Malaysian J Pathol 1998; 20(1): 1 - 10

Biology and pathological associations of the human papillomaviruses: a review

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Abstract

Historical cottontail rabbit papillomavirus studies raised early indications of a mammalian DNA oncogenic virus. Today, molecular cloning recognises numerous animal and human papillomaviruses (HPVs) and the development of ***in vitro*** transformation assays has escalated oncological research in HPVs. Currently, their detection and typing in tissues is usually by Southern blotting, ***in-situ*** hybridization and polymerase chain reaction methods.

The complete papillomavirus virion constitutes a protein coat (capsid) surrounding a circular, double-stranded DNA organised into coding and non-coding regions. 8 early (El-E8) open reading frames (ORFs) and 2 late (L1, L2) ORFs have been identified in the coding region of all papillomaviruses. The early ORFs encode proteins which interact with the host genome to produce new viral DNA while late ORFs are activated only after viral DNA replication and encode for viral capsid proteins.

All papillomaviruses are obligatory intranuclear organisms with specific tropism for keratinocytes. Three possible courses of events can follow papillomaviruses entry into cells: (1) viral DNA are maintained as intranuclear, extrachromosomal, circular DNA episomes, which replicates synchronously with the host cell, establishing a latent infection; (2) conversion from latent into productive infection with assembly of complete infective virions; (3) integration of viral DNA into host cellular genome, a phenomenon seen in HPV infections associated with malignant transformation.

Human papillomaviruses (HPVs) essentially induce skin and mucosal epithelial lesions. Various skin warts are well known to be HPV-associated (HPVs 1, 2, 3, 7 and 10). Besides HPVs *3* and 10, HPVs 5, 8, 17 and 20 have been recovered from ***Epidermodysplasia verruciformis*** lesions. Anogenital condyloma acuminatum, strongly linked with HPVs 6 and 11 are probably sexually transmitted. The same HPVs, demonstrable in recurrent juvenile laryngeal papillomas, are probably transmitted by passage through an infected birth canal.

HPVs described in uterine cervical lesions are generally categorized into those associated with high (16, 18), intermediate (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68) and low (6, 11, 26, 40, 42, 43, 44, 53, 54, 55, 62, 66) risk of cervical squamous carcinoma. Cervical adenocarcinoma, clear cell carcinoma and small cell neuroendocrine carcinoma have also been linked to HPVs, especially HPV18.

Other lesions reported to be HPV-associated are: papillomas, dysplasia and carcinomas in the nasal cavity (HPV 6, 11, 57); squamous papilloma, condyloma acuminatum, and verruca vulgaris of the oral cavity (HPV 6, 11), oral focal epithelial hyperplasia (HPV 13, 32); warty lip lesions (HPV 2): and conjunctival papillomas (HPV 6, 11).

Key words: human papillomavirus, carcinoma cervix, dysplasia, papilloma, condyloma.

A HISTORICAL PERSPECTIVE OF THE PAPILLOMAVIRUSES

The papillomaviruses are a group of DNA viruses which induce warts (papillomas) in humans and animals. The viral aetiology of human skin warts was suggested by Ciuffo who demonstrated the transmission of the warts through cell-free infiltrates in 1907.[1] In 1933, Shope described the first papillomavirus, the cottont'ail rabbit papillomavirus (CRPV).[2] The virus, recovered from naturally occurring cutaneous papillomas in cottontail rabbits, produced papillomas in domestic rabbits, many of which became malignant. This provided one-of the first experimental examples of a mammalian DNA oncogenic virus. Further progress in the study and characterisation of papillomaviruses was then hampered by the lack of a tissue culture system for laboratory propagation of the virus. Only limited information from study of viral particles obtained from warts was available and up to the 1960s, the prevailing belief was that there was only a single

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type of human papillomavirus and the nature of the infected epithelium determined the morphology and behaviour of the lesion.[3] From the late 1970s molecular cloning techniques of viral DNA emerged, allowing for the recognition of a multitude of animal and human papillomaviruses.[4,5] Since the 1980s, research interest on the papillomavirus has escalated with the development of ***in vitro*** transformation assays. The recognition that papillomaviruses, including some human papillomaviruses (HPV), can transform cells in culture has spurred a lot of interest in this area. Large epidemiological and molecular-based studies since then have strongly linked cervical cancer with some types of human papillomavirus.[6-12] In 1995, the World Health Organisation's International Agency for Research on Cancer (IARC) consensus panel concluded that there was compelling evidence, from both biological and epidemiological standpoints, to consider certain papillomaviruses as carcinogenic in humans.[13] At the National Institutes of Health Consensus Development Conference in 1996, it was concluded that cervical cancer is causally related to infection with the human papillomavirus.[14]

CLASSIFICATION

Since early days, the genus ***Papillomavirus*** together with the genus ***Polyomavirus,*** constituted the family ***Papovaviridae.*** "Papova" is derived from the first two alphabets of the names of the viruses first grouped together to form this family, the rabbit papillomavirus, mouse polyomavirus and simian vacuolating virus (SV 40). This family of viruses has in common the following properties; a small size, non-enveloped virion, icosahedral capsid, double-stranded circular DNA genome and the requirement of the host cellular nucleus as the site of multiplication.[15] This classification has long been debated and recent biological and molecular biological studies have revealed fundamental differences in the genomic organisation of the two genera, papillomaviruses and polyomaviruses. These are now considered subfamilies rather than genera within the Papovavirus family.[15]

The classification of papillomaviruses is based on the (1) host species infected and (2) genomic homology of the species-specific virus. Although, papillomaviruses are fairly ubiquitous and have been described in man, other higher vertebrates (rabbits, cattle, horses, dogs, sheep, elk, deer, the harvest mouse etc), and occasional avian species, namely the parrot and chafinch,[16] they are remarkably species-specific. One notable exception is the occurrence of sarcoid in horses as a result of bovine papillomavirus (BPV) transmission from cattle.[17,18] To date there is no known natural transmission of human papillomavirus to any other species or vice versa animal papillomavirus to humans.

In most animal species, only a single papillomavirus type has been described, with the exception of the bovine papillomavirus where there are 6 known types.[19] This is probably due more to a lack of studies conducted on animal papillomaviruses than a true lack of multiple papillomavirus types in animals. In humans, more than 80 HPV types have been designated[20] since the first report on the human papillomavirus in 1965[21] and the cloning of the first HPV in 1980.[22] Unlike other viruses, the antigenic similarity of the papillomavirus capsid proteins precludes usage of serological methods for typing and papillomaviruses are genotyped instead. In the earlier days, a new distinct HPV type was defined when the isolate exhibited less than 50% homology with genomes of known HPV types by DNA hybridisation under stringent condition. Although this method of establishing genetic identity could be done more rapidly it was not always accurate.[24] More recently, a more specific system which compares the nucleotide sequences of the E6, E7 and L1 open reading frames of the new isolate with that of known types is used for characterising novel HPV types. It was decided at the Papillomavirus Nomenclature Committee Workshop held in Seattle in 1991, that prior to a previously unidentified isolate being designated as a new HPV type, its entire genome had to be cloned. Furthermore, the unidentified isolate should share <90% homology in the E6, E7 and L1 nucleotide sequences with established HPV types.[25] Isolates showing 90-98% homology should be designated as subtypes while those with more than 98% homology are variants of the established HPV types.[26]

BIOLOGY OF PAPILLOMAVIRUSES

Virion Structure

Papillomaviruses are small, non-enveloped viruses with an icosahedral shape which measures about 52-55 nm in diameter.[15] The complete virion, the infective unit, is made up of a protein coat (capsid) surrounding the DNA genome. Cryoelectron microscopy with 3-D image reconstruction reveals the capsid to be structurally made up of 72 pentameric capsomeres arranged

on a T=7 surface lattice.[27] Biochemically, the capsid is composed of 2 proteins, a major (L1) and a minor (L2) protein.'' The genome which constitutes about 12% of the virion weight is made up of circular, double-stranded DNA of about 8000 nucleotide base pairs. Sequencing of the viral genome shows that the genomic organisation of the various papillomaviruses is remarkably similar. Also, all the viral genes are located on one DNA strand which serves as the template for transcription in a clockwise direction.[28,29]

Genomic Structure

The papillomavirus genome (Fig. 1) is essentially made up of coding and non-coding regions.[15] Located on the single coding strand of the papillomavirus genome, the genetic information of the coding region is potentially protein coding and the potential protein coding regions are referred to as open reading frames (ORF). 8 early (E) open reading frames (E1-E8) and 2 late (L) open reading frames (L1, L2) have been identified in the coding region of all papillomaviruses, irrespective of species. The early region ORFs encode proteins essential to the early life cycle of the virus.[30] These proteins interact with the host genome and program the host cell to produce new viral DNA. In contrast, the ORFs of the papillomavirus late region are activated only after viral DNA replication has occurred. These are expressed exclusively in productive infections and encode for the viral capsid proteins.[31] A summary of the functions of the papillomaviral ORFs is shown in Table 1.

The non-coding region is variously referred to as the long control region (LCR), upstream regulatory region (URR) or simply as the non-coding region. The LCR sits upstream of the early ORFs. The size of this region varies slightly among the different papillomaviruses. Its function is still not well understood except that it is a regulatory region of papillomaviral genetic expression. While 7 transcriptional promoters have been identified in the BPV 1 LCR, to date only a few promoters have been identified and studied in the human papillomaviruses.[32]

NATURAL HISTORY OF HPV INFECTION

All papillomaviruses, including the human papillomaviruses, are obligatory intranuclear organisms, which exhibit a specific tropism for keratinocytes. Little is known about the attachment and entry into host cells, transportation to the host cell nucleus and subsequent capsid uncoating of the papillomaviruses. Nonetheless,

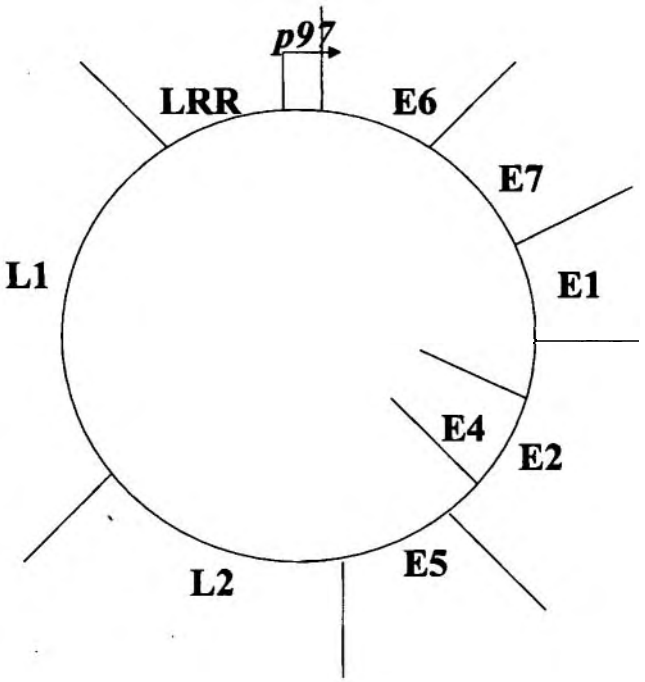

FIG. 1: Schematic representation of the circular genomic organisation of HPV16. The early (E) and late (L) genes and the non-coding long control region (LRR) are indicated. The only transcriptional promotor mapped for HPV 16, designated p97 is shown in the diagram. (Adapted from Fields Virology[15]).

it is clear that papillomaviruses must reach mitotically active cells for establishment of an infection. This implies that papillomaviral infection commences in the basal layer of squamous epithelium; the viruses presumably reaching it through breaks in the surface epithelium.

Three possible courses of events can follow entry of papillomaviruses into basal cells. In the first instance, the viral DNA can be maintained as intranuclear, extrachromosomal, circular DNA episomes, which replicates synchronously with the host cell, establishing a latent infection. As the viral DNA does not interfere with the host cell division, latently infected cells are morphologically normal. In latent infections, only early and not late ORFs are expressed.

Under certain circumstances, there is conversion from a latent into a productive infection with assembly of complete infective virions. The signals which trigger this conversion are still unclear although in humans, immunosuppressed states appear to predispose to this change.[33] When productive infection supervenes, viral DNA replication is no longer in tandem with cellular division. The number of episomal viral DNA progressively increases from the basal cell layer to the surface. As the promoter of the capsid-encoding late ORFs is only active in terminally differentiated superficial epithelium, viral capsid proteins are only formed in these layers. Consequently, complete infective virions composed of capsid proteins surrounding episomal DNA are also found only in these layers.[15] Transmission of infection seems to be via contact with the superficial layers of the epithelium where the infective agents reside. Unlike latent infections, morphological changes are seen in the squamous epithelium during productive infections and these changes serve as useful clues to the underlying aetiology. With the increased viral DNA replication, host cellular mitotic activity is simultaneously increased and this results in thickening of the epithelium. The increased viral replication also appears to interfere with the mitotic spindle and cytokinesis of the host cell leading to multinucleation and cytologic atypia. The perinuclear halos characteristic of koilocytes is attributed in part to E4 protein and possibly its role in breakdown of cytokeratin. In human infections, productive infections are most commonly seen in infections with low risk HPVs and result in formation of squamous papillomas (warts) or low grade dysplastic lesions e.g low grade squamous intraepithelial lesions in the uterine cervix.

Table 1: **Papillomavirus open reading frames and their functions**

Open Reading Frame	Function
E1	Initiation of viral DNA replication
E2	Transcription regulation of other ORF, auxillary role in viral DNA replication
E3	No known function
E4	Disrupts cytokeratin cytoskeleton
E5	Membrane associated transforming protein which interacts with growth factor receptors
E6	Transforming protein of HPVs which degrades p53
E7	Transforming protein of HPVs which binds to the retinoblastoma protein
E8	No known function
L1	Major capsid protein
L2	Minor capsid protein

A third possible outcome of human papillomavirus infection is integration of viral DNA into the host cellular genome. This is usually seen in mfections with HPVs asssociated with high risk of malignant transformation.[34,35] When this event occurs, the circular episomal viral DNA linearises prior to splicing into the linear host cellular DNA. The site of integration of the HPV DNA into the host cellular DNA is not constant and varies in each case. In contrast, the break of the circular HPV DNA consistently occurs at the site of the E1 and E2 ORFs although the reasons for such an event are currently poorly understood. As a result, the integration site into the host cell DNA always involves the E1 and E2 ORFs of the HPV.[34,36] Full-length E2 proteins have been shown to repress the promoters of E6 and E7 transcription located within the LCR in the episomal form of the virus.[37-40] In the integrated form, due to the linearisation of the HPV DNA and the breakage at the site of E1 and E2 ORFs, there is loss of E2 control which leads to an over-expression of E6 and E7 proteins. Increased E6 proteins result in upregulated degradation of the negative cell cycle regulator p53 while increased E7 displaces the positive cell cycle regulator E2F-1 from an inactivating bond with the hypopshosphorylated pRB. These events cumulatively result in unbridled cell proliferation, loss

of ability and time to repair damaged DNA and eventual malignant transformation.

DETECTION OF HPV

Today, diagnosis is almost entirely dependent on nucleic acid hybridisation. Unlike other viruses, electron microscopic examination is unreliable as viral particles are not always present even in histologically diagnosed warts. Similarly, serological examination is thwarted by the large number of HPV types.

Many DNA hybridisation assays have been used to detect HPV. These techniques include the Southern blot, which has long served as the "gold standard" for HPV detection. Apart from providing a good positive internal control for the specificity of the annealing reaction, the Southern blot also allows identification of the HPV type. Besides Southern blotting, dot blot, slot-blot, "reverse blot", filter-in-siru hybridisations have all been used for HPV DNA detection.

More recently, tissue in-situ hybridisation (ISH) employing isotopic and non-isotopic probes gave rise for the first time to topographical localisation of HPV in tissue sections (Fig. 2).[41,42] The importance of topographical localisation using tissue ISH which cannot be undermined, is however being continuously challenged by the highly sensitive and specific polymerase chain reaction (PCR) technique. The PCR amplification of segments of the viral genome which demonstrates a 10^4 times sensitivity beyond all the earlier techniques is now the preferred method especially in epidemiological investigation of HPV infections.

CLINICAL ASSOCLATIONS AND PATHOLOGY OF HUMAN PAPILLOMAVIRUS INFECTIONS

Human papillomaviruses (HPV) essentially induce epithelia1lesions involving skin and mucosae. Infections are commonly acquired by contact with infected epithelium through skin abrasions, sexual intercourse or passage through an infected birth canal. A summary list of lesions with their associated HPV types is shown in Table 2.

Skin warts

HPV induced skin warts are most commonly found in older children and young adults. Although these squamous papillomas can be found on any part of the skin, they are most usual on the hands, feet and face. In a special category are the warts found on the hands of butchers and

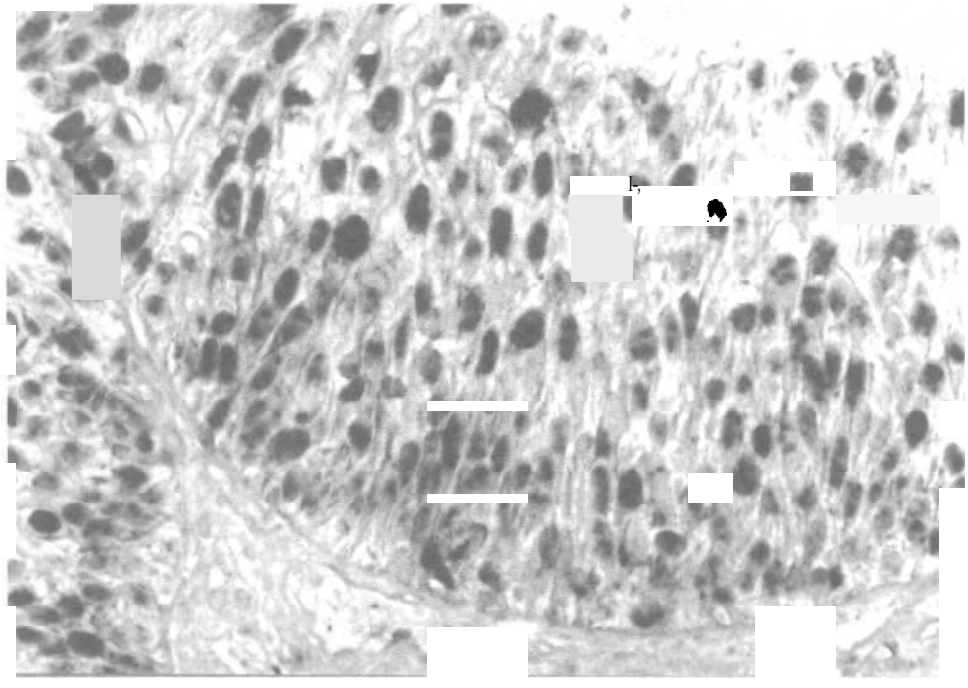

FIG. 2: Photomicrograph of a laryngeal papilloma showing positive nuclear signals. In-situ hybridization using digoxigenin-labelled DNA probe against HPV6 and NBT/BCIP detection system. X 300.

Table 2: HPV associated lesions

Clinical Lesion		HPV types
Cutaneous Lesions	***Skin warts***	
	Plantar warts	1
	Common warts	2
	Flat warts	3,10
	Butcher's warts	*7*
	Epidermodysplasia verruciformis	3,10 5,8,17,20
Mucosal Lesions	*Anogenital warts (condyloma acuminatum)*	6,11
	Anogenital carcinoma especially cervical carcinoma	
	Associated with high risk	16,18
	Associated with intermediate risk	31,33,35,39,45,51,52,56,58,59, 68, some unnamed types
	Associated with low risk	6,11,26,40,42,43,44,53,54,55, 62,66, some unnamed types
	Aerodigestive Lesions	
	Respiratory lesions	
	Recurrent respiratory papillomatosis	6,11
	Nasal papillomas, dysplasia, carcinoma	*57*
	Oral lesions	
	Focal epithelia1 hyperplasia	13,32
	Lip vermillion warts	2
	Squamous papilloma	6,11
	Condyloma acuminatum	6,11
	Verruca vulgaris	6,11
	Conjunctival Papillomas	6,11

meat handlers ("butcher's warts"). As expected by the species-specificity of papillomaviruses, these lesions are also due to HPV, and are not zoonotic in origin. Although not absolutely correlated, certain HPV types are associated with particular cutaneous lesions. HPV 1 is commonly associated with plantar warts, HPV2 with common skin warts, HPV 3 and 10 with flat warts and HPV *7* with the butcher's warts. Clinical outcome is not spectacular and most cutaneous warts spontaneously regress after a few years. This has been attributed to the eventual mounting of an adequate host cell mediated immune response.

Epidermodysplasia verruciformis

Epidermodysplasia verruciformis (EV) is an inheritable disorder whereby patients develop multiple cutaneous warts that fail to regress but instead exhibit a potential for malignant transformation. The majority of EV patients have defects in cell-mediated immunity.[43,44] Although it has been suggested that these patients have a rare recessive genetic condition which renders them immunologically defective and compromised in their ability to resolve their warts, the precise mode of inheritance of EV and its pathogenesis is still not well worked out. Most of the cutaneous warts found in EV patients are flat or present as reddish macules. Many HPV types including HPV 3 and 10, which commonly induce flat warts in normal people are also encountered in the lesions of EV patients but the more distinctive types recovered from EV lesions and rarely from non-EV population are HPV 5, 8, 17 and 20.[45] Approximately 30% of the patients exhibit malig-

nant tranformation of their warts, especially those lesions located in sun-exposed areas. The ensuing Bowen's disease (in-situ carcinoma) or invasive squamous cell carcinoma usually occurs after some years. From the epidemiological observations, it seems likely that at least three factors i.e. genetically determined deficient cell mediated immunity, HPV and ultraviolet radiation, are involved in pathogenesis of EV and the malignant predisposition of the warts.

Anogenital warts (Condyloma acuminatum)

Exophytic warts (condyloma acuminatum) usually arise in the vagina and vulva of the female and the penis and scrotum of the male. The HPV types most commonly associated with condyloma acuminatum are 6 and 11 and these now show strong evidence of sexual transmission.[46] Like cutaneous warts, many condylomas also regress spontaneously probably as a result of cell-mediated immunity. In the same line, condylomas can increase in numbers in immunosuppressed states e.g superimposed HIV-infection.[47]

Anogenital carcinomas

The most important genital carcinoma associated with HPV is carcinoma of the uterine cervix. It is generally believed that there is now compelling evidence that HPV is aetiologically associated with cervical carcinoma. At the Consensus Development Conference of the National Institutes of Health in 1996, it was concluded that cervical cancer is aetiologically related to HPV infection.[14]

About 30 HPV types have been described in cervical lesions. [48-51] In general, genital HPVs can be divided into 3 categories, those associated with high (16, 18), intermediate (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68 and a few unnamed types) and low (6, 11, 26, 40, 42, 43, 44, 53, 54, 55, 62, 66 and a few unnamed types) risk of cervical carcinoma. However, the prevalence of these HPV types in cervical cancers may vary from community to community. In Malaysia, the pattern is not yet clear and studies are on-going at various research institutions.[52,53]

Unlike the strong belief that HPVs are associated with cervical cancers, its association with cervical precancerous squamous lesions remains to be clarified. In part, the uncertainty is compounded by controversy over the nomenclature and definition of these lesions. Currently many classification systems are in practice, such as the traditional WHO system of dysplasia, the Richart system of cervical intraepithelial neoplasia (CIN)[54] and more recently, the Bethesda system which introduced the concept of low and high grade squamous intraepitheial lesions (LSIL and HSIL).[55] A development in the Bethesda system that has profound significance on the HPV-precancer story is the inclusion of condylomas into the classification of pre-invasive cervical squamous cell cancer for the first time. To many histopathologists, equating condylomas with CIN I defies the basic concept of the morphological definition of "mild dysplasia" or its Richart equivalent. This contradiction needs to be addressed before information from various studies can be interpreted meaningfully.

All the 30 or so types of genital HPV can occur throughout the genital epithelium. Notwithstanding the above mentioned controversy over nomenclature, invasive carcinomas and high grade pre-invasive lesions, e.g. CIN II and III in the cervix, are most commonly associated with the high risk, less frequently with the intermediate risk and rarely with low risk HPV types. On the contrary, condylomas and low grade pre-invasive lesions are on the whole associated with low risk HPVs. Although HPVs can affect any part of the genital epithelium, HPV associated cancers are most commonly encountered in the uterine cervix, especially the squamo-columnar transformation zone of the uterine cervix. This is probably explained by the fact that in the female genital tract, the cervical squamo-columnar transformation zone is a highly unstable region of rapid cell turnover that predisposes it to adjunctive carcinogens like HPVs.

Besides squamous cell carcinoma HPVs, in particular HPV 18, also appear to be associated with adenocarcinoma, clear cell carcinoma and small cell neuroendocrine carcinoma in the uterine cervix.[56-60] Less commonly, HPVs are also recovered from Bowen's disease and squamous cell carcinoma of the anal skin and other parts of the female genital tract e.g. vulva, vagina and perineum. A Malaysian study has demonstrated HPV in carcinoma of the penis.[61] It is also interesting to note that, whenever data is available, both the male and female sexual counterparts have often been found to share the same HPV types and this observation lends added support for the sexual transmission of HPVs. [62]

Aerodigestive lesions

HPV 6 and 11, usually found in condylomas and low grade pre-cancerous genital tract lesions, are also associated with recurrent respiratory papil-

lomatosis (juvenile laryngeal papillomatosis).[63-65] Most commonly sited on the vocal cord, these papillomas are also found in the nose, oral cavity, tracheo-bronchial tract and in the lungs. Although onset in adulthood is known, most papillomas fust present by 10 years of age and it is envisaged that the HPVs are probably transmitted by passage through an infected birth canal. Although usually benign, these papillomas are often recurrent. Their main clinical significance lie in their potential for lethal respiratory obstruction. Also rarely, these papillomas can undergo malignant transformation.[66,67]

Apart from HPV 6 and 11, HPV 57 has recently been described to be an important type occurring in the nasal cavity and is associated with papillomas, dysplasia and carcinomas in the nasal cavity.[68] In the oral cavity, HPV has been shown to be associated with several distinct clinical entities. Focal epithelia1 hyperplasia (FEH), which occurs mainly in children and present ~ . ~ Warty lesions occurring on the lip vermilion are associated with HPV 2.[71] The other lesions arising in the oral mucosa, including the usually solitary squamous papilloma, frequently multiple cauliflower-like condyloma acuminatum, and the highly keratinised verruca vulgaris, are associated with genital HPV 6 and 11.[72,73]

Other HPV associated lesions

Conjunctival papillomas are relatively rare lesions and are also mainly associated with HPV 6 and 11.[74] HPVs have also been reported in cancers of the urethra, oesophagus, colon, urinary bladder and the prostate, however their significance in the causation of these lesions is still largely speculative.[75-79]

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