



RESEARCH ARTICLE

HUMAN PAPILLOMA VIRUS AND HEAD AND NECK CANCER- A REVIEW

Hari Shanker. A¹, Subhalakshmi. Sen¹, Chetana Chandrasekhar¹, Sunitha Carnelio¹Ambika Nalwar²,
and Monica C. Solomon*¹

¹Department of Oral Pathology and Microbiology, MCODS, Manipal

²Department of Public Health Dentistry, Bapuji Dental College

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ABSTRACT

Head and neck cancer is very frequent around the world about 6%. Cancers in oropharyngeal region associated with Human Papilloma Virus (HPV) type 16 oncogenic virus are presently on steep rise. HPV are small oncogenic DNA virus, nearly 200 types of which have been found till now. HPV can cause the abnormal growth of cells, but only the high-risk types (16, 18, 31, 33, 35 etc.) lead to cancer. The biology of HPV related oropharyngeal carcinomas is discrete with p53 degradation, RB pathway inactivation, p16 up regulation. Usually HPV related head and neck carcinomas are with good prognosis as they are more responsive to chemotherapy and radiotherapy than HPV negative

Better understanding of the role of this virus in head and neck cancers would open avenues for the application of target specific therapies in the management of HPV associated cancers.

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INTRODUCTION

Head and neck squamous cell carcinoma (HNSCC) is 6th most common cancer worldwide [X. Dufoura *et al* 2012, T. Ramqvist *et al* 2010, J. Nemes *et al* 2006]. Its varying rates of incidence and mortality across nations have kept researchers in a continuous quest for answers. Countries like China, India, Philippines, Hungary and France are known to have high risk association with cancer [S. Syrjanen 2005]. Despite improved diagnostic and treatment methods, the prognosis has not been satisfactory [C. Miller *et al* 1996]. Head and neck cancers involve the oral cavity, paranasal sinuses, tongue, and oro and naso pharynx [S. Syrjanen 2005]. Etiology of this disease is varied and has been linked to many local and environmental factors. Though tobacco, alcohol and environmental factors are well established etiologic agents of HNSCC [C. Miller *et al* 1996, S. Marur *et al* 2010, J. Massano *et al* 2006], recently a fraction of about 15%-20% have been attributed to other factors [M. Gillison *et al* 2000] like viruses mainly Human Papilloma Virus (HPV). Though HPV has been previously studied for its implication in cervical cancer, the detection of HPV in oral cavity suggests its possible role in the etiology of head and neck cancer. It has been found that there is a 14 fold increase in the incidence of oropharyngeal carcinoma in patients with HPV detected in their oral cavity [E. Martin *et al* 2013]. Syrjanen *et al.* for the first time in 1983 proposed the association of HPV in HNSCC [S. Syrjanen 2005] which was later supported by various authors based on evidence like the well-assessed broad epitheliotropism of HPV [D. Miller *et al* 2012], morphological similarities between oropharyngeal and genital epithelia, the ability of immortalizing human oral keratinocytes [D. Miller *et al* 2012, X. Wang *et al* 2012] in vitro and the strongly established etiological role of high risk (HR) HPV in cervical SCC.

HPV

HPV is a small environment resistant non-enveloped virus of 8kb. It has a circular and double stranded DNA [T. Ramqvist *et al* 2010, M. Longworth *et al* 2004, C. Luo *et al* 2007] and an outer capsid 55nm in thickness, comprising of about 8000 nucleotide base pairs [J. Nemes *et al* 2006, J. Haedicke *et al* 2013, C. Chung *et al* 2009, M. Lazarczyk *et al* 2009]. HPV genomes reveal a well-preserved general organisation. There are 7-9 [M. Lazarczyk *et al* 2009] or 8 Open Reading Frames (ORF) or proteins [T. Garnett *et al* 2006] grouped based on genotypes. Functionally the genome of HPV has 3 regions [A. Venuti *et al* 2011], the 1st non coding region is Upregulatory region (URR) or Long Control Region (LCR) which mainly regulates the transcription of E6 and E7 [A. Venuti *et al* 2011, F. Thierry *et al* 2008]. The 2nd region is an early region having ORFL: E1, E2, E4, E5, E6 along with E7 all of which encode no proteins taking part in viral replication and oncogenesis [A. Venuti *et al* 2011] [Table 1]. L1 and L2 structural proteins encoded in the late region form the 3rd part of viral genome [A. Venuti *et al* 2011]. Almost all the HPV detected till now have been classified into α , β , γ , μ , Nu [A. Venuti *et al* 2011] based on their tissue tropism and gene sequencing [J. Haedicke *et al* 2013]. the alpha type is known for its malignant potential and its predilection for mucosal tissue. The malignant types of HPV are classified as low risk and high risk type. Low risk type (6, 11, 42, 43, 44, 51, 61, 70, 72, 81) [J. Haedicke *et al* 2013] have known to cause condylomas [C. Miller *et al* 1996] and benign cervical lesions and are rarely seen in malignant types [C. Miller *et al* 1996], while the high risk type (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66) [J. Haedicke *et al* 2013] are known to be primary cause in cervical carcinomas [C. Miller *et al* 1996]. Other HPV types like 26, 53 and 66 are known to have potential high risk with a not well known

* Corresponding author: **Monica C. Solomon**

Department of Oral Pathology and Microbiology, MCODS, Manipal

cervical oncogenic potential [J Baseman *et al* 2005]. Both high-risk and low-risk types of HPV can cause the growth of abnormal cells, but only the high-risk types lead to cancer as only the E7 protein encoded by high-risk HPVs can immortalize human epithelial cells [C. Miller *et al* 1996] by loss of cell cycle regulation. It is important to note, that in the genital tract the large majority of high risk HPV infections regress without clinical manifestations and do not cause cancer [J. Fernandes *et al* 2012].

the viral antigen region essential to initiate host response. Some invitro studies on integrins showed 6, 4, 1 involvement, of which 6 played a dominant role in secondary binding of HPV [D. Miller *et al* 2012].

There are 3 modes of entry with convincing evidences. These include clathrin mediated, caveolar mediated and clathrin caveolar independent pathway which involves tetraspanin enriched microdomans [G. Spoden *et al* 2013].

Table 1 Showing the role of the various HPV oncoproteins

Protein	Functions
E1	Replication of viral DNA [J. Fernandes <i>et al</i> 2012], maintenance of viral episome in keratinocyte [D. Miller <i>et al</i> 2012].
E2	Segregation of viral genomes [L. Feller <i>et al</i> 2009], control of viral transcription, DNA replication - McPhillips <i>et al</i> (2006); Kadaja <i>et al</i> (2009) [J. Fernandes <i>et al</i> 2012], repress the expression of early promoter genomes [D. Miller <i>et al</i> 2012], controls the actions of E6-E7 either by transcriptional control or direct control [T. Garnett <i>et al</i> 2006].
E4	Seen in differentiated layers of epithelium, help in HPV genome amplification, regulate the expression of late genes, control the virus maturation, disrupts organisation of intermediate filaments and facilitating the release of virions [J. Fernandes <i>et al</i> 2012]. No role in transformation.
E5	Apoptotic inhibition, transcription factors upregulation, evading immunosurveillance [A. Venuti <i>et al</i> 2011]]. Co-relating clinical manifestation E5 is classified into alpha, beta, gamma, delta [A. Venuti <i>et al</i> 2011]]. Enhanced EGFR signalling [D. Miller <i>et al</i> 2012].
E6	E6 degrades p53, DNA damage and genomic instability, immortalisation Activate the expression of telomerase, inactivation of pro apoptotic factors Bax, Bac [C. Chung <i>et al</i> 2009, E. Yim <i>et al</i> 2005].
E7	Coded more by high risk than low risk type of HPV [E. Yim <i>et al</i> 2005]. Bind to pRb gene and inactivates it [C. Chung <i>et al</i> 2009, E. Yim <i>et al</i> 2005, S. Best <i>et al</i> 2012, C. Fakhry <i>et al</i> 2006, P. Lassen <i>et al</i> 2008, B. Kumar <i>et al</i> 2007], acts on proteins like p107, p130 CDK inhibitors causing cell proliferation [C. Chung <i>et al</i> 2009].
L1 major capsid protein	Contains the major factor vital for adhesion to cell surface receptors [J. Fernandes <i>et al</i> 2012]. It induce the production of neutralizing type-specific antibodies against the virus [J. Fernandes <i>et al</i> 2012, J. Icenogle <i>et al</i> 1995]. Essential for virion assembly, stability, uncoating [J. Icenogle <i>et al</i> 1995].
L2 minor capsid protein	L2 contributes to the binding of virion in the cell receptor [J. Icenogle <i>et al</i> 1995, R. Finnen <i>et al</i> 2003], favouring its uptake, transport to the nucleus and delivery of viral DNA to replication centres. Besides, L2 helps the packaging of viral DNA into capsids [R. Finnen <i>et al</i> 2003].

Mechanism of entry

HPV viruses have shown tropism towards basal layer of epithelium [D. Miller *et al* 2012, J. Haedicke *et al* 2013, T. Garnett *et al* 2006, S. Best *et al* 2012], which may be the only cell within which the virus infection can be established [D. Miller *et al* 2012]. It is thought that infection occurs at sites of injury in the proliferating basal layer of epithelial surfaces [D. Miller *et al* 2012, L. Feller *et al* 2009]. This proliferation due to micro trauma induces basal cell migration and enhanced cell division therefore increasing the probability of a productive infection. The virus for its entry into the cells of epithelium has to first gain attachment to the cell. Majority of papilloma viruses use heparan sulphate proteoglycans (HSPG’s) [J. Haedicke *et al* 2013], as attachment receptor, mainly syndecans and glypicans [D. Miller *et al* 2012] along with perlecan which are seen in abundance in extra cellular matrix (ECM) [M. Sapp *et al* 2009]. The virus particles also infect the cells by attaching themselves to ECM components that are secreted by the keratinocytes. Syndecan-1, an isotype of HSPG expressed predominantly in epithelia, is used for initial attachment and also to promote conformational changes in the viral capsid [M. Sapp *et al* 2009]. Initial changes are L1 dependant and later secondary changes are attributed to L2. The L2 terminus gets exposed immediately after the capsid interacts with the HSPG [M. Sapp *et al* 2009]. After initial conformational changes in L1 there is reduced affinity of capsid with heparan sulphates occurring simultaneously with L2 changes [M. Sapp *et al* 2009]. These interactions are thought to initiate attachment and be essential for infectious internalization but not directly responsible for mediating virion internalization [M. Sapp *et al* 2009]. There is a hypothesis stating that these interactions may result in inaccessibility of

The present model of cellular entry is micropinocytosis which includes actin dependent pathway which is independent of clathrin, caveolin, cholesterol and dynamin [J. Haedicke *et al* 2013, G. Spoden *et al* 2013]. HPV16 after binding to cells colocalize along the CD63 and CD151 transmembrane tetraspanins but their role is not completely elucidated [G. Spoden *et al* 2013].

Life cycle of HPV

Immediately after the entry of virus into epithelial cell, the life cycle of HPV is initiated [J. Fernandes *et al* 2012]. Life cycle of HPV is divided into maintenance and differentiation phase [J. Fernandes *et al* 2012].

Maintenance phase

HPV enters the epithelium through exposed microlesions [D. Miller *et al* 2012, J. Fernandes *et al* 2012]. They attach themselves to the cells in basal layer and infect the cell when keratinocytes migrate into wound. The affinity of this virus to basal cells is attributed mainly to the specific receptors which are characteristic to the basal layer. As the cell is infected by virus, activation of cascade of viral gene expression occurs resulting in formation of 20-100 viral DNA extrachromosomal copies per cell [M. Longworth *et al* 2004]. This number is maintained throughout the course of infection [M. Longworth *et al* 2004]. The infection is very mild in initial stage of infection when there is minimal expression of viral protein [M. Longworth *et al* 2004, J. Fernandes *et al* 2012]. This helps in immune evasion and containment of infection [J. Fernandes *et al* 2012]. The basal layer which is in continuous turnover forms reservoir for HPV. The viral proteins E1, E2, E6, E7, have predominant role in episomal maintenance and the protein E2 is the key regulator in viral DNA regulation as it

has both positive and negative control abilities [J. Fernandes *et al* 2012]. It regulates E6, E7, E1, and also itself. It blocks transcription factors and regulates viral DNA replication. The HPV along with the maturing epithelial cells replicate productively and the suprabasal cells begin to express E6 and E7. These E6, E7, proteins deregulate cell cycle by modifying cellular environment and cell growth regulatory pathways [D. Miller *et al* 2012, C. Fakhry *et al* 2006].

Differentiation dependant pathway

There is a low expression of viral proteins in the maintenance phase but as the epithelial cell matures the HPV goes into cycle of productive replication. This virus even after the epithelial cell reaches the terminal differentiation phase remains active. In the differentiated epithelial cell the viral genome is amplified from 50-200 in initial phase to several thousand copies [J. Fernandes *et al* 2012]. Late viral functions are activated by E1, E4, E5 of which E5 plays role in initial infection binding to epidermal derived growth factor receptor (EDGFR) [C. Chung *et al* 2009], platelet derived growth factor receptor (PDGFR), colony stimulating factor1 (CSF) and promote cell proliferation. This E5 is frequently deleted after viral incorporation into host genome [C. Chung *et al* 2009, A. Venuti *et al* 2011]. Thus it can be hypothesised that E5 has minimal or no role in late stage of infections. The epithelial cells as they progress into the granular layer, late proteins like L1 and L2 are expressed. L1 binds to L2 with disulphide bonds and forms the viral capsule which protects the virus from adverse conditions.

Pathophysiology

Human immune system usually clears the virus from the body or it remains undetected causing latent infections [S. Best *et al* 2012]. According to Richardson *et. al* (2003) the HR-HPV lasts for 12-18 months in body. The main risk of HR-HPV is its tendency to cause malignant transformation [J. Fernandes *et al* 2012]. After cervical cancer HPV infects H&N region at different anatomical locations. Mucosal lining of tonsil and base of tongue are considered to be the most common site. HPV in cutaneous sites cause warts along with certain malignancies like Epidermodysplasia Verruciformis (EV) [M. Lazarczyk *et al* 2009]. Moreover persistent infections with HPV-16&18 can sometimes result in malignancies. 20% of oral cancers and 6-60% of oropharyngeal cancers are attributed to HPV [E. Martin *et al* 2013].

Immune response by host

It is a well-known fact that HPV can remain undetected by the host for a long period by escaping the immune response [T. Garnett *et al* 2006]. But if detected the human body reacts by both humoral and cell mediated response [S. Best *et al* 2012]. The virus avoids the host defence mechanism by remaining dormant in basal cell and follows keratinocyte cell differentiation cycle. Replication of virus occurs mainly in terminally differentiated cells. In case of insufficient quantities of viral protein, HPV causes necrosis and apoptosis and the main pro-inflammatory cytokines required for activation of central signals are not produced which result in less or no inflammatory response [J. Fernandes *et al* 2012]. Humoral response in HPV are mainly regulated by immunoglobulins IgA and IgG the antibodies secreted by B-lymphocytes. Dendritic cells (DC), natural killer cells (NKC) T-Lymphocyte

secreting cytokines including interferon (INF) and tumour necrosis factor (TNF) act as a part of cell mediated response [S. Best *et al* 2012].

Langerhans cell (LC) a type of DC is the first to encounter this virus in the basement membrane of the mucosal epithelium [J. Fernandes *et al* 2012]. Previously many studies stated that host immune response was incapable of detecting the virus because of a low density in distribution of the virus. Later Hurbert *et.al* conducted studies using organoleptic culture model and showed that human immune response was able to detect and respond to the virus and it was the evading mechanism of virus which protects it from the immune system [T. Garnett *et al* 2006].

Immune system evading mechanism

HPV is capable of evading immune mechanism of host. For this usually it follows two mechanisms, adapting to keratinocyte differentiation cycle and by the inhibitory effects of the virus [S. Best *et al* 2012].

Viral adaptation to keratinocyte differentiation

Life cycle of virus is usually dependant of the differentiation of keratinocyte. The virus affects the cell which is in terminal differentiating stage, progressing to apoptosis thus escaping the immune mechanism. The HPV infects the basal cells and matures along with them. In mature cells they express E6, E7 oncoproteins which play a vital role in viral replication. E6 inhibits the cell from entering the apoptotic cycle while E7 provides the virus with necessary cellular replication required for viral DNA replication. The virus in the oral cavity shows predilection towards the deep tonsillar crypts which are immune privileged sites, where the action of T-lymphocytes is usually inhibited. Hence the virus shirks the immune mechanism.

Role of viral oncoproteins in immune evasion

E1 – most conserved of all HPV positive oncoproteins. They are expressed in less amount in HPV infections [D. Miller *et al* 2012]. It is thought that the main function of E1 is to maintain viral episome in undifferentiated keratinocyte.

E2 – mainly involved in regulating viral DNA transcription and viral genome segregation [L. Feller *et al* 2009]. The interaction of E1 and E2 is responsible for adhesion of E1 to origin of replication by stimulating DNA replication

E5 – its hydrophobic property makes it difficult to be detected. Though it remains for short period its role is crucial in immune evasion [A. Venuti *et al* 2011]. It reacts with MHC 1 and it reduces its surface expression [J. Fernandes *et al* 2012] it is also thought to react with p21 suppressing it so depressed apoptotic activity [T. Garnett *et al* 2006].

E6 – This is the main oncoprotein of HPV which interferes with p53 tumour suppressor protein. It binds to it by E6 associated protein [E. Yim *et al* 2005] (E6AP) targeting its degradation and inactivation of MDM2 [J. Fernandes *et al* 2012]. When the infected cell enters the unscheduled S phase, it prevents cell entering into apoptosis [M. Lazarczyk *et al* 2009]. E6 also acts on proapoptotic factors like Bax, Bak and deactivate them along with p53 and finally results in loss of apoptosis.

E7 – the main target of E7 is pRb gene along with it the oncoprotein also acts on pocket proteins like p107 and p130. In normal conditions pRb acts on histone deacetylase, binds to E2F transcription factor in G1 cell cycle. E7 ubiquitinates pRb causing its deregulation and thus resulting in unregulated G1/S phase of cell cycle. The action of E7 on p107 and p130 inhibitors of CDK like p21^{CIP1} p27^{KIP1} results in cellular proliferation and carcinogenesis [C. Chung *et al* 2009].

DETECTION

HPV in oral cavity were first detected by Syrjanen *et al* [S. Syrjanen 2005]. The gold standard for detection of HPV is PCR detection of E6. Real time PCR, type specific DNA insitu hybridisation and detection of HPV specific antibodies by IHC are other known methods. PCR is most commonly used but recently PCR and reverse transcriptase PCR are seen to be used the most.

Prognosis

It is now known widely that HPV positive cancers have better prognosis than other mutation induced cancers [M. Sugiyama *et al* 2007]. They respond better to chemotherapy and radiotherapy [J. Rautava *et al* 2012]. The main reasons explaining better prognosis include, the unstable nature of HPV genome, HPV positive cells having the ability to be easily induced to apoptosis and the treatment resulting in localised improvement of immunity and viral eradication. A study conducted by Caihua and Marist *et al* stated that the combined detection of HPV 16 DNA in HNSCC and immune staining with E6, E7 were clinically valuable markers of better prognosis [C. Liang *et al* 2012].

Histological picture:

HPV induced oropharyngeal cancers have been characterised by presence of non-keratinised basaloid type of histology along with moderate eosinophilic staining and some comedo type of necrosis with mild mitotic activity. There may be some variations with rare histologic type including basaloid undifferentiated adenosquamous carcinoma.

Vaccination

Two types of vaccines, bivalent and quadrivalent vaccine have currently been licenced to be used for prevention of HPV in USA. Bivalent vaccine acts against type 16, 18 while quadrivalent acts on 6, 11 along with 16 and 18. L1 protein which has tendency to ensemble to form VLP, forms the base of these vaccines. The vaccine now used is mainly for prophylactic purposes and cannot be used in previously infected or persistent infections. Apart from vaccines many antiviral and immune modulating agents are being evaluated for their role as a treatment option for viral induced oral lesion. The role of cytokines in treatment of cancer as for IFN and its role in suppression of E6, 7 gene transcripts of HPV is being considered.

CONCLUSION

HPV is a virus that causes precancerous lesions or cancer. The incidence of HPV associated oropharyngeal cancer has increased over decades. Currently there are no screening methods similar to a Papanicolaou test for detecting cell changes caused by HPV infection in oropharyngeal tissues. There is a need for noninvasive methods that can detect HPV

at an early stage. Data show the HPV vaccinations are safe and highly effective in preventing a lasting infection of the HPV types they target. However it is not known how long a single series of HPV vaccination will last, and if revaccination is required, then how often. A vaccine can only prevent infection, not cure an existing one. Therefore further research needs to be conducted to prevent and cure the HPV associated diseases.

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