Organization of the HPV life cycle

Most work on HPVs has centred on the analysis of the high-risk HPV types and in particular on HPV16, which is the primary cause of cervical cancer. From these studies and from the analysis of related HPV types (including HPV11 and HPV1), a general pattern of viral gene expression has been worked out (Fig. 1) that can, with modification, be applied to human papillomaviruses from other groups.

3.1. Infection and uncoating

Initial infection requires access of infectious particles to cells in the basal layer, which for some HPV types is thought to require a break in the stratified epithelium. Such breaks may not be readily apparent, and may occur under conditions where the skin is exposed to water or is abraded (e.g., swimming pool surfaces), or is subjected to other environments where micro traumas may develop. It has been suggested that for a lesion to be maintained, the virus must infect an epithelial stem cell (Egawa, 2003; Schmitt et al., 1996). In cutaneous skin, such stem cells are abundant within the hair follicle, and for viruses of the B1 supergroup (which are prevalent but which cause inapparent infections), the hair follicles may represent an important site of entry. Several studies have shown that DNA of viruses from the B1 supergroup can be readily amplified by PCR from plucked

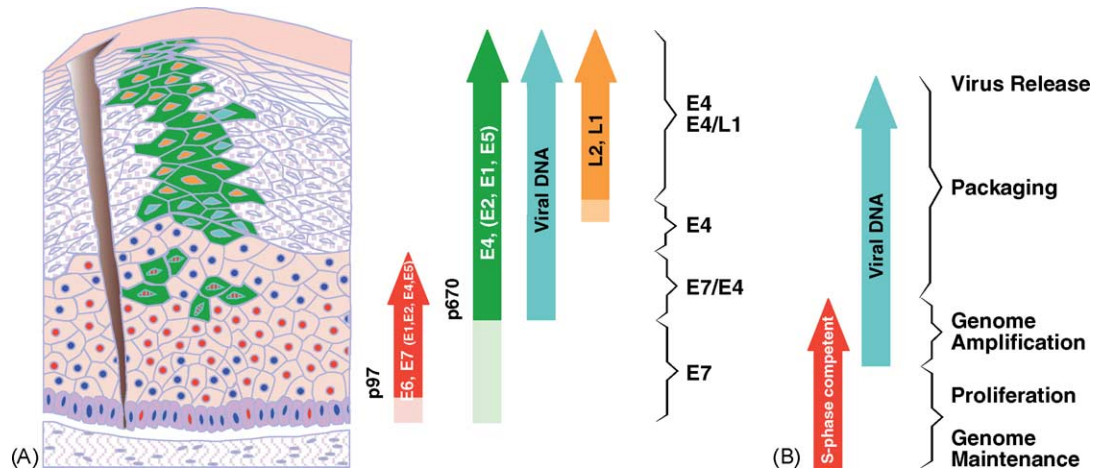


Fig. 1. Life cycle organization during productive infection by HPV types from supergroup A. (A) Diagrammatic representation of the skin to reveal the pattern of HPV16 gene expression as the infected cell migrates towards the epithelial surface. Other supergroup A viruses, such as HPV2 and HPV11 follow a similar pattern. After infection (in this case through a cut), the viral genome is maintained as a low copy number episome. During epithelial differentiation, the p97 promoter directs expression of the E6 and E7 genes necessary for S-phase entry (red). The p670 promoter is up-regulated in the higher epithelial layers, and viral replication proteins (E1, E2, E4, E5) increase in abundance (green), facilitating amplification of viral genomes (blue). Changes in mRNA splicing allow E4 to persist into the upper epithelial layers where the viral capsid proteins (yellow) are found. (B) Cells in the lower epithelial layers are S-phase competent. Viral genome amplification begins in these cells but ceases once the cells lose their ability to express S-phase proteins. Although amplified viral genomes can be detected throughout the upper epithelial layers, cells that are actively supporting genome amplification appear confined to a region where E7 expression coincides with the high-level expression of E4, and probably also, an increase in the abundance of E1 and E2. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

hair follicles (Boxman et al., 2001). For high-risk mucosal viruses such as HPV16, the formation of cervical lesions may be facilitated by infection of columnar cells, which can subsequently go on to form the basal layer of the stratified epithelium of the transformation zone. Controversy exists as to the nature of the cell surface receptor that allows initial attachment of the virus to the cell, although most studies have suggested a dependence on the presence of heparin sulphate (Giroglou et al., 2001; Joyce et al., 1999). Recent work has suggested that the internalization of bound virions is a slow process with a half-life of hours rather than minutes, and that it occurs through the endocytosis of clathrin coated vesicles (Culp and Christensen, 2004; Day et al., 2003; Selinka et al., 2002). Papillomavirus uncoating may be facilitated by the disruption of intracapsomeric disulphide bonds in the reducing environment of the cell (Li et al., 1998) allowing viral DNA to be transported into the nucleus.

3.2. Genome maintenance

Following infection and uncoating, it is thought that the virus maintains its genome as a low copy number episome in the basal cells of the epithelium. The pattern of viral gene expression in these cells is not well defined, but it is generally thought that the viral E1 and E2 proteins are expressed in order to maintain the viral DNA as an episome (Wilson et al., 2002) and to facilitate the correct segregation of genomes during cell division (You et al., 2004). Failure to express the full length E1 protein in the context of the HPV31 genome, prevents episomal maintenance, and in cultured epithelial

cells leads to the integration of viral genomes into the host cell chromosome (Fratini et al., 1996). Whether the viral ‘transforming’ proteins (E6 and E7) are also expressed in cells of the basal layer is not certain (Crum et al., 1988), although it appears that initial infection is followed by a proliferative-phase that results in an increase in the number of basal cells harbouring viral episomes. The number of viral genomes, and the pattern of viral gene expression in cell lines derived from low-grade cervical lesions appears to reflect those found in the basal layer of naturally-occurring lesions. It has been suggested that the viral genome is maintained in the basal layer at around 10–200 copies per cell, and that viral early proteins (E6, E7, E1 and E2) are expressed at low level (De Geest et al., 1993; Stanley et al., 1989). The contribution of E6 and E7 to basal cell proliferation during *in vivo* infection is currently uncertain, and it has been suggested that expression of E1 (and possibly also E2) may be sufficient for the basal maintenance of viral episomes (Zhang et al., 1999).

3.3. Proliferative-phase

In uninfected epithelium, basal cells exit the cell cycle soon after migrating into the suprabasal cell layers and undergo a process of terminal differentiation. Changes include the physical cross-linking of keratin intermediate filaments, the formation of cornified envelopes, and the secretion of lipids, which together allow the epithelial surface to form a physical barrier against the environment (Madison, 2003). During papillomavirus infection, E7 (and presumably also

E6) is expressed in these cells, the restraint on cell cycle progression is abolished and normal terminal differentiation is retarded (Sherman et al., 1997). E6 and E7 are thought to work together to achieve these effects, and in lesions caused by high-risk HPV types (such as HPV16), the two proteins are expressed from a bicistronic mRNA (Stacey et al., 2000) expressed from the viral early promoter (p97). Both E6 and E7 have functions that stimulate cell cycle progression and both can associate with regulators of the cell cycle (Munger et al., 2001). The association of E7 with members of the pocket protein family such as pRb is well characterized. pRb is a negative regulator of the cell cycle that normally prevents S-phase entry by associating with the E2F family of transcription factors. E7 binding to pRb displaces E2F, irrespective of the presence of external growth factors, and leads to the expression of proteins necessary for DNA replication. E7 can also associate with other proteins involved in cell proliferation, including histone deacetylases (Longworth and Laimins, 2004), components of the AP-1 transcription complex (Antinore et al., 1996), and the cyclin-dependent kinase inhibitors p21 and p27 (Funk et al., 1997). Despite the ability of E7 to stimulate cell proliferation, during productive infection only a subset of cells in the parabasal layers are mitotically active. The expression of cyclin E is absolutely necessary for S-phase entry, and is expressed during natural infection as a result of E7 expression and disruption of the E2F/pRb complex. In differentiating epithelial cells, however, the high levels of cyclin-dependent kinase inhibitors (p21cip1 and p27kip1) can lead to the formation of inactive complexes that contain E7, cyclinE/cdk2 and either p21 or p27 (Noya et al., 2001). It appears that during natural infection, the ability of E7 to stimulate S-phase progression is limited to the subset of differentiated cells with low levels of p21/p27, or which express high enough levels of E7 to overcome the block to S-phase entry. The viral E6 protein complements the role of E7, and is thought to prevent the induction of apoptosis in response to unscheduled S-phase entry mediated by E7. Although the association of E6 with p53, and the inactivation of p53-mediated growth suppression and/or apoptosis has been well documented, E6 can also associate with other pro-apoptotic proteins including Bak (Thomas and Banks, 1998) and Bax (Li and Dou, 2000). As a consequence, the presence of E6 is considered a predisposing factor in the development of HPV-associated cancers, allowing the accumulation of chance errors in host cell DNA to go unchecked. The E6 protein of high-risk HPV types can also stimulate cell proliferation independently of E7 through its C-terminal PDZ-ligand domain (Thomas et al., 2002). E6-PDZ binding is sufficient to mediate suprabasal cell proliferation (Nguyen et al., 2003a,b) and may contribute to the development of metastatic tumours by disrupting normal cell adhesion. In addition to E6 and E7, it is thought that the other viral early proteins (i.e., E1, E2, E4 and E5) are expressed prior to the onset of genome amplification in order to ensure maintenance of the viral episome at low copy-number (Middleton et al., 2003).

3.4. Genome amplification

For the production of infectious virions, papillomaviruses must amplify their viral genomes and package them into infectious particles. For supergroup A, HPV types such as HPV16, HPV11 or HPV2, this occurs in the mid or upper epithelial layers following an increase in activity of the late (differentiation dependent) promoter. The late promoter resides within the E7 open reading frame and it is thought that its up-regulation leads to increased expression of proteins involved in viral DNA replication (i.e., E1, E2, E4 and E5) without directly affecting expression of the E6 and E7 proteins that are necessary for S-phase entry (Middleton et al., 2003). Amplification of viral genomes begins in a subset of cells in the proliferative compartment and requires expression of all viral early gene products including E4 (Peh et al., 2004) and E5 (Fehrmann et al., 2003; Genther et al., 2003), whose roles in replication are not yet fully understood. The binding of E2 to the HPV upstream regulatory region is necessary for viral DNA replication, and recruits the E1 DNA helicase to the viral origin of replication. The assembly of the E1/E2 initiation complex on the viral origin is analogous to the formation of the complex between cellular initiation proteins (cdc6 and MCMs) on cellular origins, and may allow the replication of viral genomes to proceed in the absence of cellular DNA synthesis. Throughout the virus life cycle, the relative levels of different viral proteins are controlled by promoter usage and by differential splice site selection, with an increase in the level of E1 and E2 allowing an increase in viral copy number in the upper epithelial layers (Ozbun and Meyers, 1998a). The molecular mechanisms that lead to activation of the late promoter and up-regulation of E1/E2 expression are not yet well understood, and it remains possible that this promoter is constitutively active at all stages during the productive cycle. Current models suggest that a modest increase in promoter activation during differentiation may lead to an increase in the level of E1 and E2 (and also E4 and E5), and a subsequent increase in genome copy number. The newly replicated genomes would serve as templates for the further expression of E1 and E2, which would facilitate additional amplification of viral genomes and in turn, further expression of the E1 and E2 replication proteins (Middleton et al., 2003).

3.5. Virus synthesis

Papillomaviruses encode two structural proteins that are expressed in the upper layers of infected tissue once viral genome amplification has been completed (Ozbun and Meyers, 1998b). L2 is a minor coat protein that like L1 is produced in a subset of the cells that express E4 (Doorbar et al., 1997). The major capsid protein (L1) is expressed after L2 allowing the assembly of infectious particles in the upper layers of the epithelium (Florin et al., 2002). Papillomavirus particles comprise an approximately 8000 base pair genome within a capsid that contains 360 copies of the L1 protein,

and probably 12 copies of L2, organized into a 72 capsomere icosahedral shell (Modis et al., 2002). The L2 protein accumulates at nuclear structures known as PML bodies during virus assembly (possibly through association with the transcription factor Daxx (Becker et al., 2004)) and recruits L1 to these domains. It has been suggested that PML bodies may be the sites of papillomavirus DNA replication (Day et al., 1998; Swindle et al., 1999), and that capsid proteins accumulate at these sites to facilitate packaging. Although virus like particles can assemble in the absence of L2, the L2 protein is thought to enhance packaging (Stauffer et al., 1998; Zhou et al., 1993) and infectivity (Roden et al., 2001). To be successful, the virus must eventually escape from the infected skin cell and survive extra-cellularly prior to re-infection. Papillomaviruses are non-lytic, and are not released until the infected cells reach the epithelial surface. Papillomaviruses are resistant to desiccation (Roden et al., 1997) and their extra-cellular survival may be enhanced if they are shed from the epithelial surface within a cornified squame (Bryan and Brown, 2001). The intracellular retention of papillomavirus antigens until the cell reaches the uppermost epithelial layers may compromise the immune detection of the virus, particularly as the virus also has molecular mechanisms that limit the presentation of viral epitopes to the immune system in the lower epithelial layers (Ashrafi et al., 2002; Marchetti et al., 2002; Matthews et al., 2003). Although the expression of viral proteins can inhibit expression of differentiation markers preventing the formation of normal cornified squames (Doorbar et al., 1997), it has also been suggested that the viral E4 protein may contribute directly to virus egress in the upper epithelial layer by disturbing keratin integrity (Doorbar et al., 1991; Wang et al., 2004) and by affecting the assembly of the cornified envelope (Bryan and Brown, 2000; Lehr et al., 2004).

4. Life cycle organization amongst HPVs of different type

Although all papillomaviruses must follow the broad pattern of events described above in order to produce infectious virions, different strategies of productive infection are apparent between the different evolutionary groups. Human papillomaviruses from the B2 supergroup such as HPV4 for instance, do not contain the LXCXE motif necessary for pRB association (Munger et al., 2001) in their E7 protein, suggesting that at a molecular level they may operate differently from viruses of supergroup A, such as HPV2, which cause lesions at similar sites. Similarly, the E4 protein of HPV4 appears to lack the classical keratin binding motif that is present in HPV1 (E supergroup) and HPV2 (A supergroup) (Doorbar, 1996), despite infecting cutaneous epithelium, and sharing the same requirements as other HPV types regarding escape from the cornified squame. Comparative analysis of papillomaviruses of different types has shown the E1 and L1 regions to be the most highly conserved (de Villiers et al., 2004). It

appears that these ORFs are fundamental for the survival of all papillomaviruses, and that they were probably present in the ancestor of modern papillomaviruses. Despite the diversity amongst papillomaviruses, it appears that viruses from related evolutionary groups share certain similarities. This can be illustrated by comparing papillomaviruses contained within the E supergroup (such as HPV 1) with those from the A group (such as HPV2), both of which cause verrucas (Fig. 2). In the former group, which includes canine oral papillomavirus (COPV), genome amplification begins as soon as cells leave the basal layer, without the intervening proliferative-phase characteristic of viruses such as HPV2 or HPV11 (Middleton et al., 2003). It has been speculated that these differences may reflect differences in transmission routes of the different HPV types and the need to produce the appropriate number of virus particles to allow infection without stimulating immunity against infection. It is equally possible that the co-evolution of papillomaviruses with their hosts has led to adoption of different general strategies to achieve the same goal.

5. Regression of lesions and virus latency

Although genome amplification and packaging is necessary for the formation of new virions, infection can have other outcomes. Experimental inoculation of rabbits with ROPV, or the inoculation of dogs with COPV generally leads to the development of lesions that can persist for weeks rather than years (Christensen et al., 2000; Nicholls et al., 2001). Lesions produced by ROPV and COPV resemble in many respects those produced by HPVs, and these viruses have been proposed as models to study mucosal HPV infection in humans. Between weeks 8 and 12, lymphocyte infiltration and lesion regression takes place, and by 16 weeks, the infected area has the appearance of uninfected epithelium (Nicholls et al., 2001). A similar pattern has been reported in cattle (Knowles et al., 1996) and may also occur in humans under some circumstances (Coleman et al., 1994). The importance of the immune system in controlling the spread of HPV-associated disease is well established, and patients with immune defects are particularly susceptible to infection, and can develop widespread lesions that are refractory to treatment. HPV infections are a particular problem in renal transplant and other immunosuppressed patients, in HIV infected individuals, and in some patients with genetic defects that affect immune cell function. The failure of patients suffering from Epidermodysplasia Verruciformis (Ramos et al., 2002) or certain forms of severe combined immunodeficiency syndrome (Laffort et al., 2004) to control infection implicates a role for specific pathways of immune regulation. The inapparent infections associated with viruses of the B1 supergroup may resemble infections by other HPV types once they have been brought under control by the host immune system (Stern, 2005). The frequent detection of HPV16 DNA in cervical lesions in the absence of any obvious disease, may be explained by its

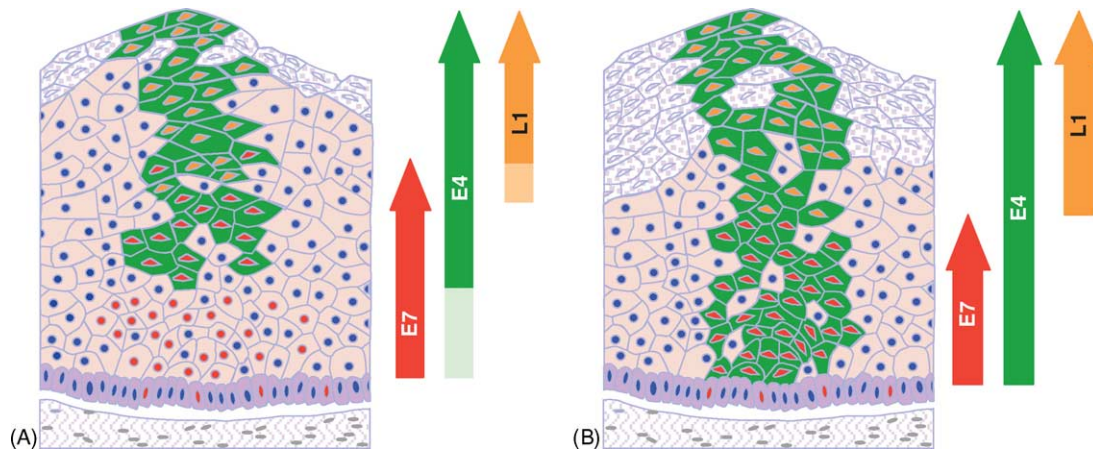


Fig. 2. Differences in life cycle organization amongst evolutionarily distinct HPV Types. When compared to HPVs from supergroup A such as HPV2 or HPV11 (left panel labelled A), those from supergroup E such as HPV1 or HPV63 (right panel labelled B), begin their productive cycle close to the basal layer. In lesions caused by these HPV types, there is no separate compartment where E7 can be detected in the absence of E4, and viral genome amplification begins in the parabasal cell layers (Peh et al., 2002).

presence in a latent state, with only very few cells (if any) able to support the productive cycle during epithelial cell differentiation. Following immune regression, papillomavirus DNA is thought to remain in the basal epithelial cells and to be reactivated when levels of immunosurveillance decline. The pattern of viral gene expression in basal cell layers during latent infection may be similar to the expression pattern in these layers during productive infection. It has been suggested that latent gene expression is restricted to E1 and E2 and that during this phase of the virus life cycle, the E6 and E7 genes are not required (Zhang et al., 1999).

6. Productive infection, abortive infection and HPV-associated cancers

In the absence of regression, lesions may persist, and may in some instances progress to cancer. A common characteristic of tumour viruses is their ability to cause tumours

at sites where their productive life cycle cannot be completed. This general characteristic appears to hold true for papillomavirus-associated cancers, such as those caused by cottontail rabbit papillomavirus (CRPV) in domestic rabbits, and by BPV 1 in horses (Campo, 2002). High-risk HPVs from supergroup A have been associated with human cervical cancers (Walboomers et al., 1999) whereas viruses from the B supergroup (particularly B1) have been implicated in the development of non-melanoma skin cancer (Harwood et al., 2004). When compared to the prevalence of HPV infections in the general population, the number of lesions that progress to cancer is very low. High-risk papillomaviruses from the A supergroup infect genital sites in men and women and cause flat lesions at cervical sites. In women, who do not successfully resolve their infection, such lesions can progress to cervical intraepithelial neoplasia grade 1 (CIN1), and may progress further to CIN2, CIN3 and cancer (Steenbergen, 2005; Gross and Barrasso, 1997; Peto et al., 2004). Low-grade cervical lesions (CIN1) resemble productive infections

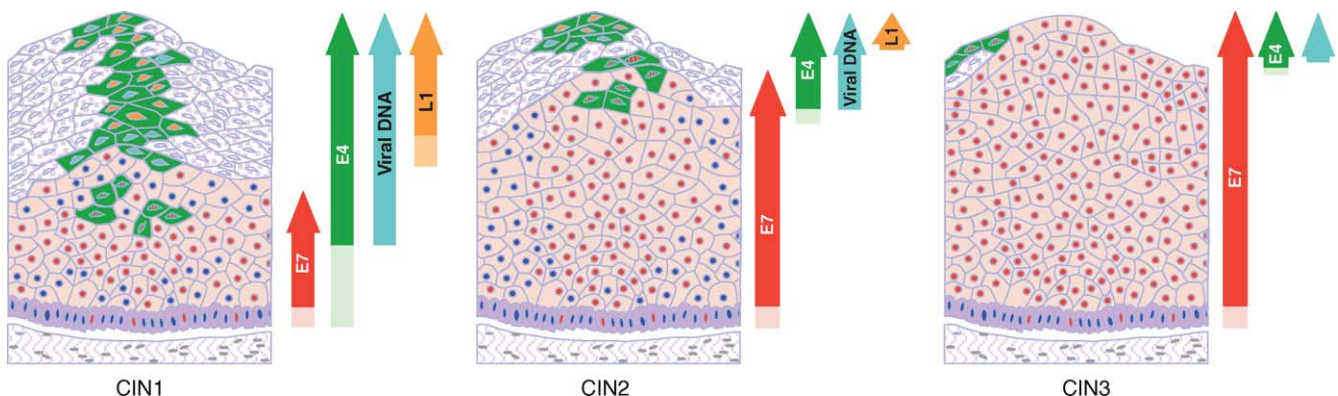


Fig. 3. Changes in the HPV16 life cycle during the development of cervical cancer. During progression from cervical intraepithelial neoplasia grade 1 (CIN1) to CIN3, normal regulation of the papillomavirus life cycle is lost. CIN1s generally resemble productive lesions caused by other supergroup A HPV types, and express virus coat proteins at the epithelial surface. In CIN2 and CIN3 lesions, the order of life cycle events is unchanged, but the extent of E7 expression is increased.

caused by related HPV types, while high-grade lesions such as CIN2 and CIN3 have a more extensive proliferative-phase, with the productive stages of the virus life cycle being supported only poorly (Middleton et al., 2003). The key event in the progression of productive lesions to high-grade neoplasia may result from a deregulation in the expression of the viral transforming proteins (E6 and E7), which leads to increased cell proliferation in the lower epithelial layers and an inability to repair secondary mutations in the host cell DNA (von Knebel Doeberitz, 2002). It is generally thought that the transformation zone is a particularly susceptible site for cervical cancer to develop. It appears that high-risk HPV types such as HPV16 cannot reliably complete their life cycle at this site, occasionally leading to abortive infection (Fig. 3). The progression from CIN3 to cancer usually occurs in lesions that contain integrated copies of the viral genome in which E7 expression is elevated. Thereafter, retention of the E6 and E7 genes and loss of the E2 and E4 genes, which can exert a negative effect on cell growth, usually accompanies the development of invasive cervical cancer. Although other HPV types such as those from the B1 supergroup are also associated with human cancers (i.e., non-melanoma skin cancer), in these cases, the integration of HPV sequences into host cell genome is not necessary, and viral sequences are not universally retained (Jablonska and Majewski, 1994; Harwood et al., 2004).

7. Life cycle organization amongst animal papillomaviruses

The general concepts that relate to the life cycles of human papillomaviruses appear to be applicable to the animal systems that are used to study infection (Peh et al., 2002). In many instances, animal papillomaviruses fall into evolutionary groups that contain no human members suggesting that they have been following an evolutionary path that is distinct from that followed by the HPVs (de Villiers et al., 2004). An exception to this are viruses from the B supergroup, which are widespread in animals (Antonsson and Hansson, 2002), and viruses from the E supergroup, which have been found in rabbits, cats and dogs. The medically important viruses from the A supergroup appear restricted to primates. With the exception of bovine papillomaviruses (BPV), the natural history of infection in animals has not been extensively studied. BPV1 and BPV2 are fibropapillomaviruses and produce lesions that have underlying dermal involvement. BPV1 causes cutaneous warts in its natural host, but induces fibroblastic tumours in horses. Although these viruses have been extensively studied because of their ability to transform cells in culture, they are evolutionarily distinct from the papillomaviruses that cause tumours in humans. The second important group of bovine papillomaviruses includes BPV4, which can induce gastrointestinal tumours that can progress to cancer in cattle that have bracken fern in their diet (Campo, 2002). As with other papillomavirus-induced cancers, it is thought that infection leads to an expansion in the number

of dividing cells, and that in the presence of co-carcinogens (quercetin in bracken fern), the accumulation of secondary genetic changes can lead to cancer. In the case of human viruses from the B1 supergroup, the co-factor has been identified as UV light, whereas for HPV-associated cervical lesions, metabolites from smoking present an increased risk of cancer progression.

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References

- Antinore MJ, Birrer MJ, Patel D, Nader L, McCance DJ. The human papillomavirus type 16 E7 gene product interacts with and transactivates the AP1 family of transcription factors. *EMBO J* 1996;15:1950–60.
- Antonsson A, Hansson BG. Healthy skin of many animal species harbors papillomaviruses, which are closely related to their human counterparts. *J Virol* 2002;76:12537–42.
- Ashrafi GH, Tsirimonaki E, Marchetti B, O'Brien PM, Sibbet GJ, Andrew L, et al. Down-regulation of MHC class I by bovine papillomavirus E5 oncoproteins. *Oncogene* 2002;21:248–59.
- Becker KA, Florin L, Sapp C, Maul GG, Sapp M. Nuclear localization but not PML protein is required for incorporation of the papillomavirus minor capsid protein L2 into virus-like particles. *J Virol* 2004;78:1121–8.
- Bernard HU. The clinical importance of the nomenclature, evolution and taxonomy of human papillomaviruses. *J Clin Virol* 2005;32S:S1–S6.
- Bosch FX, Lorincz A, Munoz N, Meijer CJ, Shah KV. The causal relation between human papillomavirus and cervical cancer. *J Clin Pathol* 2002;55:244–65.
- Boxman IL, Russell A, Mulder LH, Bavinck JN, ter Schegget J, Green A. Association between epidermodysplasia verruciformis-associated human papillomavirus DNA in plucked eyebrow hair and solar keratoses. *J Invest Dermatol* 2001;117:1108–12.
- Brentjens MH, Yeung-Yue KA, Lee PC, Tyring SK. Human papillomavirus: a review. *Dermatol Clin* 2002;20:315–31.
- Bryan JT, Brown DR. Association of the human papillomavirus type 11 E1(E4) protein with cornified cell envelopes derived from infected genital epithelium. *Virology* 2000;277:262–9.
- Bryan JT, Brown DR. Transmission of human papillomavirus type 11 infection by desquamated cornified cells. *Virology* 2001;281:35–42.
- Campo MS. Animal models of papillomavirus pathogenesis. *Virus Res* 2002;89:249–61.
- Christensen ND, Cladel NM, Reed CA, Han R. Rabbit oral papillomavirus complete genome sequence and immunity following genital infection. *Virology* 2000;269:451–61.
- Coleman N, Birley HDL, Renton AM, Hanna NF, Ryait BK, Byrne M, et al. Immunological events in regressing genital warts. *Am J Clin Pathol* 1994;102:768–74.
- Crum CP, Nuovo G, Friedman D, Silverstein SJ. Accumulation of RNA homologous to human papillomavirus type 16 open reading frames in genital precancers. *J Virol* 1988;62:84–90.

- Culp TD, Christensen ND. Kinetics of in vitro adsorption and entry of papillomavirus virions. *Virology* 2004;319:152–61.
- Day PM, Lowy DR, Schiller JT. Papillomaviruses infect cells via a clathrin-dependent pathway. *Virology* 2003;307:1–11.
- Day PM, Roden RBS, 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;72:142–50.
- De Geest K, Turyk ME, Hosken MI, Hudson JB, Laimins LA, Wilbanks GD. Growth and differentiation of human papillomavirus type 31b positive human cervical cell lines. *Gynecol Oncol* 1993;49:303–10.
- de Villiers EM, Fauquet C, Broker TR, Bernard HU, zur Hausen H. Classification of papillomaviruses. *Virology* 2004;324:17–27.
- Doorbar J. The E4 proteins and their role in the viral life cycle. In: Lacey C, editor. *Papillomavirus reviews: current research on papillomaviruses*. Leeds: Leeds Medical Information, Leeds University Press; 1996. p. 31–8.
- 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;352:824–7.
- Doorbar J, Foo C, Coleman N, Medcalf E, Hartley O, Prospero T, et al. Characterisation of events during the late stages of HPV16 infection in vivo using high affinity synthetic fabs to E4. *Virology* 1997;238:40–52.
- Egawa K. Do human papillomaviruses target epidermal stem cells? *Dermatology* 2003;207:251–4.
- 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;77:2819–31.
- Florin L, Sapp C, Streeck RE, Sapp M. Assembly and translocation of papillomavirus capsid proteins. *J Virol* 2002;76:10009–14.
- Frattini MG, Lim HB, Laimins LA. In vitro synthesis of oncogenic human papillomaviruses requires episomal genomes for differentiation-dependent late gene expression. *Proc Natl Acad Sci* 1996;93:3062–7.
- Funk JO, Waga S, Harry JB, Espling E, Stillman B, Galloway DA. Inhibition of CDK activity and PCNA-dependent DNA replication by p21 is blocked by interaction with the HPV16 E7 oncoprotein. *Genes and Development* 1997;11:2090–100.
- Genther SM, Sterling S, Duensing S, Munger 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;77:2832–42.
- Giroglou T, Florin L, Schafer F, Streeck RE, Sapp M. Human papillomavirus infection requires cell surface heparan sulfate. *J Virol* 2001;75:1565–70.
- Gross GE, Barrasso R, editors. *Human papillomavirus infection: a clinical atlas*. Berlin: Ullstein Mosby; 1997.
- Harwood CA, Suretheran T, Sasieni P, Proby CM, Bordea C, Leigh IM, et al. Increased risk of skin cancer associated with the presence of epidermodysplasia verruciformis human papillomavirus types in normal skin. *Br J Dermatol* 2004;150:949–57.
- Jablonska S, Majewski S. Epidermodysplasia verruciformis: immunological and clinical aspects. In: zur Hausen H, editor. *Human pathogenic papillomaviruses*. Heidelberg: Springer-Verlag; 1994.
- Joyce JG, Tung JS, Przysiecki CT, Cook JC, Lehman ED, Sands JA, et al. The L1 major capsid protein of human papillomavirus type 11 recombinant virus-like particles interacts with heparin and cell-surface glycosaminoglycans on human keratinocytes. *J Biol Chem* 1999;274:5810–22.
- Knowles G, O'Neil BW, Campo MS. Phenotypical characterization of lymphocytes infiltrating regressing papillomas. *J Virol* 1996;70:8451–8.
- Laffort C, Le Deist F, Favre M, Caillat-Zucman S, Radford-Weiss I, Debre M, et al. Severe cutaneous papillomavirus disease after haemopoietic stem-cell transplantation in patients with severe combined immune deficiency caused by common gamma cytokine receptor subunit or JAK-3 deficiency. *Lancet* 2004;363:2051–4.
- Lehr E, Hohl D, Huber M, Brown D. Infection with human papillomavirus alters expression of the small proline rich proteins 2 and 3. *J Med Virol* 2004;72:478–83.
- Li B, Dou QP. Bax degradation by the ubiquitin/proteasome-dependent pathway: involvement in tumor survival and progression. *Proc Natl Acad Sci* 2000;97:3850–5.
- Li M, Beard P, Estes PA, Lyon MK, Garcea RL. Intercapsomeric disulfide bonds in papillomavirus assembly and disassembly. *J Virol* 1998;72:2160–7.
- Longworth MS, Laimins LA. The binding of histone deacetylases and the integrity of zinc finger-like motifs of the E7 protein are essential for the life cycle of human papillomavirus type 31. *J Virol* 2004;78:3533–41.
- Madison KC. Barrier function of the skin: “la raison d’être” of the epidermis. *J Invest Dermatol* 2003;121:231–41.
- Marchetti B, Ashrafi GH, Tsirimonaki E, O'Brien PM, Campo MS. The bovine papillomavirus oncoprotein E5 retains MHC class I molecules in the Golgi apparatus and prevents their transport to the cell surface. *Oncogene* 2002;21:7808–16.
- Matthews K, Leong CM, Baxter L, Inglis E, Yun K, Backstrom 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;77:8378–85.
- Middleton K, Peh W, Southern SA, Griffin HM, Sotlar K, Nakahara T, et al. Organisation of the human papillomavirus productive cycle during neoplastic progression provides a basis for the selection of diagnostic markers. *J Virol* 2003;77:10186–201.
- Modis Y, Trus BL, Harrison SC. Atomic model of the papillomavirus capsid. *EMBO J* 2002;21:4754–62.
- Munger K, Basile JR, Duensing S, Eichten A, Gonzalez SL, Grace M, et al. Biological activities and molecular targets of the human papillomavirus E7 oncoprotein. *Oncogene* 2001;20:7888–98.
- Myers G, Bernard H-U, Delius H, Favre M, Icenogel J, Van Ranst M, Wheeler C, editors. *Human papillomaviruses a compilation and analysis of nucleic acid and amino acid sequences*. 1st ed. Los Alamos, USA: Theoretical Biology and biophysics group T-10, Los Alamos National Laboratory; 1994.
- Nguyen ML, Nguyen MM, Lee D, Griep AE, Lambert PF. The PDZ ligand domain of the human papillomavirus type 16 E6 protein is required for E6's induction of epithelial hyperplasia in vivo. *J Virol* 2003a;77:6957–64.
- Nguyen MM, Nguyen ML, Caruana G, Bernstein A, Lambert PF, Griep AE. Requirement of PDZ-containing proteins for cell cycle regulation and differentiation in the mouse lens epithelium. *Mol Cell Biol* 2003b;23:8970–81.
- 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;283:31–9.
- Noya F, Chien W-M, Broker TR, Chow LT. p21cip1 degradation in differentiated keratinocytes is abrogated by costabilization with cyclin E induced by human papillomavirus E7. *J Virol* 2001;75:6121–34.
- Ozbun MA, Meyers C. Human papillomavirus type 31b E1 and E2 transcript expression correlates with vegetative viral genome amplification. *Virology* 1998a;248:218–30.
- Ozbun MA, Meyers C. Temporal usage of multiple promoters during the life cycle of human papillomavirus type 31b. *J Virol* 1998b;72:2715–22.
- 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 (CRPV) productive cycle in vivo. *J Virol* 2004;78:2142–51.
- Peh WL, Middleton K, Christensen N, Nicholls P, Egawa K, Sotlar K, et al. Life cycle heterogeneity in animal models of human papillomavirus-associated disease. *J Virol* 2002;76:10401–16.
- Peto J, Gilham C, Fletcher O, Matthews FE. The cervical cancer epidemic that screening has prevented in the UK. *Lancet* 2004;364:249–56.

- 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;32:579–81.
- Roden RB, Day PM, Bronzo BK, Yutzy WH, Yang Y, Lowy DR, et al. Positively charged termini of the L2 minor capsid protein are necessary for papillomavirus infection. *J Virol* 2001;75:10493–7.
- Roden RB, Lowy DR, Schiller JT. Papillomavirus is resistant to desiccation. *J Infect Dis* 1997;176:1076–9.
- 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;70:1912–22.
- Selinka HC, Giroglou T, Sapp M. Analysis of the infectious entry pathway of human papillomavirus type 33 pseudovirions. *Virology* 2002;299:279–87.
- Sherman L, Jackman A, Itzhaki H, Stoppler MC, Koval D, Schlegel R. Inhibition of serum- and calcium-induced differentiation of human keratinocytes by HPV16 E6 oncoprotein: role of p53 inactivation. *Virology* 1997;237:296–306.
- Stacey SN, Jordan D, Williamson AJ, Brown M, Coote JH, Arrand JR. Leaky scanning is the predominant mechanism for translation of human papillomavirus type 16 E7 oncoprotein from E6/E7 bicistronic mRNA. *J Virol* 2000;74:7284–97.
- Stanley MA, Browne HM, Appleby M, Minson AC. Properties of a non-tumorigenic human cervical keratinocyte cell line. *Int J Cancer* 1989;43:672–6.
- Stauffer Y, Raj K, Masternak K, Beard P. Infectious human papillomavirus type 18 pseudovirions. *J Mol Biol* 1998;283:529–36.
- Steenbergen RDM, de Wilde J, Wilting, SM, Brink AATP, Snijders PJF, Meijer CJLM. HPV-mediated transformation of the anogenital tract. *J Clin Virol* 2005;32S:S25–S33.
- Stern PL. Immune control of human papillomavirus (HPV) associated anogenital disease and potential for vaccination. *J Clin Virol* 2005;32S:S72–S81.
- Swindle CS, Zou N, Van Tine BA, Shaw GM, Engler JA, Chow LT. Human papillomavirus DNA replication compartments in a transient DNA replication system. *J Virol* 1999;73:1001–9.
- Thomas M, Banks L. Inhibition of bak-induced apoptosis by HPV-18 E6. *Oncogene* 1998;17:2943–54.
- Thomas M, Laura R, Hepner K, Guccione E, Sawyers C, Lasky L, et al. Oncogenic human papillomavirus E6 proteins target the MAGI-2 and MAGI-3 proteins for degradation. *Oncogene* 2002;21:5088–96.
- von Knebel Doeberitz M. New markers for cervical dysplasia to visualise the genomic chaos created by aberrant oncogenic papillomavirus infections. *Eur J Cancer* 2002;38:2229–42.
- Walboomers J, Jacobs M, Manos MM, Bosch F, Kummer J, Shah K, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J Pathol* 1999;189:12–9.
- Wang Q, Griffin H, Southern S, Jackson D, Martin A, McIntosh P, et al. Functional analysis of the human papillomavirus type 16 E1E4 protein provides a mechanism for in vivo and in vitro keratin filament re-organisation. *J Virol* 2004;78:821–33.
- Wilson VG, West M, Woytek K, Rangasamy D. Papillomavirus E1 proteins: form, function, and features. *Virus Genes* 2002;24:275–90.
- You J, Croyle JL, Nishimura A, Ozato K, Howley PM. Interaction of the bovine papillomavirus E2 protein with Brd4 tethers the viral DNA to host mitotic chromosomes. *Cell* 2004;117:349–60.
- 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;263:388–94.
- Zhou J, Stenzel DJ, Sun XY, Frazer IH. Synthesis and assembly of infectious bovine papillomavirus particles in vitro. *J Gen Virol* 1993;74:763–8.