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Review Article

HPV in Oral Squamous Cell Carcinoma: Where in the Maze?

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Abstract

Head and neck squamous cell carcinoma is a significant cause of cancer worldwide and ranks 6th among all malignancies. Oral squamous cell carcinoma is one of the most common cancers in Indian subcontinent. The path that brings people to oral cancers has two distinct etiologies: one through tobacco and alcohol, other via the human papilloma virus (HPV). There is growing evidence that HPV-16 may act as a co-carcinogen, along with tobacco, in the causation of oral cancers. It has been identified as an etiologic agent for subset of oral squamous cell carcinomas, specifically those arising from the oropharynx, including base of tongue and tonsils. The two most harmful and cancer causing human papilloma viruses are HPV-16 & HPV-18. In the oral environment it is HPV-16 that we are concerned with. Oral squamous cell carcinoma associated with HPV-16 have prognostic significance as they have been found to have better outcomes, being more responsive to radiotherapy and showing higher survival rates. This article highlights the role of HPV-16 in the etiopathogenesis of oral squamous cell carcinoma and presents various diagnostic aids which can be used to detect HPV.

Key words: Human Papillomavirus;E6 Protein;E7 Oncoprotein;HPV Genome;Immortalization; HPV Vaccines;Squamous Cell Carcinoma.

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Introduction

The high risk human papilloma viruses (HPV) are reported to be significant independent risk factors for oral squamous cell carcinoma (OSCC).[1] The HPV virion is approximately 55 nm in diameter and consists of a closed circular double stranded DNA genome, with a size of almost 8000 bp.[2] Oral Squamous Cell Carcinoma represents 12% of all cancers in men and 8% of all cancers in the female population. Its incidence in the Indian subcontinent is among the highest in the world.[3] The term "head and neck cancer" has been widely adopted in the recent literature and includes lesions at several anatomical sites: lip, oral cavity, nose and paranasal sinuses, nasopharynx, oropharynx, hypopharynx, and larynx.[1,4] Head and neck malignancies are characterized by a multiphasic and multifactorial etiopathogenesis.[1,5,6]

Oral human papilloma virus infection, like alcohol and tobacco, is now recognized to play a role in the pathogenesis of head and neck squamous cell carcinomas (HNSCC).[7] At least 16 HPV DNA genotypes (1, 2, 3, 4, 6, 7, 10, 11,13, 16, 18, 31, 32, 33, 35, 57) have been isolated from oral lesions.[6,7] Most of them are low-risk HPVs (i.e. 6, 11, 13, 32) and are associated with benign proliferative epithelial lesions of the oral cavity (e.g. squamous papilloma, condyloma acuminatum, verruca vulgaris and focal epithelial hyperplasia). In addition, high-risk HPV genotypes (i.e. 16, 18, 31, 33, 35) have been reported to be associated with epithelial dysplasia and oral squamous cell carcinoma.[8–12]

The HPV cannot be cultured in vitro and detection of the virus relies on molecular analysis of HPV-DNA sequence using nucleic acid probes.[13,14] Three types of nucleic acid hybridization methods for HPV detection exist. These include direct nucleic acid probe methods-like Southern blot and In-Situ Hybridization, hybridization signal amplification which is an extension of direct probe techniques with sensitivity boost innovations-like multimeric layering of reporter molecules on DNA probes (Gen Point), and target amplification methods, most notably PCR.[13] Target amplification has been confirmed as the most sensitive of all DNA analysis techniques and real-time quantitative PCR as the most sensitive of the target amplification methods[14] with a one target nucleic acid sequence detection limit.[13,15]

The HPV genome is approximately 8000 bp in length and encodes eight open reading frames (ORFs), which are transcribed as polycistronic mRNAs.[16,17] HPVs have a genome that is divided into three regions: an

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early region (E), a late region (L), and a non-coding long control region (LCR). The E region encodes six non-structural proteins: E1, E2, E4, E5, E6, and E7. The L region encodes two structural proteins: L1 and L2 (Fig 1).[18] The E1, E2, E4, and E5 proteins are required for viral DNA replication, the E6 and E7 proteins cooperate to transform and immortalize cells, and the L1 and L2 proteins are needed for the production of viral particle.[19-21]

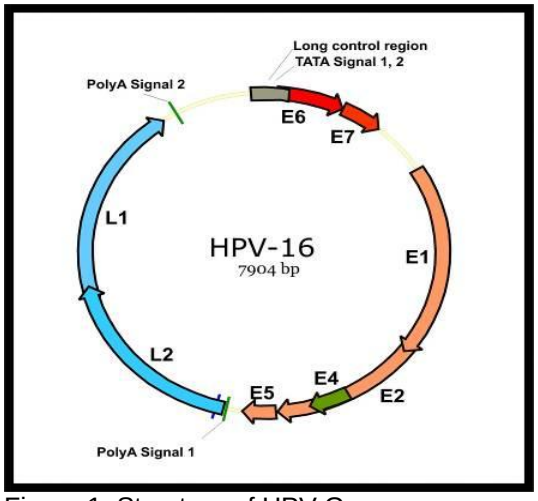

Figure 1: Structure of HPV Genome

Molecular structure and role of E6, E7 Oncoproteins

The high-risk HPVs (e.g., HPV16 and HPV18) are known to be tumorigenic in human epithelial tissues.[22] HPV must adhere to a specific receptor protein on the keratinocytes membrane. Once the virus entered into the cell, it transforms itself of its protein coat and the viral DNA may then utilize host cell themselves. These viruses elaborate early gene proteins (E) that are able to regulate the host cell cycle, or mitotic capabilities.[1] Cellular interactions of E6 and E7 oncoproteins and their synergy in induction of cell immortalization.[23] E6 activates telomerase and SRC kinases, and inhibits p53 and BAK. E7 inhibits pRb, with consequent release of E2F.[23] E2F activates the expression of several host genes involved in the cell cycle progression, and the E6/E7-inactivated p53 and pRb-related proteins permit the cell to escape normal check points (Table 1)[23], with subsequent loss of DNA replication[16] and up regulation of p16, which is inactivated by E7. In addition, E7 stimulates cyclins A and E, inactivates CKIs p21 and p27 and induces centriole amplification. E6 and E7 synergize in cell immortalization (dotted lines); E6 prevents apoptosis induced by high E2F levels, while E7 shields E6 from inhibition by p16 genes expressed during HPV infection.[23] The simultaneous effects of loss of both p53 and pRb function may lead to the malignant transformation of epithelial cells[16] (Fig. 2).[1,23]

High-risk HPV E6 protein

The most important function of high-risk E6 is binding of the tumor suppressor p53, a DNA-binding protein expressed in response to DNA damage or unscheduled induction of DNA replication, resulting in cell cycle arrest or apoptosis. Since HPV depends on the cellular DNA synthesis machinery and must stimulate S-phase progression for the replication of its genome, over expression of p53 inhibits viral replication.[23,24] Furthermore, E6 oncoproteins inhibit degradation of SRC-family kinases by E6-AP, stimulating mitotic activity.[23,25] As well as their effects on p53 protein, high-risk E6 proteins activate telomerase, an enzyme responsible for replicating telomeric DNA at the ends of chromosomes. E6 shows a remarkable pleiotropism in binding other host-cell proteins, leading to substantial functional consequences for E6-expressing cells, although they are not fully understood at present.

High-risk HPV E7 protein

E7 proteins are primarily localized in the nucleus, where they associate with retinoblastoma gene product (pRb) to facilitate progression into the S-phase of the cell cycle.[23,26] Besides pRb, E7 interacts with two other members of the pRb family, p107 and p130, which also negatively regulate E2F transcription. In addition, high-risk E7 stimulates the S-phase genes cyclin E and cyclin A, interacts with cyclin-kinase complexes and abrogates the inhibitory activities of cyclin-dependent kinase inhibitors (CKIs), such as p21CIP-1/WAF-1 and p27KIP-1. These interactions are a major factor in growth stimulation of HPV-infected cells, uncoupling cyclin-dependent kinase activity from CKIs and interfering with the ability of p53 to induce G1 growth arrest following DNA damage.[23,25]

Cytological and Histopathological Examination

Different methods are used to detect specific types of HPV DNA in lesions and shows varying sensitivity and specificity.[1] The detection of HPV in the oral mucosa may be done by cytology and histological examination.[1]

Gene product	Description
E1	Helicase function; essential for viral replication and control of gene transcription
E2	Viral transcription factor; essential for viral replication and control of gene transcription
E4	Interaction with cytoskeleton proteins; viral assembly
E5	Growth stimulation by interaction with growth factor receptors; down regulation surface HLA class I molecules
E6	Cell immortalization; p53-degradation; telomerase activation; anti-apoptotic effect; induction of genomic instability
E7	Cell immortalization; interaction with pRb and pRb-associated pocket proteins; transactivation of E2F-dependent promoters; induction of genomic instability
L1	Major Capsid protein
L2	Minor capsid protein; role in recruiting viral genomes for encapsidation; involvement in nuclear transport of viral DNA

Table 1: Overview of HPV gene products (E) early and (L) late.

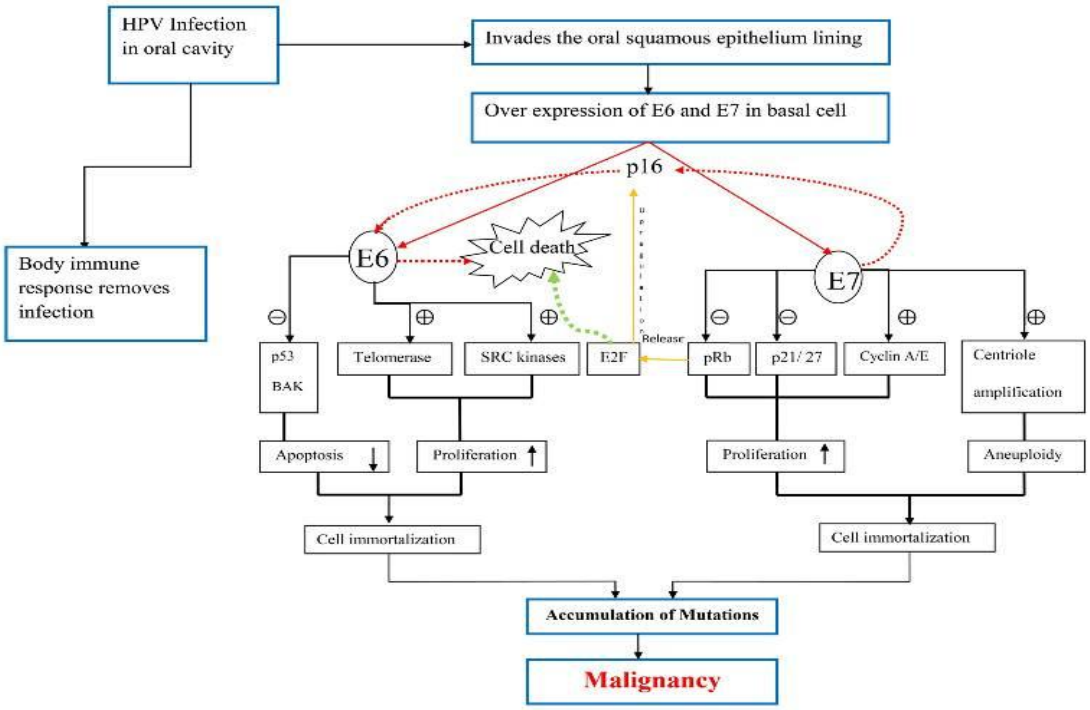

Figure 2: Mechanism of Malignancy Associated With HPV

In 1951, a Canadian cytologist Ernest Ayre described and demonstrated squamous epithelial cells with a 'perinuclear halo' in smears from the uterine cervix. These 'halo cells' were described as mononucleated or binucleated squamous cells with hyperchromatic nuclei and peri-nuclear 'clearing' (Fig 3). Ayre proposed that these squamous cells with 'halo' were 'precancer cells and some long standing infection or inflammation – viral or some other type – was responsible for the appearance of these cells.[3,27] Koilocytes (Histologic markers for HPV Presence) in histologic sections is thought to represent a cytopathic effect of HPV, and is considered to give a clue to the diagnosis of HPV infection. Presence of koilocytes has been documented in smears and tissue sections of OSCC cases (Fig 4).[1,3,28] High prevalence of koilocytes (koilocytosis) has been reported in OSCC cases, and it has been found to be equally distributed among different tumor grades.[3,29] Many studies have further confirmed this histologic evidence of HPV by performing molecular biology techniques like in-situ hybridization and Polymerase Chain Reaction (PCR), to demonstrate HPV DNA in those lesions.[3,30,31]

HPV Vaccines for Head and Neck Squamous Cell Carcinoma

Conceptually, there are three approaches to HPV vaccine development.

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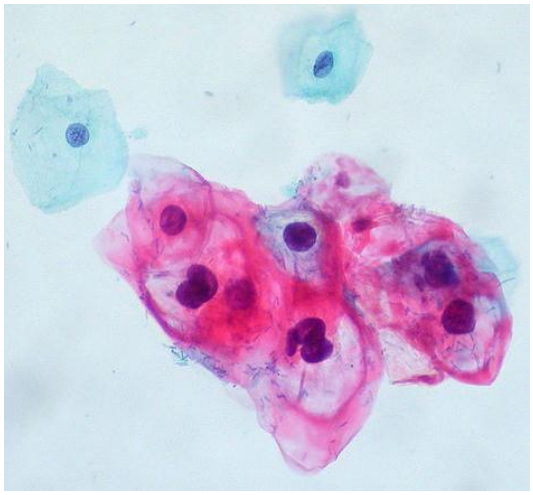

Figure 3: The hematoxylin and eosin stained photomicrograph at high power showing group of koilocytes on the bottom right is accompanied by two normal intermediate squamous cells at the top and left.

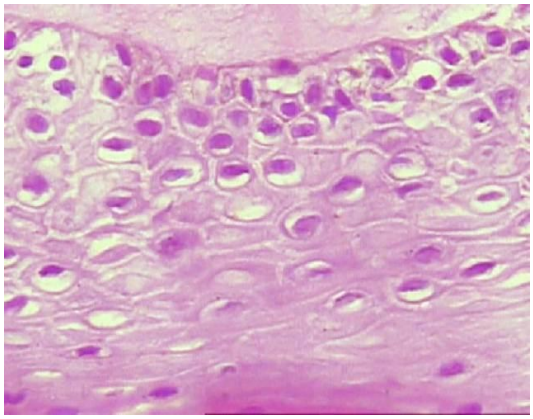

Figure 4: The photomicrograph with hematoxylin and Eosin stained high power section showing squamous epithelial layer showing koilocytosis[1]

The first approach seeks to block HPV-induced neoplasia by preventing the virus from establishing infection in the epithelium; mainly through the induction of neutralizing antibody via viral capsid protein.[32] A prophylactic vaccine should stimulate the complete neutralization of free virus upon exposure before infection can occur. However, this may be of less benefit to those individuals who already have an established infection and dysplasia. The second approach of vaccine development addresses the needs of these patients by attempting to induce a cellular immune response (both CD4+ and CD8+) which may be able to both prevent and induce the regression of approach is known as antigen-specific immunotherapy, in which effector cells, particularly T-cells, are primed against HPV antigen epitopes known to be expressed by the neoplastic cells (tumor-specific antigens) such as E6 and E7.[32] Finally, the third approach seeks to combine prophylaxis and therapy in one vaccine, in an attempt to provide total coverage for people who are newly exposed to high-risk virus and for people with current infections and dysplasia.[32]

Conclusion

HPV may play a role in the carcinogenesis of head and neck malignancies. Molecular mechanisms have been able to recognize its ability to disrupt key cellular elements responsible for the regulation of cell division and apoptosis. Review of literature reveals that HPV may be a risk factor for some head and neck malignancies, but not in all cases. In order to face the challenge of cancers caused by HPV infection, it is necessary to further unravel the viral life cycle and gain more insights in the molecular mechanisms of HPV-induced oncogenesis.

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