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Review Article

Role of Human Papilloma Virus (HPV) in Potentially Malignant and Malignant Lesions of Head and Neck

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Abstract

The sixth most common cancer diagnosed worldwide are head and neck cancer (HNC). In 2008, 633,000 new cases were diagnosed out of which 355,000 cases resulted in mortality. Early detection of precancerous lesions remains a challenge to clinicians. In 1983, evidence of Human Papilloma Virus (HPV) infection was identified in a subset of oropharyngeal carcinomas (OPC). Thus, confirming HPV as a causative agent in HNC. HPV is now recognized as an important risk factor for the development of oropharyngeal cancers, and results of previous studies have demonstrated epidemiological and molecular evidence of the presence of the HPV genome in pre-malignant oral lesions in SCC tissues, especially of the subtypes of HPV-16 and -18. There is close to no evidence linking HPV to carcinoma. However, this review focuses on the role of HPV in oral oncology and to comment on the HPV DNA reported frequencies potentially malignant oral lesions.

Keywords: Human Papilloma Virus (HPV); Malignant Lesion; Malignancy

Introduction

The sixth most common cancer diagnosed worldwide are head and neck cancer (HNC) reportedly. The incidence of oral cancer is high owing to the habit of tobacco chewing followed by alcohol, betel quid consumption. Nonetheless, most of the population develops oral cancer even without the role of risk factors, implying that additional factors like genetic predisposition, viral agents, diet etc. may play a role in oral cancer [1]. The incidence rate of oropharyngeal cancers has been increasing notably, particularly those of the tonsils and base of the tongue, which is suggestive of an association with HPV [2].

In the1970s, Professor Haraldzur Hausen first recognized a causative association between human papilloma virus (HPV) and cancer making a Nobel Prize winning discovery of identifying the presence of HPV in cervical cancer. Thenceforth, an evidence of HPV infection was identified in a subset of oropharyngeal carcinomas (OPC Human Papilloma virus (HPV) in 1983, which is one of the most common sexually transmitted infections which may play a role in the head and neck cancer pathogenesis. HPV is now recognized as an important risk factor for the development of oropharyngeal cancers, and results of previous studies have demonstrated epidemiological and molecular evidence of the presence of the HPV genome in pre-malignant oral lesions in SCC tissues, especially of the subtypes of HPV-16 and -18. There is close to no evidence linking HPV to carcinoma. However, this review focuses on the role of HPV in oral oncology and to comment on the HPV DNA reported frequencies potentially malignant oral lesions [3].

Human papillomavirus genome

Human Papilloma Virus are a member of the new Papilloma viridae family, possessing a small diameter (50 μM) and a genome

of approximately 7200 - 8000 base pairs (5.2 x 10 dalton molecular weight), with a covering of an iso-exahedric capsid without envelope that consists of 72 capsomeres. It is a heterogeneous group of viral agents infecting epithelia, with an intra-nuclear mode of replication. Capsid proteins are constituted by a major capsid protein L1 of ~54.000 daltons molecular weight and a minor capsid protein L2 of ~76.000 daltons molecular weight [4].

L2, a highly type-specific protein, is used as a target in the immunohistochemical typing of HPV infection consequently. The viral DNA guanine-cytosine content is comparable to human host cells, ranging between 42% and 43%, ranging between 42.6% and 50% [4].

Structural characteristics

Molecular biology techniques aid in the characterization of the entire HPV genome, in which the profile of their gene expression recognizes three different functional regions. Extending approximately 45% of the genome, is the first region (Early or 'E' region) which codifies early functional proteins. The second region (Late or 'L') extends for around 40% of the viral DNA, codifying late structural proteins. Containing sequences regulating gene transcription, the third region (Long Control Region or 'LCR') performs exclusively regulatory functions (Table 1). The two first codifying regions incorporate nucleotide sequences defined as 'Open Reading Frames' (ORFs), with the potential for transcription of specific mRNA [5].

Proteins	Function
pE1	Initiating DNA replication and transcription
pE2	Controlling DNA replication and transcription (ORFs E6- E7)
pE3-pE8	Not still clear
pE4	Disrupting the cytoskeleton
pE5	Interacting with cellular proteins (EGFR)
pE6	Degrading p53
pE7	Binding Rb proteins
pL1	Capsid major structural protein
PL2	Capsid minor structural protein

Table 1: HPV genoma and codified proteins functions.

These genes de-stimulate the tumor suppressor function, regulating the functions of the p21, p53 and pRb proteins which results in apoptosis, DNA repair and cell cycle control leading to cellular immortalization. The non-coding, long control region (LCR) consists of binding sites for the E1 and E2 gene products, located just above the promoter sequence 97 (P 97) controlling the transcription of the E6 and E7 oncogenes [6].

The LCR is made up of a DNA replication origin and several binding sites for viral and host proteins to regulate viral DNA replication. Two major promoters marked are P97 and P670. HPV can assimilate into the host cell chromosome in a random pattern. During this integration, the double-stranded circular DNA opens, disturbing the function of the E2 gene [7].

Pathogenesis

Adherence to a specific receptor protein on the keratinocyte membrane is required for HPV. After the virus enters 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) regulating the host cell cycle, or mitotic capabilities. The most important are E6 and E7 proteins, which bind two host proteins that are regulators of the keratinocytes at the time of cell division. E6 binds to a molecule that arrests cell division, a protein designated p53. However, it is degraded once it is bound, abrogating the inhibition of keratinocytes mitosis. Similarly, E7 binds a protein termed Rb and thus, troubling the cell cycle regulation.

Classified on the bases of their infection in epithelial cells and the ability to effect cellular transformation for e.g. HPV 1 is responsible for the infection in cutaneous cell whereas HPV 6, 11, 18 for mucosal epithelial cells of the oral cavity, uterine cervix, oropharynx, and anogenital tract. The potentially oncogenic HPV is divided into high and low-risk types. The high-risk HPV such as 16, 18, 31, 33, 35, 52, 58, 59, 68, 73, and 82 are responsible for malignancies while the low-risk sub types (6, 11, 40, 42, 43, 44, 54, 61, 70, 72 and 81) are rarely found in carcinoma, connecting frequently with benign and potentially malignant lesions of the head and neck [7].

HPVs are mostly transmitted by close contact, especially sexually but vertical spread and self-inoculation are also recognized routes of infection [5]. After HPV inoculation, three mechanisms of infection can occur:

- Through plasmid replication, occurring in the cells of lower epithelium and is subdivided into two phases; a) amplification of viral DNA up to 50 to 400 couples/diploid genome and b) maintenance of a constant number of couples for several cell generations.

- Through vegetative replication, occurring in cells that differentiate from the epithelium and involves a link between cell differentiation and viral expression of the gene [5].

Human papillomavirus (HPV) infects the basal cells of the stratified squamous epithelia which become exposed after wounding or micro trauma. Virus particles are produced only in the surface of the epithelium, from where they are shed into the surroundings to infect new target cells. In the nuclei of basal cells, the viral genome remains as a low-copy plasmid. Expression of viral proteins is regulated by differentiation of the infected cells during their movement toward the epithelial surface. At an early stage of the infection, viral DNA is replicated, along with cellular DNA, during cell division at S-phase. At some point, a switch from stable replication (genome maintenance) to vegetative viral DNA replication will occur to allow the production of genomes for packaging into virions encapsulated by L1 and L2 [7]. Thousands of virus particles are produced per cell. After viral integration of high-risk HPVs, the expression of E6 and E7 genes are permanently upregulated. HPV E6 binds and degrades p53, which leads to inhibition of apoptosis. HPV E7 protein binds and degrades the retinoblastoma tumor suppressor protein, pRB, which disrupts its interaction with the transcription factor E2F. The release and activation of E2F results in expression of S-phase genes and cell-cycle progression. Upregulation of p16 is induced by HPV-mediated disruption of E7, leading to cellular accumulation of p16 [7].

Productive replication consists of virus being expelled from the epithelial cells at the time of desquamation and is transmitted by direct contact (especially genital warts), or by indirect contact. HPVs are characterized by a special trophism for squamous epithelial cells, keratinocytes. The synthesis of viral DNA and the expression of viral genes are linked to the keratinocyte level of differentiation [4].

The normal viral replication cycle is a highly regulated process which depends on some viral proteins codified by the viral genome as well as the degree of differentiation of the infected cell. Infection usually starts in the basal and para-basal cells of squamous epithelium changing keratinocytes from the basal layer to the surface of the epithelium, which provides a suitable micro-environment for productive cell replication, responsible for transforming the keratinocyte into a permissive cell [4]. During the initial phase of infection, when the virus colonizes basal and para-basal cells of the epithelium, the viral genome then undergoes episomal replication as it is present as an extra-chromosomal fragment of circular DNA. By this stage of episomal or early replication, relatively few copies of viral DNA (20-200) per host cell are present, acting as a reservoir of infected cells, which are morphologically indistinguishable from non-infected cells, and are responsible for the latent status of infection [4].

The viral genes are expressed sequentially from early genes to late genes when the infection becomes productive, following the epithelial squamous differentiation, which starts from basal and para-basal cells, where early portions of the viral genome are more active and proceeding to higher epithelial layers along with the formation of the complete virion [4].

HPV and oral oncology

Benign skin and mucosal lesions like ordinary warts, squamous cell papillomas, focal epithelial hyperplasia, Bowen's papillomatosis, condylomas etc. are caused by LR-HPV (i.e. HPV types 2, 4, 11, 13, 32) whereas HR-HPV (i.e. 16, 18, 31, 33,35, 58) are responsible for potentially or explicitly malignant lesions such as cervical intraepithelial neoplasms, cervical, giant condylomas of Bruschke and Lowenstein, penile and vulvar carcinomas etc [4]. In oral potentially malignant lesions (PML) the HPV DNA prevalence ranges from 0% to 85% with HPV 16 or 18 genotypes encountering a higher prevalence [4].

Chair side investigations

- Oral visual and tactile examination (VTE) is estimated to detect about 59 - 99% of these cancers [8]. Oral VTE may be carried out by clinicians helping in the detection of malignant and premalignant lesions located in the oral cavity, floor of the mouth and the ventral and lateral sides of the tongue which give rise to 2/3rd of oral cancers.

- Blood plasma uses a non-invasive method, is found to be an attractive medium for the detection of infection by HPV. The

only fluid that has an indirect contact with all the organs is the blood. However, no studies performed on patients with leukoplakia have made the association of the rate of detection of HPV in blood plasma with that of the tissue [9].

- Saliva also employs a non-invasive method for collecting material which is easy to perform in any population. It is found to be adequate for the detection of HPV[i] and it can present cells of different regions of the oral mucosa. It can facilitate HPV analysis since it is known that HPV presents a predilection for the oropharyngeal region and tonsillar pillar areas of the oral cavity [9].

- Tissue is present by the proliferation of cells in the basal and parabasal layer of the epidermis or mucosa, establishing HPV infection in the more superficial layers of the epithelium. It is thus, the best source for analyzing the rate of the epitheliotropic virus infection [9].

- Biopsies samples when obtained, were divided into two parts: one half was stored in formol and the other part was conserved in liquid nitrogen. The part embedded in formol was used to perform routine histopathological examinations whereas the liquid nitrogen part was used later for the detection of DNA of HPV. Hence proving to be functional in diagnosis as well as treatment [9].

Detection techniques of virus genome

HPV is uncultivable as direct investigation by immunological analysis or electron microscopy are not specifically useful owing to the reason antigenic viral components not always being present in the infected tissues. Definitive diagnosis of HPV infection is now done by molecular biology methods, for e.g. techniques of amplification or hybridization as histopathology can only be deemed as suggestive [4].

Cytological and histopathological examination

The detection of HPV in the oral mucosa may be done by cytology and histological examination. On this basis HPV infection is characterized by koilocytosis, nuclear dysplasia, perinuclear cytoplasmic haloes, binucleation and atypical immature metaplasia. A drawback is that it shows limited sensitivity and not all patients who have HR-HPV types in oral exfoliated cells are detected with HPV DNA in the primary tumor [6].

In situ hybridization (ISH) and immunohistochemistry

ISH techniques utilize type-specific radioactively labeled DNA probes, complementary to HPV DNA sequences which are used for detecting viruses in the premalignant and malignant lesions of the head and neck. These tests only detect the virus when it is present in more than 10 copies of the viral DNA per cell [6].

DNA extraction

Analysis of preneoplastic and neoplastic specimens was performed on six adjacent 5 mm sections of each paraffin embedded tissue, with the first section being stained with hematoxylin and eosin to visualize the extent of the abnormal cells. The remaining slices were collected in a sterile 1.5 mL microcentrifuge tube. DNA was extracted by a method generating high-quality and high-quantity DNA from paraffin-embedded tissues [1]. Normal specimens were initially placed in 2 mL neutral phosphate buffered saline in sterile 5mL centrifuge tubes. Samples were centrifuged at 2000 rpm for 15 minutes, DNA was extracted from the collected cell pellets after the DNA Extraction procedure. To avoid cross-contamination at any step of the procedure, paraffin-embedded compounds were cut between samples and were subsequently subjected to DNA extraction and PCR analysis [1].

Southern blotting

SB is assay used for the detection of HPV DNA that has been standardized for a long time. It not only differentiates between episomal and integrated DNA but also detects up to 0.1 copy of viral DNA per cell. This method has some technical variability, requiring a significant amount of DNA [6].

Polymerase chain reaction (PCR)

As PCR detects the virus in less than 1 copy of the viral DNA per cell it is regarded as a highly sensitive detection method for specific subtypes of HPV. A majority of studies used the primers MY09/11 for the purpose of detecting HPV [6].

Quantitative PCR

Employs a fluorescent probe that helps in measuring the degree of fluorescence in the reaction mixture. The advantages of quantitative PCR in premalignant and malignant lesions of the oral cavity were found by Hal., *et al* [6].

Hybrid capture II

The Hybrid Capture II technique is a nucleic acid hybridization assay along with signal amplification which employs microplate chemiluminescent detection. Detection of HPV DNA is carried out by using probes of 13 high risks HPV genotypes (16, 18, 31,33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) [6].

Gene expression: DNA microarray

It is a compilation of microscopic DNA spots on a solid surface by covalent attachment to a chemical matrix. A DNA probe is used labeled with either a fluorescent tag or a radioisotope. The probe is applied to the fragment of DNA or RNA to be studied. With the rules of base pairing (A to T, C to G) "stick" to its complementary sequence, it has been made possible to miniaturize methods of probe detection for DNA allowing detection of several thousand DNA or RNA sequences in one experiment. DNA microarrays have been used successfully to identify global patterns of gene expression in different human neoplasia, including head and neck cancer [6].

RFLP (Restriction Fragment Length Polymorphism) analysis

Restriction fragment length polymorphism (RFLP) is a type of polymorphism which results from a variation in DNA sequence that can be recognized by various restriction enzymes. These are bacterial enzymes that are used by scientists to cut DNA molecules at known locations. RFLPs can be utilized as markers on genetic maps [10].

Nested PCR (NPCR)

HPV genomic DNA is detected with a highly sensitive NPCR assay. To avoid false-negative results due to low DNA yield or low number of viral copies, extracted DNA integrity is confirmed by amplifying a 523-bp fragment of p53 exons 5–6, which is larger than the HPV fragments obtained by the PCR [1].

Dot blotting

Infected specimens, which are identified by the NPCR assay, are further analyzed by DB, using type-specific oligomer probes [1].

Nonisotopic in situ hybridization

In an additional signal-enhancing step, labeling by biotinyltyramide is applied, according to the manufacturer's directions. Visualization is carried out using diaminobenzidine tetrahydrochloride [1].

Enzyme-Linked immunosorbent assay (ELISA)

The ELISA test, developed in 1971, evaluated the interactions between antibodies and antigens, and due to its simplicity and reliability, has been widely use in the field of oncology. ELISA assay is used to detect the presence of both IgG and IgM against HPV [11]. Antibodies against HPV are a marker of prior exposure to the virus. The detection of antibodies in serum, serology, can be used to study the epidemiology of HPV. Another important use of serology is to test the immunogenicity of vaccines [12].

Conclusion

OSCC risk factors include tobacco, alcohol, ultraviolet rays etc. that are mostly responsible. However, many cases have unidentifiable risk factors. An oral malignant potential of HPV infection in oropharyngeal carcinoma is likely, particularly the association of high frequency of HPV found in oral cancers involving base of the tongue, in those occurring in younger patients and without the prior history of exposure to the usual risk factors. Still further research is required to standardize a particular protocol for screening of patients with OSCC for HPV as well as to determine a specific and universal method/assay for analysis.

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