Academicia Globe:
Inderscience Research

ISSN: 2776-1010 Volume 3, Issue 11, Nov., 2022

DETECTION AND CHARACTERIZATION OF HIGH RISK HUMAN PAPILLOMA VIRUS E2 AND E6 GENES FROM CERVICAL CANCER TISSUES

Dr. Hasan Al-hayali
Cyprus International University- Department of Bioengineering
Isfahan University of Technology- University of Isfahan -Faculty of Biological Sciences and Technology-Department of Cell and Molecular Biology and Microbiology
ORCID . https://orcid.org/0000-0001-7909-0878
hasanalialhayali@gmail.com

Abstract
Cervical cancer that is caused by the human papillomavirus (HPV) is becoming more common. It is crucial to determine a tumor's HPV status accurately since HPV+ versus HPV- cancers represent two distinct biological and clinical entities with various therapeutic approaches. Oncoproteins E6 and E7, which interfere with cellular senescence and ultimately promote carcinogenesis, are encoded by high-risk HPV subtypes. Utilizing this well-established carcinogenic pathway, current HPV detection techniques identify HPV at various biological stages. A summary of the review on HPV replication, pathogenesis, epidemiology, and detection technologies, as well as their current or prospective future roles in management and prognostication, is provided in this review article.

Keywords: human papillomavirus, replication and diagnostic methods

Introduction

The biggest burden of cervical cancer is seen in poorer nations, such as India, where cytology-based screening systems face significant challenges. Since Pap-based algorithms call for many visits, high-quality cytology necessitates skilled workers and sophisticated equipment. Infection with a high-risk human papillomavirus (HPV), typically HPV type 16 (HPV16) or HPV18, is the primary cause of cervical cancer, and gene transfer experiments have identified the E6 and E7 genes as the main viral oncogenes. In order to address the constraints associated with cytologic cervical screening, molecular methods for viral detection were developed (1) using the evidence that HPV is the primary and essential cause of cervical cancer. Women at risk for cervical neoplasia can be identified with HPV DNA testing without the inherent subjectivity of cervical cytologic evaluation. Therefore, primary screening with HPV testing has been advised (2). HPV can appear in cervical cells in episomal, integrated, or both of these forms (figure 1). The regulatory E2 protein's ability to limit the expression of oncogenes through negative feedback can be lost as a result of viral integration in the human genome, which frequently occurs in the viral E1 or E2 area. According to several research, triage and screening methods based on HPV could be supported by the identification of integrated viral forms (3).

The key determinants of infection in women are the number of sexual partners, the age at which sexual activity was first initiated, and the likelihood that at least 1 of those partners was an HPV carrier as determined by his sexual behavior patterns, according to epidemiological studies looking into risk

Academicia Globe:
Inderscience Research

ISSN: 2776-1010 Volume 3, Issue 11, Nov., 2022

factors for infection (4). Early epidemiological studies used questionnaires to examine the sexual behavior of the husbands or sexual partners of women with and without cervical cancer to learn more about the role of men in women's HPV infection. Additionally, more recent research had been able to identify the presence of HPV DNA in exfoliated cells from the distal urethra, coronal sulcus, and penile shaft (5).

The majority of research to date has concentrated on how well the self-test can identify the presence or absence of HPV. Few studies have taken into account indicators of the test's acceptability or evaluated how the women who take it feel about self-testing. Women have been shown to not only have legitimately unpleasant screening test or result-receiving experiences, but there is also a widespread lack of knowledge about the connection between cervical cancer and HPV. Women have not been specifically questioned in these studies if they think the test is acceptable. This is due to the fact that there has never been a comparable alternative before. Women were asked to conduct a self-test as well as have a medical expert conduct the test in the study that is being discussed here, and the acceptability of the two approaches was compared. The women's expressed intent to use the self-test again in the future is a sign that it is acceptable (6).

A single HPV genotype can be amplified using type-specific primers for HPV detection by PCR, or a wide range of HPV genotypes can be amplified using consensus/general PCR primer pairs. A region that is conserved across several HPV genotypes, such as the L1 capsid gene, is identified by general primers. There aren't many commercially available HPV detection systems in the United States. The first-generation Hybrid Capture Tube test is the only HPV DNA test authorized by the U.S. Food and Drug Administration and has been used as an HPV detection test (1). Recently, a second-generation assay with improved kit efficiency and analytical sensitivity was created. The U.S. Food and Drug Administration is now considering this new test, known as the Hybrid Capture II test (HC II). The foundation of the hybrid capture method is the creation of RNA-DNA hybrids between complementary unlabeled HPV RNA probes and HPV DNA that may be present in clinical specimens. Antihybrid antibodies bind to and immobilize the RNADNA hybrids (2). A monoclonal antibody reagent that is attached to alkaline phosphatase is used to react with immobilized hybrids, and the complexes are then identified using a chemiluminescent substrate reaction (3-4).

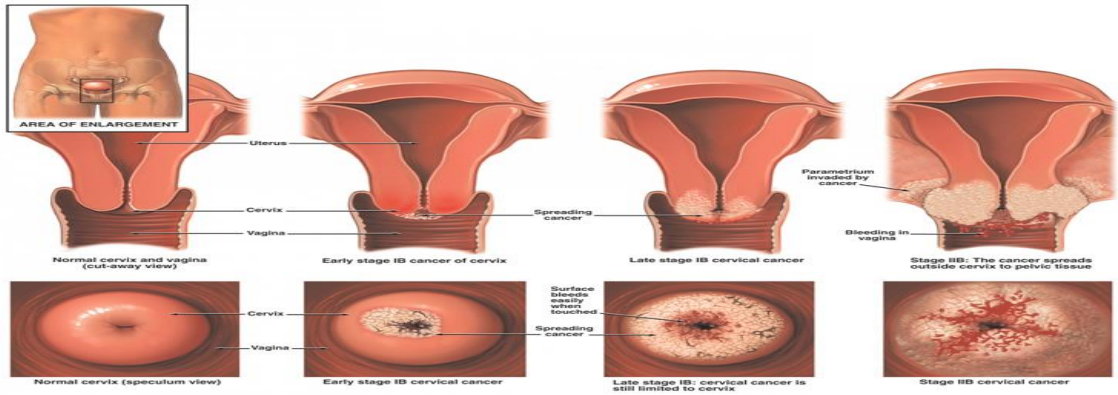

Fig .1. Different stages of cervical cancer progression (1)

Academicia Globe:
Inderscience Research

ISSN: 2776-1010 Volume 3, Issue 11, Nov., 2022

Traditional cell cultures cannot grow HPV, and serological assays offer only a limited degree of accuracy (7). Serology is not suitable for differentiating between current and previous infections since HPV infection is followed by a humoral immune response against the primary capsid protein with antibodies being detectable for many years. Consequently, the identification of viral nucleic acid is necessary for a precise diagnosis of HPV infection. Recent research has revealed an unexpectedly high incidence of HPV-DNA and different HPV genotypes in the same patient. The effectiveness of extensive community-based HPV screening studies also depends on the precision and prognostic significance of the diagnostic assays employed. Although reliable quantitative viral load measurements in clinical samples are currently technically challenging, correct HPV genotyping is necessary for the classification of patients into low-risk or high-risk groups. HPV viral load may also be a useful predictor of illness. The following objectives were developed to identify and characterize the HPV in cervical histology sample data based on the background information mentioned above (8).

Human Papillomavirus

The differentiation of the host cells directly affects how HPVs proliferate because they are only epitheliotropic. Normal squamous epithelial cells mature as stratified epithelium in the basal layers and divide as stem cells of transient amplifying cells. After dividing, one of the daughter cells migrates upward and begins to undergo terminal differentiation, whereas the other one remains in the basal layer and keeps cycling slowly and self-renewing. The productive papillomavirus infection starts when infectious virions, most commonly through micro-wounds, infiltrate cells of the basal layer. The viral genome is retained in these infected cells as a stable episome at a low copy number, acting as a reservoir for the development of a productive wart (6-8).

Replication of HPV:

The early HPV genes E1 and E2 facilitate viral DNA segregation and replication, allowing infected cells to remain in the lesion for an extended length of time. High-level amplification of the viral genome occurs as infected daughter cells move toward the epithelial surface and release viral late gene products to start the HPV life cycle's vegetative phase. Viral DNA is packed into capsids and discharged as progeny virions to re-establish infection in the epithelium's outer layers. The additional early genes E6 and E7 are necessary to coordinate the host cell environment so that it is favorable for viral DNA replication because, with the exception of the viral helicase E1, viral DNA replication is virtually entirely dependent on host replication factors. E6 and E7 cause an unscheduled re-entry into the cell cycle's S-phase in these suprabasal post-mitotic cells, activating the host replication machinery required for viral genome amplification before virion formation (9).

It is only partially understood how these proteins operate normally during the virus life cycle and how they behave. By interacting with and degrading members of the Rb family, the E7 protein propels cells into the S-phase10. This includes pRb itself and the p130 protein, which is important in the control of terminal differentiation for the high-risk HPV strains (10-12). Therefore, regardless of the presence of exogenous growth factors, E7 interferes with the interaction between pRb and the E2F family of

ISSN: 2776-1010 Volume 3, Issue 11, Nov., 2022

transcription factors. A significant number of cellular proteins necessary for DNA replication, including DNA polymerase and thymidine kinase, are then transactivated by E2F. The Bethesda system's cytopathologic classification of cervical smears continues to serve as the foundation for the present screening, which includes colposcopy and histological sampling. The Papanicolaou test primarily protects women in wealthy nations. However, there have been reports of false-negative rates of 5 to 50%, indicating that this test is not flawless (13). Thus, considerable technical advancements over the past few years have increased the likelihood of enhancing traditional cytology. Liquid-based cytology in particular has enhanced background, fixation, staining, and sampling, ensuring a more representative sample with a marked increase in sensitivity (14).

Pathogenesis of HPV:

Cervical intraepithelial neoplasia (CIN) is a sign of HPV infection, and cervical cancer is thought to develop from these lesions after a protracted period of persistent infection (14). In contrast to CIN III (severe dysplasia, carcinoma in situ) and invasive cancer lesions, which frequently exhibit high levels of E6 and E7 expression, viral DNA is typically integrated into host cell genomes in CIN I (mild dysplasia) and CIN II (moderate dysplasia) lesions, where viral genomes replicate episomally. The transition from pre-neoplastic lesions to invasive carcinoma is thought to be accompanied by integration of high-risk HPV genomes, which is thought to represent a major event in the etiology of cervical cancer (15). On the other hand, The frequency of integration, clearly distinguishes between various HPV strains. Compared to HPV types 31 and 33, HPV 16, 18, and 45 are identified in the integrated state much more frequently. Additionally, compared to precancers caused by HPV types 31 and 33, precancers caused by HPV types 18, 16, and 45 proceed to invasive cervical cancer in a significantly shorter amount of time. Integration, however, is an abnormal phase of the HPV life cycle marked by significant deletions in the viral DNA and unchecked production of the E6 and E7 oncoproteins (16). As a result, it is a byproduct of viral infection that can provide a selective advantage to the host cell while providing no obvious benefit to the virus (17).

High Risk of HPV:

Infection with the human papilloma virus (HPV), whose DNA has been detected in nearly all cases of invasive cervical cancer, is the leading risk factor for the development of cervical cancer (17). Cervical cancer has an infectious origin since HPV is a sexually transmitted illness. At some point in their lives, at least 50% of sexually active men and women contract HPV. However, 3-10% of women with HPV infection develop chronic infections and are at a high risk of getting cervical cancer. The majority of women with HPV infection will not develop cancer, and the infection typically disappears spontaneously (18). While HPV 16 tends to predominate in squamous cell carcinoma, HPV 18 appears to play an equally significant role in adenocarcinoma (19). It has received a lot of attention how high-risk E6 proteins interact with PDZ domain-containing proteins such DLG, Scribble, MAGI1-3, and MUPP-1. E6 can specifically target and degrade PDZ-domain proteins (20). Cervical tumors and their precursor lesions have changed DLG levels and intracellular localization (21-22). E6's ability to

ISSN: 2776-1010 Volume 3, Issue 11, Nov., 2022

transform in several tissue culture-based experiments and to cause tumors in mice is compromised when its C-terminal domain mediates its connection with PDZ-domain proteins (23), but not its ability to become immortal (25).

The Prevalence of HPV:

According to estimates, 11.4 percent of all females worldwide have HPV infection. However, prevalence varies significantly between nations, from 2% in South Vietnam to 43% in Zimbabwe (25). 7.9 percent of people in India have HPV infection, which is less than the global average. Despite this, South Asia has the greatest proportion of invasive cervical cancer cases that can be directly linked to HPV infection (26). The odds ratios (OR) linking HPV infection with cervical cancer are among the highest seen in any disease, and the incidence of HPV DNA is much higher in people with invasive carcinoma than in those with normal cytology (27-30).

The likelihood of contracting HPV depends on a person's level of sexual activity. Early initiation of sexual activity, several partners, unprotected sex, and sex with uncircumcised men have all been linked to an increased risk of HPV infection (24). For instance, compared to women with a single lifetime partner, those who have more than three sexual partners had a 94% higher risk of developing cervical cancer (30). Smoking, using oral contraceptives, having a large number of children, and having other sexually transmitted illnesses like HIV, Herpes, Chlamydia, gonorrhea, and syphilis are some of these (31). For instance, compared to women without children, those with high parity (three or more births) have a 51 percent higher risk of developing cervical cancer. economic evaluation Although there are interventions and preventive measures available, it is uncertain if they are financially viable in a developing nation like India (26).

In order to execute the most suitable and practical intervention options, further research is needed to evaluate the cost-effectiveness of different interventions. In particular, research is required to create effective and affordable HPV diagnostics and better cryotherapy equipment (27) At least six nonstructural proteins, E1, E2, E4, E5, E6, and E7, are encoded by human papilloma viruses. E4 is required for the HPV life cycle's productive phase, however because it is expressed at such high levels in infected cells, its suitability as a therapeutic target may be in doubt. E5 needs more research before it can be considered as a target for therapeutic intervention because its function is still mostly unclear. In contrast, both mucosal and cutaneous HPV infections are effective therapeutic targets for the E1 and E2 proteins. Therapeutics with the potential to interfere with the E1-E2 interface, their binding to DNA, or the E1 helicase ATPase function would likely be very efficient viral life cycle inhibitors with low levels of toxicity (32).

These proteins, however, would not be appropriate therapy targets for HPV-induced malignancies because they are frequently lost during the course of the disease. The E6 and E7 proteins, on the other hand, are constitutively expressed in cervical malignancies and cellular lines descended from them (26–29), are crucial for maintaining the altered phenotype (57–59), and are required for the typical viral life cycle (60–63). They both also work enzymatically by attracting components of the ubiquitin proteasome pathways to some of their respective biological target proteins, and they are both present

ISSN: 2776-1010 Volume 3, Issue 11, Nov., 2022

in modest levels. Due to all of these factors, these two proteins are the preferred targets for the therapy of HPV infection and HPV-induced cancer (33).

Diagnosis

The E6 and E7 genes of HPV subtypes include sequence variants that serve as the foundation for type-specific PCR tests. Approximately 100 bp in the E7 ORF are the target of 14 type-specific PCRs for high-risk HPV types (HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -66, and -68). In order to find inhibitory compounds, internal control primers are provided. Depending on the HPV type, these assays have an analytical sensitivity of 10 to 200 HPV copies per sample. Since the need for numerous PCR amplifications for each sample limits throughput, type-specific PCRs are now mostly used in research applications. But longitudinal studies of virgin women who started having sex during the research period give the most convincing evidence that genital forms of HPV are mostly spread through sex. In a population-based cohort research in Denmark, all the women who maintained their virginity during the study period tested negative for both serum HPV-16 antibodies and HPV DNA at enrollment and at every subsequent visit. The study included 105 monogamous women and 100 virgins (34). Only a small percentage of people who started having sex during the research period had positive tests for HPV DNA or serum HPV-16 antibodies (35).

Since HPV DNA has been found in 99.7% of examined cervical cancer cases and has been demonstrated to have greater sensitivity (84-100%) than the conventional Pap test, molecular detection of HPV DNA or RNA is presently the gold standard for identifying HPV viruses (36). The most widely used HPV tests nowadays are based on direct hybridization or DNA-based amplification methods, and there are a number of molecular assays available for detecting HPV viruses (37). These tests, however, need multiple operations and high-precision devices. Additionally, the typical PCR amplification process and product analysis can be laborious and time-consuming. Therefore, it's critical to create a quick, cheap, and simple method for HPV early detection. A new technique called loop-mediated isothermal amplification (LAMP) has recently been developed to amplify nucleic acids in an isothermal environment. Utilizing readily available laboratory equipment, such as a water bath or heating block, the reaction can be accomplished in less than an hour. The enzyme Bst polymerase, which possesses strand displacement activity, and a four-primer set that was created particularly to recognize six unique locations on the target gene are used in this approach to achieve high specificity, efficiency, and speed (38). In addition, loop primers have subsequently been added to the LAMP reaction to improve it. They cut the reaction time in half from the original LAMP approach and may improve the assay's sensitivity and specificity (40).

Due to the buildup of a white precipitate of magnesium pyrophosphate in the reaction tube, which takes less time to perform than other tests, the presence of the target sequence may be seen clearly with the naked eye (31). Therefore, using nested PCR as the gold standard, the loop-mediated isothermal amplification method for the detection of HPV types 16, 18, 45, and 58 will be developed and assessed to determine the best conditions for known clinical specimens. Broadly speaking, mucosotropic HPV types can be detected using general primer PCR techniques. This enables precise HPV typing since the

ISSN: 2776-1010 Volume 3, Issue 11, Nov., 2022

primers anneal to a highly homologous area of the HPV types. The GP5+/GP6+ and MY09/MY11 PCR systems are the most frequently used and clinically assessed generic primer PCR assays (32). After amplification with generic and consensus primers, many techniques have been employed to determine HPV genotypes. Using dot blot or microtiter plate formats, they include sequencing analysis, restriction fragment length polymorphism, and hybridization with type-specific probes (33).

Now that high throughput rapid sequencing techniques for PCR products are available, they can be used for regular clinical analysis (40). However, when a clinical sample comprises numerous HPV genotypes, sequence determination is not appropriate. Sequences that make up a small percentage of the total PCR result could go unnoticed. In turn, this might understate the frequency of infections with certain HPV genotypes, with significant ramifications for immunization or subsequent research (41). This was confirmed in a recent study from our team that compared reverse hybridization and sequencing analysis of SPF10 PCR results in 166 cervical scrapes that tested positive for HPV (22).

All samples included HPV genotypes that were compatible. Only 2% of the samples underwent direct sequence analysis, although 25% of the samples underwent reverse hybridization revealed numerous types. Multiple HPV genotypes are frequently seen in a variety of patient populations. Multiple HPV genotypes can be found in up to 35% of HPV-positive samples from patients with severe cytological diseases and more than 50% of HIV-positive patients (42) but are less common in patients with cancer (43).

Hybridization with one or more oligonucleotide probes is a frequent technique for determining the sequencing of PCR products. Type-specific probes can be used to confirm type-specific PCR results. Southern blotting is the original technique, in which a PCR product is electrophoresed before being transferred to a membrane and then being hybridized to a tagged probe (44). The epidermis (cutaneous kinds), the linings of the upper respiratory tract, and the anogenital tract are all epithelial tissues that are infected by tiny DNA viruses called HPVs (mucosotropic types) (figure 2) (45).

The distinction between low- and high-risk strains of HPV is based on how well they are able to promote malignant transformation. This idea was inspired by observations made more than 20 years ago that certain HPV types were more frequently found in cancers than in benign lesions. This idea was then supported by a large number of studies that showed that these risk categories reflect the viruses' inherent and varied capacities to affect the stability and proliferation of the infected cell's genome. In vitro cell transformation by the E6 and E7 proteins has been tested using a variety of assays. Primary human keratinocytes, the virus's natural host, primary rodent cells, such as rat embryo fibroblasts, neonatal mouse or rat kidney cells, and established rodent cell lines, such as NIH 3T3, were also frequently employed. Immortality, the development of foci and growth on soft agar, cell multiplication, and differentiation provided proof of transformation (44-46).

Academicia Globe:
Inderscience Research

ISSN: 2776-1010 Volume 3, Issue 11, Nov., 2022

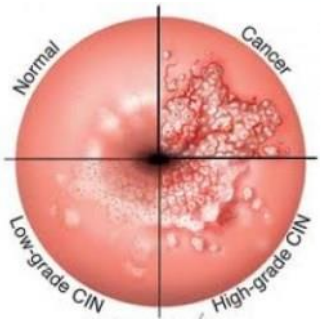

Fig 2 Morphological difference of normal and cancer (45)

Epidemiology:

The primary drivers among women are the number of sexual partners, the age at which sexual activity was first began, and the possibility that each of her partners was an HPV carrier, according to epidemiological research looking at risk factors for HPV infection (46). Since these behavioral characteristics persist throughout one's life, cervical cancer is definitely preceded by them. In the early epidemiological research, questions about the sexual conduct of the wives or sexual partners of patients with cervical cancer and controls were used to assess the role of men as potential HPV vectors. The HPV DNA in exfoliated cells from the distal urethra, coronal sulcus, and penile shaft may also be measured in more recent investigations (47).

These and other studies have demonstrated that a woman's risk of developing cervical cancer may be predicted by both her own sexual conduct and her husband's sexual behavior. The population of female sex workers is significant in the maintenance and spread of HPV infections in populations where female monogamy is prevalent. Furthermore, it has been demonstrated that the likelihood that a woman is an HPV carrier and her risk of acquiring cervical cancer are connected to the presence of HPV DNA in her partner's penis or urethra (48).

Male circumcision has been proven to protect males from being HPV carriers and their wives from having cervical cancer more recently (48) These findings supported observations made over a century ago (49) regarding HPV infections and a scientific theory developed approximately 30 years ago that male sexual activity is a major factor in the occurrence of cervical cancer (50).

Some studies reviewed research on the molecular genetics of cervical cancer and noted various cancer-induction processes. You may think of E6 and E7's impacts on host regulatory proteins as HPV-related processes. The results of viral integration and their particular influence on the integration sites may have an extra effect. The third mechanism—which might or might not be connected to HPV—is the buildup of the genetic damage to cells required for tumor formation. The observations of recurring heterozygosity losses and recurrent amplifications in a significant portion of cervical carcinomas clearly support the existence of this mechanism (51).

Experiments showing that the tumorigenicity of HeLa cells could be suppressed by fusion with healthy fibroblasts or keratinocytes or that the tumorigenicity of SiHa cells was suppressed by the introduction of chromosome 11 via microcell transfer technology also point to the involvement of unidentified tumor

ISSN: 2776-1010 Volume 3, Issue 11, Nov., 2022

suppressor genes (52). Similar to this, the addition of chromosomes 3, 4, and 6 reduced the longevity of Hela and Sitta cells (53). The goal of this discussion is not to evaluate the literature, but it is apparent that the biology of cervical cancer in connection to HPV has evolved into a model of virally mediated oncogenesis. According to frequently published research, the viral DNA found in epidemiological studies is not a passing infection of the malignant tissue but rather a biologically significant relationship (54).

Prior to the last few decades, our knowledge of cancer pathways was incomplete and frequently inaccurate. Arguments based on molecular biology assumptions did not hold up well in the face of such ambiguity (56). However, given the wealth of information currently accessible, it is possible to create a reasonable picture of the potential pathways by which cancer may arise and the potential roles of HPV infection as a catalyst. As a result, we can claim with some degree of certainty that it is possible for HPV to cause cervical cancer and that the general processes by which HPV may do so can be described with some degree of precision. Naturally, there are still many details to be discovered, but compared to the conjecture and crude models of just two decades ago, we are now very close to having a real grasp of how carcinogenesis occurs. Here is a discussion of some material that may shed light on key events in the development of HPV cancer (57).

The correlation between cervical cancer and HPV DNA in tissue samples seems conceivable and consistent with prior research. This covers both human and animal observations as well as in vitro and animal research. As molecular technology advances and is incorporated into epidemiological research protocols, new criteria of causality are being proposed and tested to explain why a generally benign viral process can occasionally end in cancer (58). Readers must be aware that the routes mentioned are the result of significant research using tissue culture, biopsied human tissues, and other molecular biology systems. However, as they have not been demonstrated to take place inside the pertinent precancerous and cancerous tissues of living hosts, many specifics of route changes and aberrant pathway effects are theoretical. The models are nevertheless highly convincing and factually consistent despite the numerous gaps and contradictions. We anticipate testing these molecular models on human beings in the future (59).

Almost all HPV types result in warty lesions, however only high risk varieties significantly increase the risk of developing cervical cancer. Given the significant DNA similarity and structural similarity among HPV strains, these discrepancies may seem surprising. But in many biological systems, a significant functional disparity brought on by minute genetic alterations is the norm (60). The E6 and E7 proteins, notably their abilities to interact with and change or delete important cell cycle regulatory components, play a major role in regulating variations in the carcinogenic potential of HPVs (61).

An HPV infection's course and outcome are influenced by the kind of HPV, the location in the body, and the type and timing of local cellular and tissue effects. (62) In regions of erosion, viral virions gain access to basal and parabasal cells, and viral DNA penetrates the cell nuclei. Epithelial cells' ability to proliferate in tissue is related to establishment, and when considerable tissue repair is required, the viral infection might spread broadly. Variable persistence in keratinocytes is correlated with virus type (63). Finally, viral DNA integration could take place, leading to the lifetime persistence of specific viral

ISSN: 2776-1010 Volume 3, Issue 11, Nov., 2022

genes in the cell. Low risk HPV types have a detectable infection in the cervix for just a brief period of time, but most high risk kinds have a longer-lasting infection. Such infections can occasionally continue for years or even decades, and in these circumstances, the risk of developing cancer is raised (67).

A capable and prepared immune system can control the spread of HPV infection. An inverse relationship between the degree of cervical neoplasia and the generation of interleukin 2 by peripheral blood mononuclear cells in response to HPV-16 E6 and E7 peptides has been found in in vitro studies (67) Compared to women with CIN 1 or HPV-infected women without lesions, women with CIN 3 or cancer seem to have a diminished capacity to establish a T helper cell type 1 (Th1)-mediated immune response to HPV E6/E7 (66). The host immune system may regulate HPV infection through a Th1-mediated cellular immune response, and the absence of such a response may predispose to the progression of cervical disease. The infection can spread broadly and remain if HPV reaches immature metaplastic basal stem cells that are actively proliferating. However, if the infection primarily affects the parabasal transit amplifying cells, it may become temporary or nearly persistent (67).

The quantity and types of cells that contract HPV can affect the size, histological grade, and longevity of lesions. Depending on the activity of the infected daughter cells, there may be intermittent viral genome amplification in either a transient or persistent infection. This might affect how accurately the lesion is detected by Pap or HPV DNA testing. The host-virus interactions between carcinogenic HPV types and low risk HPV types differ significantly; the former have activities that interfere more strongly with a variety of host cell cycle regulatory mechanisms. Therefore, it is important to think about the consequences of HPV varieties that cause cancer. Initially, HPV replicates to produce 25–50 genomes per cell (68).

Four multifunctional viral proteins, E1, E2, E6, and E7, are involved in the process by which this happens (66). E7's ability to get around the pRB tumour suppressor barrier is one of its essential functions. 176 E2F transcription factors are released after E7 binds to pRB and members of its family, which is important for boosting viral and host cell DNA synthesis. Additionally, E7 binds to and activates cyclin complexes that regulate cell cycle progression, such as p33-cyclin dependent kinase 2,192. E6 protein can circumvent the p53 protective regulatory pathways, which are crucial for avoiding genetic damage that could cause cancer (69). Once during each cell division, HPV genomes replicate in a stable state after attaching to host chromatin via the E2 protein (66). The loss of HPV DNA from cells through non-disjunction may be reduced as a result of this tethered theta mode of replication, and intracellular interference mechanisms that could cause apoptosis may be less likely to detect the presence of small amounts of HPV DNA in cells (55).

A typical differentiation and maturation process results in pyknotic condensed cells that slough from the tissue when cells differentiate and travel to the surface. However, in some spinous cells of virally infected tissues, unscheduled DNA replication is activated, and viral DNA replication is switched to the rolling circle mode, which results in the creation of viral offspring (11-14). The presence of punctate proliferating cell nuclear antigen tissue staining (a protein with a crucial role in DNA replication) and the presence of HPV virions in a fraction of upper layer cells can both be used to identify these reactivations of DNA synthesis. Both low-risk and high-risk HPV E7 proteins have the capacity to

ISSN: 2776-1010 Volume 3, Issue 11, Nov., 2022

encourage ad hoc DNA replication in spinous cells (70). It is thought that the degree of cell stimulation caused by E7 and the tissue site where it occurs are crucial factors in the development of cancer. Spinous cells produce p21cip1, a cyclin kinase inhibitor, in response to E7 by translating it from sequestered RNA. Because there is no preexisting mRNA for p21cip1 in basal and parabasal cells, the protein is generally created from newly induced transcripts by p53; however, p53 is inactivated by E6 and cannot produce p21cip1 (12).

Thus, basal cells lack the control advantage enjoyed by spinous cells. Interestingly, large concentrations of E7 can bind to p21cip1 and prevent it from functioning. Whether cells re-enter S phase and replicate viral DNA or whether cells prevent viral production is thought to depend on the relative concentrations of E7 and p21cip1. A mutually exclusive group of spinous cells with high levels of either E7 or p21cip1 are discovered through tissue examination (50). When E7 breaks through the p21cip1 block in a cell, the cell can develop into a koilocyte and release viral particles. The uneven expression of the HPV effect in tissues that are affected can be explained by this balance (45). The p53 protein, which is activated upon phosphorylation by DNA damage sensing proteins, is destroyed by the E6 oncoprotein as one of its primary functions. Because this chemical directly stimulates p21cip1, activated p53 shuts off the cell cycle in the G phase. Alternately, p53 may trigger an apoptotic pathway in cases of significant DNA damage or high viral replication rates. Alternative non-p53 dependent apoptotic mechanisms are similarly hampered by E7 (71).

Cells are therefore unable to stop the accumulation of genetic mutations in the case of E6-mediated p53 degradation. Despite the fact that cells have additional protective homeostatic systems, E6 and E7 have counter activities that can remove the barriers and cause cells to enter the S phase. Therefore, it would seem that an abnormal host-virus interaction is what leads to the development of malignancy. The abnormal control of E6/E7 expression could be a crucial step in this process (72). E6/E7 expression is mostly observed in developing spinous cells that have exited the cell cycle in low grade CIN lesions. Strong E6/E7 expression is observed in the proliferating cell compartments of high grade CIN lesions and cervical carcinomas. In many malignancies, HPV DNA is incorporated into the host genomes in a way that renders the E2 repressor protein inactive and promotes the overexpression of E6 and E7. Other mutations in the E2 protein or in repressor activities, which appear to allow ongoing production of the E6 and E7 oncoproteins, can be seen in cases when HPV integration is not found (55-59).

The production of chimaeric HPV mRNAs that encode the E6 and E7 proteins and have host sequences at their 3' termini is another way that E6 and E7 could be overexpressed in proliferating cells. These RNAs are frequently more stable and enable the synthesis of more proteins (63) Viral genomes in the basal cells of chronic HPV lesions keep the cells stimulated to overlook possible mounting DNA damage (71). Although the cells are still not immortal, E6 and E7 of high risk carcinogenic HPV types stimulate cells to form clones with a prolonged life span that have passed a point known as mortality 1 or M1 (26). Telomeres are associated with a key step in immortalization. Telomeres typically get shorter with each cell generation, and when they get too short, the cells die. Telomerase, which along with a capping function, can preserve and even prolong telomeres to allow cells to continue dividing, maintains telomere length (73).

ISSN: 2776-1010 Volume 3, Issue 11, Nov., 2022

A subsequent cell mutation can stabilize the telomeres when E6 activates telomerase, enabling cells to move on to the mortality 2 or M2 stage. How many additional independent mutations are necessary for immortalized cells to fully develop into cancer is unknown. A particular set of mutations induces a collection of new proteases that enables the cell to penetrate the basement membrane. A different mutation permits dermal cells to migrate (74).

In the late 1980s and early 1990s, there was controversy over the causal relationship between HPV DNA and cervical cancer and the justification for intervention measures based on this conclusion. The sensitivity and specificity of the HPV testing techniques available at the time were insufficient for use in large epidemiological studies. The generated results were challenging to understand, and the international literature openly expressed skepticism (74). The debate that was started at the time to determine whether it was appropriate to make public health judgments is still going strong today (75). The scientific publications coordinated by the IARC that included a significant number of scientists committed to HPV study are some of the landmarks that could serve as examples. It was still possible for HPV DNA to be a passenger, and superinfection of the neoplastic tissue could not be reliably ruled out as a possible alternative explanation, according to an IARC scientific report from 1989. A follow-up article examined the evidence once more in 1992 and came to the conclusion that measurement mistakes in HPV detection could account for earlier inconsistent epidemiological data. The review came to the conclusion that bias, chance, or confounding could be safely ruled out in the epidemiological studies and that the connection could be classified as causal in nature (76-78).

Similar exercises have been carried out by other relevant bodies, and they have repeatedly led to similar conclusions (79, 80). The US Department of Health and Human Services, the World Health Organization, the European Research Organization on Genital Infection and Neoplasia, the American College of Obstetricians and Gynecologists, the Centers for Disease Control, the National Cancer Institute (National Institutes of Health), the American Cancer Society (Cervical Cancer Resource Center), the American College of Obstetricians and Gynecologists, and the Health Technology Assessment of the U Individual scientists and peer-reviewed journals have frequently stated their understanding on the causal nature of the relationship between HPV and cervical cancer in addition to the official reviews by institutional groups (80-82)

References

1. Chaturvedi, A.K.; Engels, E.A.; Pfeiffer, R.M.; Hernandez, B.Y.; Xiao, W.; Kim, E.; Jiang, B.; Goodman, M.T.; Sibug-Saber, M.; Cozen, W.; et al. Human papillomavirus and rising oropharyngeal cancer incidence in the united states. J. Clin. Oncol. 2011, 29, 4294–4301.
2. Windon, M.J.; D'Souza, G.; Rettig, E.M.; Westra, W.H.; van Zante, A.; Wang, S.J.; Ryan, W.R.; Mydlarz, W.K.; Ha, P.K.; Miles, B.A.; et al. Increasing prevalence of human papillomavirus-positive oropharyngeal cancers among older adults. Cancer 2018, 124, 2993–2999.
3. D'Souza, G.; Kreimer, A.R.; Viscidi, R.; Pawlita, M.; Fakhry, C.; Koch, W.M.; Westra, W.H.; Gillison, M.L. Case-control study of human papillomavirus and oropharyngeal cancer. N. Engl. J. Med. 2007, 356, 1944–1956.

ISSN: 2776-1010 Volume 3, Issue 11, Nov., 2022

4. Ang, K.K.; Harris, J.; Wheeler, R.; Weber, R.; Rosenthal, D.I.; Nguyen-Tan, P.F.; Westra, W.H.; Chung, C.H.; Jordan, R.C.; Lu, C.; et al. Human papillomavirus and survival of patients with oropharyngeal cancer. N. Engl. J. Med. 2010, 363, 24–35.
5. Nulton, T.J.; Olex, A.L.; Dozmorov, M.; Morgan, I.M.; Windle, B. Analysis of the cancer genome atlas sequencing data reveals novel properties of the human papillomavirus 16 genome in head and neck squamous cell carcinoma. Oncotarget 2017, 8, 17684–17699.
6. Werness, B.A.; Levine, A.J.; Howley, P.M. Association of human papillomavirus types 16 and 18 e6 proteins with p53. Science 1990, 248, 76–79.
7. Psyrri, A.; DeFilippis, R.A.; Edwards, A.P.; Yates, K.E.; Manuelidis, L.; DiMaio, D. Role of the retinoblastoma pathway in senescence triggered by repression of the human papillomavirus e7 protein in cervical carcinoma cells. Cancer Res. 2004, 64, 3079–3086.
8. Serrano, M.; Hannon, G.J.; Beach, D. A new regulatory motif in cell-cycle control causing specific inhibition of cyclin d/cdk4. Nature 1993, 366, 704–707.
9. Li, Y.; Nichols, M.A.; Shay, J.W.; Xiong, Y. Transcriptional repression of the d-type cyclin-dependent kinase inhibitor p16 by the retinoblastoma susceptibility gene product prb. Cancer Res. 1994, 54, 6078–6082.
10. Agrawal, N.; Frederick, M.J.; Pickering, C.R.; Bettegowda, C.; Chang, K.; Li, R.J.; Fakhry, C.; Xie, T.X.; Zhang, J.; Wang, J.; et al. Exome sequencing of head and neck squamous cell carcinoma reveals inactivating mutations in notch1. Science 2011, 333, 1154–1157.
11. Poeta, M.L.; Manola, J.; Goldwasser, M.A.; Forastiere, A.; Benoit, N.; Califano, J.A.; Ridge, J.A.; Goodwin, J.; Kenady, D.; Saunders, J.; et al. Tp53 mutations and survival in squamous-cell carcinoma of the head and neck. N. Engl. J. Med. 2007, 357, 2552–2561.
12. Jordan, R.C.; Lingen, M.W.; Perez-Ordonez, B.; He, X.; Pickard, R.; Koluder, M.; Jiang, B.; Wakely, P.; Xiao, W.; Gillison, M.L. Validation of methods for oropharyngeal cancer hpv status determination in US cooperative group trials. Am. J. Surg. Pathol. 2012, 36, 945–954.
13. Lewis, J.S., Jr.; Beadle, B.; Bishop, J.A.; Chernock, R.D.; Colasacco, C.; Lacchetti, C.; Moncur, J.T.; Rocco, J.W.; Schwartz, M.R.; Seethala, R.R.; et al. Human papillomavirus testing in head and neck carcinomas: Guideline from the college of american pathologists. Arch. Pathol. Lab. Med. 2018, 142, 559–597.
14. Fakhry, C.; Lacchetti, C.; Rooper, L.M.; Jordan, R.C.; Rischin, D.; Sturgis, E.M.; Bell, D.; Lingen, M.W.; Harichand-Herdt, S.; Thibo, J.; et al. Human papillomavirus testing in head and neck carcinomas: Asco clinical practice guideline endorsement of the college of american pathologists guideline. J. Clin. Oncol. 2018, 36, 3152–3161.
15. Prigge, E.S.; Arbyn, M.; von Knebel Doeberitz, M.; Reuschenbach, M. Diagnostic accuracy of p16(ink4a) immunohistochemistry in oropharyngeal squamous cell carcinomas: A systematic review and meta-analysis. Int. J. Cancer 2017, 140, 1186–1198.
16. Rietbergen, M.M.; Snijders, P.J.; Beekzada, D.; Braakhuis, B.J.; Brink, A.; Heideman, D.A.; Hesselink, A.T.; Witte, B.I.; Bloemena, E.; Baatenburg-De Jong, R.J.; et al. Molecular

ISSN: 2776-1010 Volume 3, Issue 11, Nov., 2022

characterization of p16-immunopositive but hpv DNA-negative oropharyngeal carcinomas. Int. J. Cancer 2014, 134, 2366–2372.
17. Coordes, A.; Lenz, K.; Qian, X.; Lenarz, M.; Kaufmann, A.M.; Albers, A.E. Meta-analysis of survival in patients with hnscc discriminates risk depending on combined hpv and p16 status. Eur. Arch. Otorhinolaryngol. 2016, 273, 2157–2169.
18. Albers, A.E.; Qian, X.; Kaufmann, A.M.; Coordes, A. Meta analysis: Hpv and p16 pattern determines survival in patients with hnscc and identifies potential new biologic subtype. Sci. Rep. 2017, 7, 16715.
19. Chung, C.H.; Zhang, Q.; Kong, C.S.; Harris, J.; Fertig, E.J.; Harari, P.M.; Wang, D.; Redmond, K.P.; Shenouda, G.; Trotti, A.; et al. P16 protein expression and human papillomavirus status as prognostic biomarkers of nonoropharyngeal head and neck squamous cell carcinoma. J. Clin. Oncol. 2014, 32, 3930–3938.
20. Fakhry, C.; Westra, W.H.; Wang, S.J.; van Zante, A.; Zhang, Y.; Rettig, E.; Yin, L.X.; Ryan, W.R.; Ha, P.K.; Wentz, A.; et al. The prognostic role of sex, race, and human papillomavirus in oropharyngeal and nonoropharyngeal head and neck squamous cell cancer. Cancer 2017, 123, 1566–1575.
21. Lewis, J.S., Jr.; Thorstad, W.L.; Chernock, R.D.; Haughey, B.H.; Yip, J.H.; Zhang, Q.; El-Mofty, S.K. P16 positive oropharyngeal squamous cell carcinoma: An entity with a favorable prognosis regardless of tumor hpv status. Am. J. Surg. Pathol. 2010, 34, 1088–1096.
22. Rischin, D.; Young, R.J.; Fisher, R.; Fox, S.B.; Le, Q.T.; Peters, L.J.; Solomon, B.; Choi, J.; O'Sullivan, B.; Kenny, L.M.; et al. Prognostic significance of p16ink4a and human papillomavirus in patients with oropharyngeal cancer treated on trog 02.02 phase iii trial. J. Clin. Oncol. 2010, 28, 4142–4148.
23. Jung, A.C.; Briolat, J.; Millon, R.; de Reynies, A.; Rickman, D.; Thomas, E.; Abecassis, J.; Clavel, C.; Wasylyk, B. Biological and clinical relevance of transcriptionally active human papillomavirus (hpv) infection in oropharynx squamous cell carcinoma. Int. J. Cancer 2010, 126, 1882–1894.
24. Ukpo, O.C.; Flanagan, J.J.; Ma, X.J.; Luo, Y.; Thorstad, W.L.; Lewis, J.S., Jr. High-risk human papillomavirus e6/e7 mrna detection by a novel in situ hybridization assay strongly correlates with p16 expression and patient outcomes in oropharyngeal squamous cell carcinoma. Am. J. Surg. Pathol. 2011, 35, 1343–1350.
25. Bishop, J.A.; Ma, X.J.; Wang, H.; Luo, Y.; Illei, P.B.; Begum, S.; Taube, J.M.; Koch, W.M.; Westra, W.H. Detection of transcriptionally active high-risk hpv in patients with head and neck squamous cell carcinoma as visualized by a novel e6/e7 mrna in situ hybridization method. Am. J. Surg. Pathol. 2012, 36, 1874–1882.
26. Kerr, D.A.; Arora, K.S.; Mahadevan, K.K.; Hornick, J.L.; Krane, J.F.; Rivera, M.N.; Ting, D.T.; Deshpande, V.; Faquin, W.C. Performance of a branch chain rna in situ hybridization assay for the detection of high-risk human papillomavirus in head and neck squamous cell carcinoma. Am. J. Surg. Pathol. 2015, 39, 1643–1652.

ISSN: 2776-1010 Volume 3, Issue 11, Nov., 2022

27. Schache, A.G.; Liloglou, T.; Risk, J.M.; Jones, T.M.; Ma, X.J.; Wang, H.; Bui, S.; Luo, Y.; Sloan, P.; Shaw, R.J.; et al. Validation of a novel diagnostic standard in hpv-positive oropharyngeal squamous cell carcinoma. Br. J. Cancer 2013, 108, 1332–1339.
28. de Roda Husman, A.M.; Walboomers, J.M.; van den Brule, A.J.; Meijer, C.J.; Snijders, P.J. The use of general primers gp5 and gp6 elongated at their 30 ends with adjacent highly conserved sequences improves human papillomavirus detection by pcr. J. Gen. Virol. 1995, 76 Pt 4, 1057–1062.
29. Kleter, B.; van Doorn, L.J.; ter Schegget, J.; Schrauwen, L.; van Krimpen, K.; Burger, M.; ter Harmsel, B.; Quint, W. Novel short-fragment pcr assay for highly sensitive broad-spectrum detection of anogenital human papillomaviruses. Am. J. Pathol. 1998, 153, 1731–1739.
30. Ritchie, J.M.; Smith, E.M.; Summersgill, K.F.; Hoffman, H.T.; Wang, D.; Klussmann, J.P.; Turek, L.P.; Haugen, T.H. Human papillomavirus infection as a prognostic factor in carcinomas of the oral cavity and oropharynx. Int. J. Cancer 2003, 104, 336–344.
31. Li, W.; Thompson, C.H.; O'Brien, C.J.; McNeil, E.B.; Scolyer, R.A.; Cossart, Y.E.; Veness, M.J.; Walker, D.M.; Morgan, G.J.; Rose, B.R. Human papillomavirus positivity predicts favourable outcome for squamous carcinoma of the tonsil. Int. J. Cancer 2003, 106, 553–558.
32. Fukushima, K.; Ogura, H.; Watanabe, S.; Yabe, Y.; Masuda, Y. Human papillomavirus type 16 DNA detected by the polymerase chain reaction in non-cancer tissues of the head and neck. Eur. Arch. Otorhinolaryngol. 1994, 251, 109–112.
33. Zhang, Z.Y.; Sdek, P.; Cao, J.; Chen, W.T. Human papillomavirus type 16 and 18 DNA in oral squamous cell carcinoma and normal mucosa. Int. J. Oral Maxillofac. Surg. 2004, 33, 71–74.
34. van Houten, V.M.; Snijders, P.J.; van den Brekel, M.W.; Kummer, J.A.; Meijer, C.J.; van Leeuwen, B.; Denkers, F.; Smeele, L.E.; Snow, G.B.; Brakenhoff, R.H. Biological evidence that human papillomaviruses are etiologically involved in a subgroup of head and neck squamous cell carcinomas. Int. J. Cancer 2001, 93, 232–235.
35. Smeets, S.J.; Hesselink, A.T.; Speel, E.J.; Haesevoets, A.; Snijders, P.J.; Pawlita, M.; Meijer, C.J.; Braakhuis, B.J.; Leemans, C.R.; Brakenhoff, R.H. A novel algorithm for reliable detection of human papillomavirus in paraffin embedded head and neck cancer specimen. Int. J. Cancer 2007, 121, 2465–2472.
36. Yang, Z.; Gomez-Fernandez, C.; Lora Gonzalez, M.; Esebua, M.; Kerr, D.A. Hpv testing through p16 immunocytochemistry in neck-mass fna and its correlation with tissue samples. Cancer Cytopathol. 2019, 127, 458–464.
37. Jalaly, J.B.; Lewis, J.S., Jr.; Collins, B.T.; Wu, X.; Ma, X.J.; Luo, Y.; Bernadt, C.T. Correlation of p16 immunohistochemistry in fna biopsies with corresponding tissue specimens in hpv-related squamous cell carcinomas of the oropharynx. Cancer Cytopathol. 2015, 123, 723–731.
38. Xu, B.; Ghossein, R.; Lane, J.; Lin, O.; Katabi, N. The utility of p16 immunostaining in fine needle aspiration in p16-positive head and neck squamous cell carcinoma. Hum. Pathol. 2016, 54, 193–200.

ISSN: 2776-1010 Volume 3, Issue 11, Nov., 2022

39. Buonocore, D.J.; Fowle, E.; Lin, O.; Xu, B.; Katabi, N.; Cohen, J.M. Cytologic evaluation of p16 staining in head and neck squamous cell carcinoma in cytolyt versus formalin-fixed material. Cancer Cytopathol. 2019.
40. Holmes, B.J.; Maleki, Z.; Westra, W.H. The fidelity of p16 staining as a surrogate marker of human papillomavirus status in fine-needle aspirates and core biopsies of neck node metastases: Implications for hpv testing protocols. Acta Cytol. 2015, 59, 97–103.
41. Begum, S.; Gillison, M.L.; Nicol, T.L.; Westra, W.H. Detection of human papillomavirus-16 in fine-needle aspirates to determine tumor origin in patients with metastatic squamous cell carcinoma of the head and neck. Clin. Cancer Res. 2007, 13, 1186–1191.
42. Wong, K.S.; Krane, J.F.; Jo, V.Y. Heterogeneity of p16 immunohistochemistry and increased sensitivity of rna in situ hybridization in cytology specimens of hpv-related head and neck squamous cell carcinoma. Cancer Cytopathol. 2019, 127, 632–642.
43. Kerr, D.A.; Pitman, M.B.; Sweeney, B.; Arpin, R.N., 3rd; Wilbur, D.C.; Faquin, W.C. Performance of the roche cobas 4800 high-risk human papillomavirus test in cytologic preparations of squamous cell carcinoma of the head and neck. Cancer Cytopathol. 2014, 122, 167–174.
44. Guo, M.; Khanna, A.; Dhillon, J.; Patel, S.J.; Feng, J.; Williams, M.D.; Bell, D.M.; Gong, Y.; Katz, R.L.; Sturgis, E.M.; et al. Cervista hpv assays for fine-needle aspiration specimens are a valid option for human papillomavirus testing in patients with oropharyngeal carcinoma. Cancer Cytopathol. 2014, 122, 96–103.
45. Han, M.; Bernadt, C.T.; Murray, B.; Johnson, S.M.; Jalaly, J.B.; Garcia, T.; Adhikari, L.J. Aptima hr-hpv testing from diff-quick-stained fine-needle aspiration smears of oropharyngeal squamous cell carcinoma. J. Am. Soc. Cytopathol. 2016, 5, 221–226.
46. Rollo, F.; Dona, M.G.; Pellini, R.; Pichi, B.; Marandino, F.; Covello, R.; Benevolo, M. Cytology and direct human papillomavirus testing on fine needle aspirates from cervical lymph node metastases of patients with oropharyngeal squamous cell carcinoma or occult primary. Cytopathology 2018, 29, 449–454.
47. Channir, H.I.; Gronhoj Larsen, C.; Ahlborn, L.B.; van Overeem Hansen, T.; Gerds, T.A.; Charabi, B.W.; Vainer, B.; von Buchwald, C.; Lajer, C.B.; Kiss, K. Validation study of hpv DNA detection from stained fna smears by polymerase chain reaction: Improving the diagnostic workup of patients with a tumor on the neck. Cancer Cytopathol. 2016, 124, 820–827.
48. Dockter, J.; Schroder, A.; Hill, C.; Guzenski, L.; Monsonego, J.; Giachetti, C. Clinical performance of the aptima hpv assay for the detection of high-risk hpv and high-grade cervical lesions. J. Clin. Virol. 2009, 45 (1), S55–S61.
49. Dahlstrom, K.R.; Li, G.; Hussey, C.S.; Vo, J.T.; Wei, Q.; Zhao, C.; Sturgis, E.M. Circulating human papillomavirus DNA as a marker for disease extent and recurrence among patients with oropharyngeal cancer. Cancer 2015, 121, 3455–3464.
50. Mazurek, A.M.; Rutkowski, T.; Fiszer-Kierzkowska, A.; Malusecka, E.; Skladowski, K. Assessment of the total cfdna and hpv16/18 detection in plasma samples of head and neck squamous cell carcinoma patients. Oral Oncol. 2016, 54, 36–41.

ISSN: 2776-1010 Volume 3, Issue 11, Nov., 2022

51. Jensen, K.K.; Gronhoj, C.; Jensen, D.H.; von Buchwald, C. Circulating human papillomavirus DNA as a surveillance tool in head and neck squamous cell carcinoma: A systematic review and meta-analysis. Clin. Otolaryngol. 2018, 43, 1242–1249.
52. Vogelstein, B.; Kinzler, K.W. Digital pcr. Proc. Natl. Acad. Sci. USA 1999, 96, 9236–9241.
53. Hindson, C.M.; Chevillet, J.R.; Briggs, H.A.; Gallichotte, E.N.; Ruf, I.K.; Hindson, B.J.; Vessella, R.L.; Tewari, M. Absolute quantification by droplet digital pcr versus analog real-time pcr. Nat. Methods 2013, 10, 1003–1005.
54. Damerla, R.R.; Lee, N.Y.; You, D.; Soni, R.; Shah, R.; Reyngold, M.; Katabi, N.; Wu, V.; McBride, S.M.; Tsai, C.J.; et al. Detection of early human papillomavirus-associated cancers by liquid biopsy. JCO Precis. Oncol. 2019, 3, 1–17.
55. Chera, B.S.; Kumar, S.; Beaty, B.T.; Marron, D.; Jefferys, S.; Green, R.; Goldman, E.C.; Amdur, R.; Sheets, N.; Dagan, R.; et al. Rapid clearance profile of plasma circulating tumor hpv type 16 DNA during chemoradiotherapy correlates with disease control in hpv-associated oropharyngeal cancer. Clin. Cancer Res. 2019, 25, 4682–4690.
56. Economopoulou, P.; Koutsodontis, G.; Avgeris, M.; Strati, A.; Kroupis, C.; Pateras, I.; Kirodimos, E.; Giotakis, E.; Kotsantis, I.; Maragoudakis, P.; et al. Hpv16 e6/e7 expression in circulating tumor cells in oropharyngeal squamous cell cancers: A pilot study. PLoS ONE 2019, 14, e0215984.
57. Hanna, G.J.; Supplee, J.G.; Kuang, Y.; Mahmood, U.; Lau, C.J.; Haddad, R.I.; Janne, P.A.; Paweletz, C.P. Plasma hpv cell-free DNA monitoring in advanced hpv-associated oropharyngeal cancer. Ann. Oncol. 2018, 29, 1980–1986.
58. Veyer, D.; Wack, M.; Mandavit, M.; Garrigou, S.; Hans, S.; Bonfils, P.; Tartour, E.; Belec, L.; Wang-Renault, S.F.; Laurent-Puig, P.; et al. Hpv circulating tumoral DNA quantification by droplet-based digital pcr: A promising predictive and prognostic biomarker for hpv-associated oropharyngeal cancers. Int. J. Cancer 2019.
59. Lee, J.Y.; Garcia-Murillas, I.; Cutts, R.J.; De Castro, D.G.; Grove, L.; Hurley, T.; Wang, F.; Nutting, C.; Newbold, K.; Harrington, K.; et al. Predicting response to radical (chemo)radiotherapy with circulating hpv DNA in locally advanced head and neck squamous carcinoma. Br. J. Cancer 2017, 117, 876–883.
60. Cao, H.; Banh, A.; Kwok, S.; Shi, X.; Wu, S.; Krakow, T.; Khong, B.; Bavan, B.; Bala, R.; Pinsky, B.A.; et al. Quantitation of human papillomavirus DNA in plasma of oropharyngeal carcinoma patients. Int. J. Radiat. Oncol. Biol. Phys. 2012, 82, e351–e358.
61. Zumbach, K.; Hoffmann, M.; Kahn, T.; Bosch, F.; Gottschlich, S.; Gorogh, T.; Rudert, H.; Pawlita, M. Antibodies against oncoproteins e6 and e7 of human papillomavirus types 16 and 18 in patients with head-and-neck squamous-cell carcinoma. Int. J. Cancer 2000, 85, 815–818.
62. Herrero, R.; Castellsague, X.; Pawlita, M.; Lissowska, J.; Kee, F.; Balaram, P.; Rajkumar, T.; Sridhar, H.; Rose, B.; Pintos, J.; et al. Human papillomavirus and oral cancer: The international agency for research on cancer multicenter study. J. Natl. Cancer Inst. 2003, 95, 1772–1783.

ISSN: 2776-1010 Volume 3, Issue 11, Nov., 2022

63. Waterboer, T.; Sehr, P.; Michael, K.M.; Franceschi, S.; Nieland, J.D.; Joos, T.O.; Templin, M.F.; Pawlita, M. Multiplex human papillomavirus serology based on in situ-purified glutathione s-transferase fusion proteins. Clin. Chem. 2005, 51, 1845–1853.
64. Kreimer, A.R.; Johansson, M.; Waterboer, T.; Kaaks, R.; Chang-Claude, J.; Drogen, D.; Tjonneland, A.; Overvad, K.; Quiros, J.R.; Gonzalez, C.A.; et al. Evaluation of human papillomavirus antibodies and risk of subsequent head and neck cancer. J. Clin. Oncol. 2013, 31, 2708–2715.
65. Holzinger, D.; Wichmann, G.; Baboci, L.; Michel, A.; Hofler, D.; Wiesenfarth, M.; Schroeder, L.; Boscolo-Rizzo, P.; Herold-Mende, C.; Dyckhoff, G.; et al. Sensitivity and specificity of antibodies against hpv16 e6 and other early proteins for the detection of hpv16-driven oropharyngeal squamous cell carcinoma. Int. J. Cancer 2017, 140, 2748–2757.
66. Rubenstein, L.M.; Smith, E.M.; Pawlita, M.; Haugen, T.H.; Hamsikova, E.; Turek, L.P. Human papillomavirus serologic follow-up response and relationship to survival in head and neck cancer: A case-comparison study. Infect. Agent. Cancer 2011, 6, 9.
67. Fakhry, C.; Qualliotine, J.R.; Zhang, Z.; Agrawal, N.; Gaykalova, D.A.; Bishop, J.A.; Subramaniam, R.M.; Koch, W.M.; Chung, C.H.; Eisele, D.W.; et al. Serum antibodies to hpv16 early proteins warrant investigation as potential biomarkers for risk stratification and recurrence of hpv-associated oropharyngeal cancer. Cancer Prev. Res. (Phila.) 2016, 9, 135–141.
68. Schroeder, L.; Wichmann, G.; Willner, M.; Michel, A.; Wiesenfarth, M.; Flechtenmacher, C.; Gradistanac, T.; Pawlita, M.; Dietz, A.; Waterboer, T.; et al. Antibodies against human papillomaviruses as diagnostic and prognostic biomarker in patients with neck squamous cell carcinoma from unknown primary tumor. Int. J. Cancer 2018, 142, 1361–1368.
69. Turner, D.O.; Williams-Cocks, S.J.; Bullen, R.; Catmull, J.; Falk, J.; Martin, D.; Mauer, J.; Barber, A.E.; Wang, R.C.; Gerstenberger, S.L.; et al. High-risk human papillomavirus (hpv) screening and detection in healthy patient saliva samples: A pilot study. BMC Oral Health 2011, 11, 28.
70. Zhao, M.; Rosenbaum, E.; Carvalho, A.L.; Koch, W.; Jiang, W.; Sidransky, D.; Califano, J. Feasibility of quantitative pcr-based saliva rinse screening of hpv for head and neck cancer. Int. J. Cancer 2005, 117, 605–610.
71. Qureishi, A.; Ali, M.; Fraser, L.; Shah, K.A.; Moller, H.; Winter, S. Saliva testing for human papilloma virus in oropharyngeal squamous cell carcinoma: A diagnostic accuracy study. Clin. Otolaryngol. 2018, 43, 151–157.
72. Rosenthal, M.; Huang, B.; Katabi, N.; Migliacci, J.; Bryant, R.; Kaplan, S.; Blackwell, T.; Patel, S.; Yang, L.; Pei, Z.; et al. Detection of hpv related oropharyngeal cancer in oral rinse specimens. Oncotarget 2017, 8, 109393–109401.
73. SahebJamee, M.; Boorghani, M.; Ghaffari, S.R.; AtarbashiMoghadam, F.; Keyhani, A. Human papillomavirus in saliva of patients with oral squamous cell carcinoma. Med. Oral Patol. Oral Cir. Bucal. 2009, 14, e525–e528.
74. Hanna, G.J.; Lau, C.J.; Mahmood, U.; Supplee, J.G.; Mogili, A.R.; Haddad, R.I.; Janne, P.A.; Paweletz, C.P. Salivary hpv DNA informs locoregional disease status in advanced hpv-associated oropharyngeal cancer. Oral Oncol. 2019, 95, 120–126.

ISSN: 2776-1010 Volume 3, Issue 11, Nov., 2022

75. Chuang, A.Y.; Chuang, T.C.; Chang, S.; Zhou, S.; Begum, S.; Westra, W.H.; Ha, P.K.; Koch, W.M.; Califano, J.A. Presence of hpv DNA in convalescent salivary rinses is an adverse prognostic marker in head and neck squamous cell carcinoma. Oral Oncol. 2008, 44, 915–919.
76. Wang, Y.; Springer, S.; Mulvey, C.L.; Silliman, N.; Schaefer, J.; Sausen, M.; James, N.; Rettig, E.M.; Guo, T.; Pickering, C.R.; et al. Detection of somatic mutations and hpv in the saliva and plasma of patients with head and neck squamous cell carcinomas. Sci. Transl. Med. 2015, 7, 293ra104.
77. Ahn, S.M.; Chan, J.Y.; Zhang, Z.; Wang, H.; Khan, Z.; Bishop, J.A.; Westra, W.; Koch, W.M.; Califano, J.A. Saliva and plasma quantitative polymerase chain reaction-based detection and surveillance of human papillomavirus-related head and neck cancer. JAMA Otolaryngol. Head Neck Surg. 2014, 140, 846–854. [
78. Liu, C.J.; Kao, S.Y.; Tu, H.F.; Tsai, M.M.; Chang, K.W.; Lin, S.C. Increase of microrna mir-31 level in plasma could be a potential marker of oral cancer. Oral Dis. 2010, 16, 360–364.
79. Duz, M.B.; Karatas, O.F.; Guzel, E.; Turgut, N.F.; Yilmaz, M.; Creighton, C.J.; Ozen, M. Identification of mir-139-5p as a saliva biomarker for tongue squamous cell carcinoma: A pilot study. Cell. Oncol. (Dordr.) 2016, 39, 187–193.
80. Greither, T.; Vorwerk, F.; Kappler, M.; Bache, M.; Taubert, H.; Kuhnt, T.; Hey, J.; Eckert, A.W. Salivary mir-93 and mir-200a as post-radiotherapy biomarkers in head and neck squamous cell carcinoma. Oncol. Rep. 2017, 38, 1268–1275.
81. Wan, Y.; Vagenas, D.; Salazar, C.; Kenny, L.; Perry, C.; Calvopina, D.; Punyadeera, C. Salivary mirna panel to detect hpv-positive and hpv-negative head and neck cancer patients. Oncotarget 2017, 8, 99990–100001.
82. Schache, A.G.; Liloglou, T.; Risk, J.M.; Filia, A.; Jones, T.M.; Sheard, J.; Woolgar, J.A.; Helliwell, T.R.; Triantafyllou, A.; Robinson, M.; et al. Evaluation of human papilloma virus diagnostic testing in oropharyngeal squamous cell carcinoma: Sensitivity, specificity, and prognostic discrimination. Clin. Cancer Res. 2011, 17, 6262–6271.