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Papillomaviruses

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Abstract

Papillomaviruses are a large family of small deoxyribonucleic acid (DNA) tumour viruses that cause hyperproliferative warts of cutaneous and mucosal epithelium. These viruses are ubiquitous in the animal kingdom and infect reptiles, birds and mammals and probably orginated ~350 million years ago. Subsets of human papillomaviruses (HPVs), referred to as 'high-risk' types are associated with anogenital cancers (uterine cervix, vulva, vagina, anus, penis), oropharyngeal cancers, and skin cancers. The papillomavirus life cycle is strictly dependent on the terminal differentiation programme of the host cell - the keratinocyte. The virus deregulates host cell cycle control and inactivates the host cells's antiproliferative response in order to reprogramme the differentiating cells to support viral replication. In the case of high-risk viruses, this is achieved by interactions with important tumour repressor pathways. Two prophylactic vaccines (Gardasil and Cervarix) are available to control infection by the most prevalent HPV types found in anogenital and oropharyngeal cancers.

Keywords: human papillomavirus; animal papillomavirus; cervical cancer; head and neck cancer; viral oncogenes; E5; E6; E7; epidemiology; papillomas; genital warts; vaccines.

Key Concepts:

- Papillomaviruses show strong species and tissue restriction and they share a similar genetic organization.
- Papillomaviruses are small double-stranded (ds) DNA viruses that replicate as non-integrated episomes in keratinocytes and are dependent on the keratinocyte undergoing terminal differentiation in order to complete their life cycle.
- Low-risk viruses such as HPV 6 and 11 are associated with the formation of anogenital warts and laryngeal papillomaviruses that have a low-risk of progression to cancer.
- The most severe impact of human papillomavirus infection in humans is that a small proportion of infections progress to anogenital, oropharyngeal and skin cancers.
- High-risk viruses such as HPV16 and HPV18 are associated with infections of the mucosa lining the anogenital and oropharyngeal tract and HPV16 is the most prevalent genotype in cancers arising at these sites.
- A majority of HPV infections are naturally eliminated by host cell-mediated immunity and HPV infections are especially common in immunocompromised indivuals (e.g. HIV-infected patients, organ-recipient patients, predisposing genetic conditions [e.g. epidermoldysplasia verrucciformis]).
- These viruses are a common sexually transmitted disease (STD) and infection in the oropharynx is most likely via an oral-genital route.
- High-risk HPV early proteins E5, E6 and E7 are oncoproteins and the expression of E6 and E7 is retained in all cancers; E6 and E7 deregulate tumour suppressor pathways leading to host genomic instability.

- Papillomaviruses replicate as extrachromosomal plasmids in the nulei of infected cells, but during cancer progression the viral genome often becomes integrated into the host chromosome, resulting in a loss of the negative feedback control of viral oncogene expression by E2.
- Prophylactic vaccines protect against infection with vaccine-associated HPV types by inducing neutralizing antibodies that recognise epitopes on the major capsid protein.

Introduction

Papillomaviruses are a diverse and intriguing group of small deoxyribonucleic acid (DNA) viruses that cause hyperproliferative warts of cutaneous and mucosal epithelium. Numerous different papillomaviruses may infect a single species, and more than 180 *human papillomavirus* (HPV) genotypes have been identified. The most severe impact of HPV infection in humans is that a small proportion of lesions caused by the 'high-risk' genotypes progress to anogenital, oropharyngeal and skin cancers. Many other HPV infections of the mucosa and skin lead to benign but unsightly warts, although some benign lesions can cause life-threatening disease, such as laryngeal papillomatosis. During their life cycle, the assembly of new infectious papillomavirus progeny is restricted to the differentiated layers of the epithelium; a characteristic that has hampered the growth of the virus in tissue culture systems and as such has been a barrier to understanding the molecular biology of the virus life cycle. The situation has been much improved with the study of animal papillomaviruses and from the development of cell-based models that recapitulate the complete virus life cycle. A key feature of papillomavirus replication is the necessity to access the host cell's DNA synthesis machinery in order to replicate the viral DNA genome and to do so in differentiating cells the virus drives these cells to proliferate. There are distinct differences in the nature of virus - host interactions formed in these cells between the high-risk cancer-causing and the low-risk viruses and these differences explain their varied pathogenesis. The development of prophylactic vaccines to control the infection of some of the most prevalent high-risk viruses that infect the mucosa has been one of the major breakthroughs in controlling papillomavirus infection of humans.

Classification

All papillomaviruses are nonenveloped, contain ds circular DNA and assemble in the nucleus of the infected cell. The papillomavirus DNA is associated with host cell histones or histone-like proteins and is encapsidated by 72 pentameric capsomeres arranged on a skewed icosahedral lattice (Figure 1). The viral genome consists of between 7.3 and 8.0 kilobase pairs, and contains 8–10 open reading frames (ORFs) which code for early (E) and late (L) viral proteins (Figure 2). Viral transcription initiates from one DNA strand only and extensive splicing of viral ribonucleic acid (RNA) occurs. See also Viral Capsids and Envelopes: Structure and Function (DOI:10.1002/978047000015902.a0001091.pub2) and Transcriptional Gene Regulation in Eukaryotes (DOI:10.1002/9780470015902.a0002322.pub2).

Papillomaviruses belong to the *Papillomaviridae* family (de Villiers et al., 2004). The complete DNA sequence has been determined for about 183 human papillomaviruses and 130 animal papillomaviruses (http://pave.niad.nih.gov). New

papillomaviruses are being isolated and characterized each year and the rate of their discovery has increased with the use of metagenomic sequencing. All papillomavirus genomes have a very similar organization of viral genes and regulatory elements (Figure 2). The nucleotide sequences of the major coat protein (L1) have been used to establish phylogenetic trees by DNA sequence comparisons (Bernard et al., 2010). A papillomavirus type has been operationally defined as having a nucleotide sequence that differs from the homologous nucleotide sequence of every other papillomavirus type by at least 10%. Isolates of the same type are referred to as 'subtypes' or 'variants', and differ from each other usually by less than 5% at the nucleotide level. Papillomaviruses have been divided into different genera each given a letter of the Greek alphabet and subdivided further into intragenus species. The two major groups containing HPVs are represented as the Alpha (α) - papillomaviruses (which contain the majority of those types associated with mucosal infections) and the Beta (β) - papillomaviruses that contain mostly viruses found in cutaneous infections of patients with a rare genetic disorder known as epidermodysplasia verruciformis (EV) or those with other immunosuppressive disorders and conditions.

The first complete papillomavirus sequence was established for *Bovine papillomavirus type 1* (BPV-1) in 1982 (Chen et al., 1982). Complete genomic sequences are available for papillomaviruses that infect a diverse range of species including cattle, horses, rabbits, dogs, cats, rodents, sheep, bats, reptiles, marine mammals, birds, nonhuman primates. Sequence comparisons between human and animal papillomaviruses indicate that diversity between types is increased with increasing evolutionary distances between hosts. These observations suggest a hypothesis of an ancient papillomavirus–host association, originating some 350 million years ago (Bernard, 2013). See also Viral Classification and Nomenclature (DOI:10.1002/9780470015902.a0000440.pub3) and Virus Evolution (DOI:10.1002/9780470015902.a0000436.pub3).

Virus Life Cycle

The papillomavirus life cycle is confined to, and completed within, the differentiating epithelial keratinocyte, and infectious virions are produced in the fully differentiated upper layers (Doorbar et al., 2012) (Figure 2). Papillomaviruses are dependent on the host cell for provision of the enzymes and replication factors necessary to synthesize the viral DNA, but since these factors are not expressed in the post-mitotic differentiating keratinocytes an important part of the life cycle is the reactivation of their expression by stimulating cell cycle re-entry of differentiating cells. However, the virus is also dependent on cellular factors present in differentiating cells for expression of the virus late genes and assembly of new progeny and as such the virus needs to achieve a balance between cell proliferation and differentiation. The nature of the virus – host interactions engaged in this process has a bearing on the risk of infections induced by the highrisk HPV types progressing to malignancy. During the virus life cycle, the three viral oncogenes, E5, E6 and E7, are involved in the stimulation of epithelial cell hyperproliferation leading to the classic raised appearance of most warts. E6 also counteracts the host cell's antiproliferative responses to enable survival of the infected cells. The two early gene products, E1 and E2 regulate viral DNA replication and E2 also controls the transcription of the viral oncogenes. The E4 gene product supports a cellular environment conducive to viral genome amplification. Two late genes, L1 and L2, make up the coat proteins of the papillomavirus capsid and are only expressed in the upper cell layers. It has been suggested that E4 contributes to virion release from the fully differentiated, virus-laden squames by compromising cellular integrity through disruption of the cytokeratin network. A small noncoding region (400–800 bp) between the late gene L1 and the early gene E6 contains numerous regulatory elements, including the

replication origin (Figure 1). See also Cornification of the Skin: a non apoptotic cell death mechanism (DOI:10.1002/9780470015902.a0021583) and DNA Plant and Animal Virus Replication (DOI:10.1038/npg.els.0001018).

Types of Human Papillomas

Papillomas associated with HPV infections have been detected in numerous sites of the body but are confined to cutaneous and internal mucosal regions. In addition, HPV DNA has been detected in many human cancers, primarily in cervical, vulval, vaginal, penile and anal cancers, various cutaneous cancers including nonmelanoma skin cancer, and in subset of cancers of the head and neck region.

Cutaneous warts (verrucae)

Perhaps the most familiar of all HPV infections are the benign common warts of the hands and feet. Typical common hand warts (verrucae vulgaris) are often found on the backs of the hands and fingers of small children, and are dome-shaped with multiple conical projections described as papillomatosis (Figure 3). This particular morphological feature provides the descriptive name for the wart viruses. Histological features of verruca vulgaris include hyperkeratosis (thickening of the corneal layer) and papillary epidermal hyperplasia (acanthosis). The upper epidermis contains koilocytes – enlarged keratinocytes with pyknotic nuclei surrounded by a clear halo (Figure 3). Deep plantar warts (verrucae plantaris) on the soles of the feet can be found in adults as well as children. Flat warts of the hands, feet and face (verrucae plana) are also grouped with the common warts. Almost all of these infections are eliminated by host cell-mediated immunity, leading to wart regression and clearance of the papillomas. The majority of benign cutaneous warts harbour HPV genotypes from the *gamma*, *mu* (e.g. *HPV1*) and *nu* genera. A few genotypes commonly associated with skin warts (e.g. *HPV2*) are defined as α-papillomaviruses.

Ano-Genital warts (condyloma acuminata, Buschke–Löwenstein tumour, Bowen disease, bowenoid papulosis)

The most important group of HPV infections are the genital or venereal warts. These lesions have been subcategorized into flat, inverted and papillary condylomas. Disease associated with genital HPV infections also includes carcinoma *in situ* of the uterine cervix (see below). The most recognizable of the genital warts are the benign condyloma acuminata. These infections can be large and appear on the external genitalia of both sexes, as well as internally in the vaginal wall, vagina and perianal canal. The predominant HPV types of condyloma acuminatum are the so-called ‘low-risk’ HPV types 6 and 11. Giant condylomas (Buschke–Löwenstein tumour) also involve penile and anal sites and predominantly contain HPV-6 and -11. These latter lesions are locally invasive, but rarely metastasize.

Carcinoma *in situ* (includes Bowen disease and bowenoid papulosis) of anogenital sites often contain HPV DNA. Cervical intraepithelial neoplasia (CIN) associated with HPV infections of ‘intermediate to high-risk’ HPV types typically begin as

flat or inverted condylomas (condyloma planum; CIN I). Grades of CIN identify increasing numbers of undifferentiated malignant cells and a decrease in normal epithelial cell differentiation. CIN III, therefore, morphologically describes severe dysplasia and carcinoma *in situ*. More than 60 different HPV types have been found in genital infections, and approximately 30 types have been detected in anogenital cancers. The most common genotypes associated with over 70 % of cervical cancers are HPV-16 (61%) and HPV-18 (10%) (de Sanjose et al., 2010).

Oral and laryngeal papillomas

A small but significant number of patients present with papillomas of the mucosa of the ororespiratory tract. Especially difficult to cure are papillomas of the larynx that occur in children and adults; the children becoming infected *in utero* or during birth itself. Although laryngeal papillomas are associated with low-risk HPV-6 and -11, these usually benign infections can cause life-threatening disease by obstruction of airways, and from the debilitating effects of frequent treatments for recurrences. Patients suffering from recurret respiratory papillomatosis can go on to develop cancer, especially if the disease progresses into the lower airways, including the lungs.

Macular lesions

Cutaneous flat papillomas known as macular lesions appear on patients with a rare autosomal recessive disease, EV, and have yielded many different HPV types. Many of these lesions progress to malignant squamous cell carcinomas (Jablonska et al., 1972), and the predominant HPV types in such cancers are the β-papillomaviruses HPV-5 and -8. Patients with EV tend to have depressed cell-mediated immunity and although the cause of this immunosuppression is uncertain, these individuals have mutations in one of two genes (*EVER1/TMC6* and *EVER2/TCM8*) whose function is linked to zinc homeostasis which is important in T cell activation (Ramoz et al., 2002). In addition, the EV-associated HPV types are also detected in nonmelanoma skin cancers of immunosuppressed patients (de Villiers et al., 1997).

Subclinical or latent infections

An important category of HPV-associated disease is subclinical or latent infection. It is becoming increasingly clear that not all HPV infections permanently manifest as macroscopic disease. Subclinical infections represent a significant reservoir of infection for (1) host-to-host transmissions, (2) future sites for macroscopic disease following reactivation by a variety of environmental cofactors (immune suppression, carcinogens, stress-related effects, hormonal changes, exposure to ultraviolet light, wounding and coincidental interactions with other STD agents, to name a few) and (3) future sites for malignant progression following reactivation. A model of papillomavirus latency based on animal studies describes a scenario in which the viral DNA is maintained at very low copy number in a stem (or stem cell-like) cell in the basal layer accompanied by low level viral gene activity. Reactivation of latent infections, particularly those involving high-risk HPV types, upon waning of host immune surveillance may contribute to disease-burden in an aging population (Maglennon et al., 2011).

Types of Animal Papillomas

Animal papillomavirus infections parallel HPV infections in cutaneous and mucosal tissue distribution. Several broad subcategories of animal papillomas can be defined: ungulate fibropapillomas, cutaneous papillomas and oral and genital papillomas. Cutaneous and mucosal animal papillomaviruses display features in common with human counterparts, including tissue distribution, genomic organization of viral genes, viral gene functions and ultrastructural identity of capsids. It is worth noting that in s small number of animal papillomaviruses the genomic structure does deviate from normal organization and two papilloma-polyomavirus hybrids have been detected in bandicoots which contain a papillomavirus late region and a region containing polyomavirus early proteins. The genome of the first laboratory mouse papillomavirus (*Mus musculus* type 1, *MusPV1*) was isolated and sequenced in 2011 and is considered an important discovery for the development of small animal models of papillomavirus infection (Ingle et al., 2011). The earliest animal models of papillomavirus infections include bovine fibropapillomas, the Shope rabbit papilloma and canine oral papillomatosis and the study of these, as well as the more recent model of Rhesus macaque anogenital infection, has provided many important observations regarding virus infection and life cycle, pathogenesis, host immunological responses and vaccine efficiacy. See also Polyomaviruses (DOI:10.1038/npg.els.0001082).

Bovine papillomaviruses

One unique feature of BPV-1 and other ungulate fibropapillomaviruses is their ability to infect fibroblasts as well as epithelial cells. A cell culture model for viral infection, viral DNA replication and viral RNA synthesis using BPV-1 has provided considerable information on the early events of the papillomavirus life cycle and viral gene function. BPV-4 is a mucosotropic papillomavirus that causes alimentary cancers in cattle, especially following consumption of bracken fern shown to contain carcinogenic substances.

Shope rabbit papilloma

Cutaneous Shope rabbit papillomas contain *Cottontail rabbit papillomavirus type 1* and are indigenous to cottontail rabbits of the Midwestern plains states of the United States. This model system was the first to demonstrate a link between papillomavirus infection and progression to malignant squamous cell carcinoma (Rous and Beard, 1935) and continues to be of value in studies on virus–host interactions.

Canine oral papillomatosis

Dogs infected with *Canine oral papillomavirus* provide a model to examine papillomavirus infections in mucosal tissues of intact hosts. Natural transmission is common, and vaccines can be assessed for protection against both experimental and natural infection (Stanley et al., 2001).

Rhesus macaque anogenital papillomas

Rhesus macaques are the only species except humans in which mucosatropic papillomaviruses cause cervical neoplasia. *Rhesus papillomavirus type 1* (RHPV-1) is closely related to the high risk virus HPV16. The sexual transmission of RHPV-1 and disease development in the animal resemble high-risk HPV infection in all major characteristics making it an excellent model for understanding the pathogenesis, treatment and prophylaxis of genital cancer development.

Mechanism of Oncogenesis

A role for HPVs in human anogenital cancers was suggested in the 1970s by Harald zur Hausen (zur Hausen, 1977) and several years later his laboratory cloned HPV16 sequences from invasive cervical cancers (Durst et al., 1983). His discovery of high-risk HPV as the aeitological cause of cervical cancer was recognized by the award of the Nobel Prize for Physiology or Medicine in 2008. Cloning and sequencing of papillomavirus genomes has provided opportunities to test transforming functions of papillomavirus genomes as well as individual viral genes. Interestingly, high-risk HPV types immortalize cultures of human keratinocytes, whereas low-risk HPV types do not. *In vitro* cell transformation assays and animal models of carcinogenesis have been used to show that three early viral genes E5, E6 and E7 play a major role in papillomavirus-mediated transformation leading to oncogenesis (Figure 4). See also Oncogenic Viruses (DOI:10.1002/9780470015902.a0000421.pub2) and Tumour Suppressor Genes (DOI:10.1002/9780470015902.a0001475.pub2).

E5

BPV-1 was shown to transform fibroblast cell lines in the early 1960s. Molecular cloning, sequencing and mutational analysis of the BPV-1 genome identified a small, hydrophobic, 44-amino acid protein (E5) as the major transforming protein of BPV-1. The E5 proteins of HPVs show no sequence similarity with BPV-1 E5 and have only weak transforming activity, but there is a correlation between the presence of the *E5* gene in the HPV genome and carcinogenic potential. Further research has shown that the membrane-bound E5 proteins cause hyperactivation of mitogenic signalling pathways and lead to overproliferation of HPV infected cells. BPV-1 E5 protein binds to the platelet-derived growth factor receptor and activates it in the absence of ligand, whereas HPV E5 proteins stimulates the epidermal growth factor (EGF) pathway and this association involves upregulation of nondegraded EGF receptors at the cell surface. HPV E5 also modulates the trafficking of other membrane-bound proteins (e.g. FAS and MHC proteins) leading to reduced apoptosis and immune evasion. Perturbation of endocytic trafficking of proteins is likely to involve the ability of E5 to bind to the 16-kDa subunit of the vacuolar H^+-ATPase (adenosine triphosphatase) (Goldstein et al., 1991) and by acting as a viral ion channel (also known as a viroporin) (Wetherill et al., 2012). Also see Protein Kinases:Signatures in Cancer (DOI:10.1002/9780470015902.a0000659.pub2).

E6

E6 proteins are 16–19 kDa in size, contain two zinc-binding domains and are present in both the nucleus and cytoplasm. E6 functions affect global cellular transcription, inhibit keratinocyte differentiation and apoptotic pathways and induce cell

proliferation. The first potential mechanism of E6 transforming ability was the demonstration of an association of E6 with the tumour suppressor protein p53 (Werness et al., 1990). E6-associated p53 complexes are targeted for degradation via the ubiquitin pathway, a function that is dependent on E6 binding to the cellular ubiquitin ligase E6-AP (Scheffner et al., 1990). Residual p53 protein is inactivated by E6 targeting of several members of a family of transcriptional regulators (histone acetyltransferases p300/CBP, ADA3, TIP60). Loss of the apoptosis promoting protein p53 is hypothesized to relax DNA repair and thus allows for an accumulation of genetic mutations that cause immortalization and transformation. However, there is a general consensus that E6 proteins mediate their transforming activity via both p53-dependent and p53-independent mechanisms. Further research has shown that E6 engages with a number of proteins involved in cell survival pathways and include several members of a family of apoptosis-promoting proteins such as BAK (*Bcl-2* homologous antagonist/killer) and several interactions with components of the TNF (tumour necrosis factor) - mediated apoptotic pathway. E6 proteins target a number of PDZ domain-containing proteins via a short PDZ-binding peptide sequence (Kiyono et al., 1997). The most well-studied of these are interactions with discs large 1 and Scribble, both components of the cell polarity control machinery; a cellular process that is deregulated in epithelial cancers (Banks et al., 2012). E6-mediated proteasome degradation of these targets is linked to hyperproliferation and acquisition of phenotypes associated with metastasis. Potential discriminating activity between E6 proteins from high- and low-risk HPV types has been found for some of these interactions (e.g. p53 and PDZ proteins). See also Apoptosis: Molecular Mechanisms (DOI:10.1002/9780470015902.a0001150.pub2), P53 and Cell Death (DOI:10.1002/9780470015902.a0021824), The BCL-2 Family Proteins – Key Regulators and Effectors of Apoptosis (DOI:10.1002/9780470015902.a0021568) and Apoptosis and the Cell Cycle in Human Disease (DOI:10.1002/9780470015902.a0006043).

Additional actions of E6 proteins include activation of the telomerase enzyme through multiple pathways including the modulation of transcriptional repressors and activators of the enzyme. Telomerase adds repeat sequences to chromosome ends and is believed to play a role in cellular immortalization. See also Telomerase: Structure and Function (DOI:10/1002/9780470015902.a0006167.pub2) and Telomeres in Cell Function: Cancer and Ageing (DOI:10.1002/9780470015902.a0001168.pub2).

E7

Papillomavirus E7 proteins are approximately 100 amino acids in size, contain two zinc-binding motifs and a region that shows homology with other viral oncogenes. They disrupt the functions of host cell-cycle regulatory proteins, including interactions with the tumour suppressor protein pRB and other ‘pocket proteins’, p107 and p130. Together these interactions overide the G1-S checkpoint leading to reactivation of host DNA replication machinery in postmitotic, differentiated keratinocytes, and to increased viral DNA synthesis (Cheng et al., 1995). Another E7 activity is to allow host cells with DNA damage to avoid cell cycle checkpoint controls and E7 can target and inactivate the host proteins that regulate these checkpoints (e.g. claspin, allows mitotic entry of damaged cells). In fact, HPV replication is dependent on activation by E7 of the DNA damage sensing pathways such at ATM (ataxia telangiectasia-mutated). Low-risk HPV E7 proteins do not demonstrate the transforming potential of high-risk HPV E7 proteins in a manner analogous to HPV E6 proteins of high- versus low-risk HPV types. The viral protein is also linked to telomere maintenance. See also DNA damage

(DOI:10.1002/9780470015902.a0000557.pub3), Cell Cycle (DOI:10.1002/9780470015902.a0001354.pub2), Checkpoints in the Cell Cycle (DOI:10.1038/npg.els.0001355) and Cell Cycle Checkpoint Genes and Cancer (DOI:10.1038/npg.els.0006046).

Role in Cervical Cancer

Uterine cervical cancer is the second leading cause of cancer-related deaths in women worldwide with an estimated 530,000 new cases per year and more than 270,000 deaths (de Martel et al., 2012). More than 85% of these death occur in low and middle income countries. There is universal agreement now that HPVs are the central aetiological factor in this cancer; almost all cervical cancers and their precursor lesions (>99.7%) have been shown to contain HPV DNA (Walboomers et al., 1999). Besides the connection between cervical cancer and certain HPV types, these HPV types are involved in cancers of other mucosal sites, as discussed below. The mechanism(s) by which certain HPV types trigger events leading to cervical cancer have come from a variety of experimental and epidemiological studies. A summary of the most important observations is listed below.

- DNA of a subset of α–HPV types (24 types) has been found in almost all cervical cancers. Twelve of these (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59) have been defined as group 1 carcinogens by the World Health Organization, whereas some of the others are defined as only as ‘probable’ (Group 2A) or ‘possible’ (Group 2B) carcinogens because of insufficient evidence linking them to carcinogenesis (Bouvard et al., 2009) .
- Precursor lesions containing high-risk HPV DNA precede carcinoma *in situ* of the cervix, which precedes malignant metastatic cervical cancer. The progression of cervical disease from infection and devlopmenty of low grade CIN can be over many decades.
- Expression of viral oncogenes (E6 and E7) is maintained in all cancers.
- Viral DNA of high-risk HPV types in anogenital and oropharyngeal cancer cells is often integrated into the host chromosomes and is found to be an early event in high-risk HPV cervical infections (Collins et al., 2009).
- Integration of the viral DNA leads to enhanced expression and stabilization of E6 and E7 transcripts. One mechanism for this upregulation of the viral oncoproteins includes the loss of function by the viral E2 protein (gene disrupted by integration), which can negatively regulate viral E6 and E7 expression (Goodwin and DiMaio, 2000).
- In viral episomal driven cancers, E2 binding sites within the LCR are often hypermethylated and this may block E2 dependent transcriptional repression of viral oncogene transcripts.
- Viral DNA integration has been found to occur at chromosomal break points or fragile sites. Such integration may lead to potential disregulation of certain cellular oncogenes or tumour suppressor genes. Surveys of viral/host DNA integration sites have revealed the presence of hybrid viral/host mRNA and proteins.

- Particular molecular variants of HPV-16 are associated with anogenital neoplasms more frequently than are other HPV-16 variants.
- Co-infection with HPV and *Human immunodeficiency virus* (HIV) increases the risk of cervical cancer due to increased immunosuppression.

One noticeable and intriguing observation is that cervical cancer is by far the most prevalent of the HPV-associated anogenital cancers in immunocompetent patients, despite HPV infection of mucosal tissues of other genital and ororespiratory sites in both genders. Cervical cancer and its precusror lesions arise at the squamocolumnar junction, a region of the cervix where the columnar epithelium meets the squamous epithelium, and is a particularly vulnerable site for HPV infection and subsequent malignant progression. Recent research has identified a unique cell population at this site from which all cervical cancers may originate (Herfs et al., 2012).

There is a general consensus that HPVs are necessary but not sufficient for the development of cervical cancer. HPVs play a role in the development of precancerous lesions by stimulating epithelial hyperproliferation and disrupting cell cycle regulatory events, thereby setting the stage for malignant progression. Various cofactors have been suggested by epidemiological and other studies. The end result often includes chromosomal abnormalities in cervical cancer cells, which is a feature that is common to many other human tumours.

Role in Orphayngeal Cancer

A distinct subset of head and neck squamous cell carciniomas (HNSCC) are associated with high-risk HPV infections, predominantly HPV16. Most common anatomic sites of HPV-positive HNSCC are the tonsil and base of tongue, the incidence of which is increasing at a rapid rate (Chaturvedi et al., 2013). Epidemiological studies indicate that patients who develop these tumours are more likely to be young and male, and oral-genital sex is thought to be the route of transmission to this site. It is interesting that HPV-positive patients show a superior survival to HPV-negative patients when treated with radiation and chemotherapy-based therapies; with median survival rates of 131 months versus 20 months in one study conducted in the US (Ang et al., 2010). The fact that HPV-positive and -negative HNSCC tumours have distinct molecular profiles shows that these cancers develop along distinct pathways (Agrawal et al., 2011; Stransky et al., 2011). See also Molecular Genetics of Oral Cancer (DOI: 10.1002/9780470015902.a0025222).

Role in Skin Cancer

The development of non-melanoma skin cancers (basal cell carcinomas and squamous cell carcinomas) at sun-exposed sites is common in Caucasians and there is a strong link with viral load of the β-papillomaviruses associated with EV and the development of these cancers. The viral oncoproteins of several of these viruses (e.g. HPV8 and HPV38) have been shown to have transforming properties in cell-based studies and animal models of carcinogenesis. The precise contribution that HPV makes to the development of these cancers is believed to be a synergistic role with UV-irradiation. One route would be to inhibit apoptosis by the E6 oncoprotein targeting apoptotic promoting proteins such as BAK following UV irradiation; in

this way E6 helps the survival of genetically-damaged cells (Jackson et al., 2000). See also The BCL-2 Family Proteins – Key Regulators and Effectors of Apoptosis (DOI:10.1002/9780470015902.a0021568).

Epidemiology and Controls

Incidence

With regards to the natural history of cervical infections (Woodman et al., 2007), HPV is a common sexually transmitted infection and HPV infections are acquired soon after the onset of sexual relationships, with the peak in prevalence occurring in women under 25 years of age and decreasing with further increase in age.

In population–based studies the cumulative incidence of infection with genital types has been reported to be just over 40%. The main risk factors for acquiring genital infections are an early age at onset of sexual activity and a high number of sexual partners. The bulk of genital HPV disease is transient, but persistence of the virus might be a contributing factor to disease progression. Several important conclusions are: (1) men represent an important source of viral transmission; (2) the transient nature of most of the HPV infections in young adults strongly suggest an immunologically mediated response leading to regression; (3) a variety of risk factors contribute to the outcome of persistence and progression of genital HPV infections and (4) transmission must also occur from subclinical infections. Since these studies confirm HPV infection by PCR of viral DNA, it is not clear what proportion of these 'infections' represent transcriptionally active infections, or latent infections that may be activated at a later time by various cofactors, or merely represent the presence of viral DNA in an inactive, noninfectious state. Further support for a role of host immunity in the control of HPV disease stems from data obtained from immunocompromised patients. Transplant recipients on immunosuppressive therapy and patients infected with HIV have an increased incidence of HPV infections and HPV-associated anogenital cancers. See also Acquired Immune Deficiency Syndrome (AIDS) (DOI:10.1038/npg.els.0003998), Human Immunodeficiency Viruses (HIV) (DOI:10.1038/npg/els.0000417), and Immunosuppression: Use in Transplantation (DOI:10.1002/9780470015902.a0001242.pub3).

Transmission

The cellular and molecular events of natural transmission of HPV infections in humans are poorly understood. Common hand and foot warts in children are believed to spread by person-to-person contact with infected sites. Sexual intercourse is clearly indicated for genital HPV spread and oral-genital contact in oral infections. Shed virus from the upper layers of productive lesions that is rubbed into sites of microabrasion in the uninfected epithelium during intercourse is the accepted explanation for natural transmission of genital HPV infections. Target cells for new infections are basal epithelial cells exposed to virions following microtrauma to the epithelium. Studies with animal models of papillomavirus infection have used experimentally induced wounding and papillomas, which contain abundant levels of infectious virions. However, the high incidence of human genital infections in sexually active young adults, coupled with less frequent macroscopic disease

and the difficulty in detecting abundant levels of virions in many human lesions, suggest that natural transmissions may also occur from subclinical infections.

Controls

Prevention

Prevention of the spread of genital HPV infections is a topic that is seldom discussed. Condoms are ineffective protection against the spread of exophytic condylomata. Males are clearly the vector for heterosexual transmission to females, but male penile infections often are insignificant, and seldom develop into cancerous lesions. Postinfection preventive measures in developed countries include early detection by the Papanicolaou (PAP) test, which looks for abnormal cells in samples collected from the surface of the cervix (Papanicolaou, 1948). High-risk HPV genotyping is now being incorporated into cervical screening programmes to identify those women at a higher risk of developing high-grade precancerous disease. Antiviral microbicidal agents as a preventative mechanism of HPV infection are unavailable, but some lead candidates have been identified in cell and animal models, and experimental preclinical models (Roberts et al., 2007; Tenge et al., 2014).

Vaccines

Virus-like particles (VLP) assembled from the major capsid protein L1 form the basis of two prophylactic HPV vaccines: Gardasil (manufactured by Merck & Co) is a quadrivalent vaccine consisting of VLPs of HPV types, 6, 11, 16 and 18, and a bivalent vaccine Cervarix (manufactured by Glaxo Smith Kline) that consists of HPV types 16 and 18 VLPs. Both vaccines achieve excellent type-specific protection against the vaccine-associated HPVs by the induction of high-titre neutralizing antibodies that target type-sepcific conformational epitopes located on L1. Clinical trials have shown that the vaccines are highly effective at prevention of anogenital intraepithelial disease in individuals who have not been exposed to the targeted virus types at time of vaccination (Dochez et al., 2014). Gardasil is also effective at the prevention of anogenital warts. The prophylactic vaccines may protect against infection in the head and neck region. Second generation VLP-based vaccines (e.g. nonavalent vaccine V503) will broaden protection against infection by HPV types not in the current vaccine (Joura et al., 2014). Other strategies involve the development of vaccines capable of producing a more broadly cross-protective response by targetting the minor capsid protein, L2 (Alphs et al., 2008). HPV DNA and synthetic peptide-based vaccines are aimed at clearing existing existing HPV infections and HPV-associated cervical cancers (de Vos van Steenwijk et al., 2012). Other strategies are centred on boosting cell-mediated immunity to E6 and E7 antigens. See also Tumours:Immunotherapy (DOI:10.1002/9780470015902.a0000961.pub3) and Tumour Immunology (DOI:10.1002/9780470015902.a0001429.pub2).

Treatment

Treatment of HPV infections is largely ablative. Methods include topical or intralesional treatments with caustic agents such as podophyllin, podophylotoxin, trichloroacetic acid and epigallocatechin gallate. Electro-fulguration, -dessication, -cautery, carbon dioxide laser and conventional surgery are used for the management of extensive or refractory disease. Immunomodulators (interferons, interleukins and immunopotentiating agents such as Imiquimod) and photoablative therapy

have been used with some success. Overall efficacies for these treatments range from 22 to 95% for clearance of exophytic genital warts. Recurrence rates are often high, often within weeks or months after clearance . See also Antiviral Drugs (DOI:10.1002/9780470015902.a0000410.pub2).

Future perspectives

HPVs are important human pathogens, responsible for up to 660,000 deaths per year worldwide, predominantly from cervical cancer, but the incidence of cancers at other anogenital sites and in the oropharynx are increasing. Infections from low-risk HPV types are generally not life-thereatening but are often difficult and expensive to treat. Studies on viral gene function at the molecular and cellular level have provided information on the mechanisms of viral oncogenesis. In recent years, the exploitation of animal models and of more physiological cell-based models of the virus life cycle has gone some way to unravelling the many intrguing aspects of the papillomavirus life cycle, but the role of some virus-host interactions is still unclear. A more complete understanding of how this virus replicates in the upper layers of differentiating epithelium should increase the likelihood of identifying anti-viral targets. While not completely resolved, many aspects of the natural history of HPV cervical infection are understood; a similar investment of research is necessary to understand the natural history of high-risk HPV infection in the oropharynx and at other anogenital sites. The effective management of HPV-related disease has begun with the introduction of two prophylactic papillomavirus vaccines and with vaccines of increased valency and broader cross-protection at various stages of development. Improving therapies targeted towards the treatment of existing infections, premalignant and malignant disease is the next important challenge and many steps have already been taken along this road.

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FIGURE LEGENDS

Figure 1. *Structure of papillomavirus virions.* Electron micrograph of BPV-1 particles (image reproduced with permission of Robert Garcea, University of Colorado, Colorado, US). The structure of one of the particles revealed by a three-dimensional reconstruction to 9 Å. The virion is 60 nm in diamter and it is arranged in a T=7 icosahedral lattice made from 72 L1 pentamers. The L1 protein can assemble spontanously into structures that closely resemble native virions. The precise location of the L2 protein in the capsid is not fully known but most likely sits beneath each L1 pentamer and there are possibly up to 72 L2 molecules in each infectious particle (Buck et al., 2008). "Papillomavirus capsid" by Trus BL, Roden RB, Greenstone HL, Vrhel M, Schiller JT, Booy FP - Posted at http://ccr.cancer.gov/staff/gallery.asp?profileid=5637. Licensed under Public domain via Wikimedia Commons (http://commons.wikimedia.org/wiki/File:Papillomavirus_capsid.png#mediaviewer/File:Papillomavirus_capsid.png.).

Figure 2. *Papillomavirus life cycle is tightly linked to the terminal differentiation programme of keratinocytes.* Left panel, organization of the double-stranded DNA HPV genome. ORFs have been designated for the E (early) and L (late) viral genes, the noncoding regions designated LCR (long control region), the early promoter (P_E), the late promoter (P_L) activated in differentiating keratinocytes, and the position of the early and late polyadenylation sites (pA*E* and pA*L*) are as indicated. In cancers, the papillomavirus genome is often integrated into the host DNA and the site of breakage in the viral DNA most often occurs in the region containing E1 and E2 ORFs (between the arrows). Integration leads to the disruption of expression of E2 which acts as a repressor of E6 and E7 expression. ORFs are coloured according to their timing of expression in the virus life cycle as shown in the schematic of the virus life cycle. Right panel, schematic of the papillomavirus life cycle. Infectious virions enters the epithelium through microwounds and on reaching the basement membrane (BM) binds to heparin sulphate proteoglycans which leads to a conformational change in the virus coat. This event exposes sequences of the minor capsid protein L2 that are then cleaved by the enzyme furin to facilitate binding to a receptor on the cell cycle active basal keratinocyte (Kines et al., 2009). Following infection of the basal cells, the viral genome is established in the nucleus as a low copy extrachromosomal plasmid or episome. As the cell undergoes cell division, the viral genomes attach to the host chromosomes to ensure that they are segregated between daughter cells. The virally-infected cell population expands as cell proliferation is activated at the same time as cells migrate upwards from the basal layer into the intermediate layers. Re-entry of the differentiating cells into the cell cycle allows the virus to gain access to the host's replication machinery in order that the viral genome can be amplified to thousands of copies per cell. The virus prevents these replication-activated cells from dying through apoptosis by blocking the host apoptotic pathways. Once the viral genome has amplified, the capsid proteins are expressed and virions assembled in the uppermost superficial cells and the infected squames, full of infectious particles are sloughed off. The two panels (*a* and *b*) show cell cycle activity (as determined by expression of the nuclear cell cycle marker MCM7 [minichromosome maintenance protein 7], red stain, nuclei are stained blue) of cells in uninfected cutaneous skin (*a*) and in HPV-1 infected wart tissue (b). In uninfected tissue only cells of the basal layer (arrowheads) are cell cycle active whereas in virally-infeccted tissue, suprabasal cells (sb) also express MCM7 indicating S-phase reentry.

Figure 3. *Cutaneous hyperproliferative warts.* Upper panel, a typical common hand wart (verrucae vulgaris) ("Verruca" by Klaus D. Peter, Wiehl, Germany - Own work. Licensed under Creative Commons Attribution 3.0-de via Wikimedia Commons - http://commons.wikimedia.org/wiki/File:Verruca.jpg#mediaviewer/File:Verruca.jpg). Bottom panel, Haematoxylin and eosin-stained section of common hand wart. ("Verruca vulgaris" by Nephron - Own work. Licensed under Creative Commons Attribution-Share Alike 3.0 via Wikimedia Commons - http://commons.wikimedia.org/wiki/File:Verruca_vulgaris_-_low_mag.jpg#mediaviewer/File:Verruca_vulgaris_-_low_mag.jpg). Inset, shows the appearance of koilocytes which are enlarged keratinocytes with pyknotic nuclei surrounded by a clear halo (examples are indicated with arrows) found in the upper layers of the wart. They are a recognized pathognomic feature of papillomaviurs infection.

Figure 4. *HPV oncogenesis*. The three oncoproteins E5, E6 and E7 work synergistically to promote hyperproliferation of infected cells, including the deregulation of the retinoblastoma (Rb) tumour suppressor pathway by E7 targetting the pocket proteins for degradation, deregulation of growth factor signalling pathways by E5 and by E6 targeting cell polarity proteins containing PDZ domains. In response to aberrant cell proliferation, the host cell activates apoptotic pathways including those mediated by p53. To ensure survival of the infected cells E6 inactivates the apoptotic response by targeting the tumor suppressor p53 protein for proteasomal degradation. In combination with activation of the host enzyme telomerase which is necessary for the maintenance of telomeres immortal cells can emerge. Continuous expression of the viral oncoproteins (integration of the viral DNA into the host leads also to upregulation of E6 and E7 expression) in the immortal cells leads to an unstable host genetic environment in which oncogenic mutations may occur and then clonal populations of fully transformed cells emerge. Figure adapted from Moody, CA., Laimins, LA. (2010) Human papillomavirus oncoproteins: pathways to transformation. Nat. Rev. Cancer **10**:550-60. Reproduced by permission of Nature Publishing Group.

Figure 4 requires permission (obtained).