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Review

Exploring the Role of HPV - from Sexually Transmitted Infection to Oropharyngeal Carcinoma

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Abstract: Human papillomavirus (HPV), a DNA virus, causes one the most common sexually transmitted infections (STI), spreading through direct sexual contact to the anogenital and oropharyngeal regions. Although most infected individuals may be asymptomatic and naturally undergo viral clearance, in some individuals HPV causes carcinogenesis of the infected epithelia. HPV now accounts for over 70% of oropharyngeal carcinomas (OPC). More than 90% of these OPC are associated with HPV 16, one of the 14 high-risk subtypes. In recent years, HPV positive (HPV+) OPC has been one of the most rapidly rising cancers, by new cancer incidence rates, especially among men. The HPV driven OPCs present some unique infection driven cellular and molecular features, constituting distinct preventive, therapeutic and prognostic strategies, in contrast to HPV negative (HPV-) OPC. In this manuscript, we drive through the role of HPV, from a sexually transmitted infection to malignancy transformation landscape, while parsing through the significant immune response environment. In this comprehensive review we collate key literature around transmission and epidemiology of HPV, while addressing the current state of clinical management, diagnosis, prevention and screening strategies, that can help shed light on this critical area.

Keywords: human papillomavirus; sexually transmitted infection; oropharyngeal carcinoma; oral cancer; head and neck cancers

1. Background

The human papillomavirus (HPV) is estimated to be the most common; prevalent and incident, STI among adults, infecting the squamous epithelial cells and spreading through any intimate skin to skin contact, including vaginal, anal or oral sex [1,2]. Subsequent to a persistent HPV infection, it is the etiological agent for a wide range of benign and malignant conditions in humans. HPV belongs to the *Papillomaviridae* family containing more than 400 types isolated from humans, birds, non-human mammals and reptiles [3,4]. These are epitheliotropic viruses and have a potential to initiate the process of squamous epithelial neoplasia – benign and malignant. Infectious agents are the cause for 13% of global cancer burden. HPV is one of the key drivers of this global, infection-caused, cancer burden [5].

Orogenital, epithelial warts and their potential infectivity have been described since the Greek and Roman era. The contagious nature of cutaneous warts was first reported by Payne (1891). Orogenital condyloma - a late 19th century terminology for genital wart was first linked with sexual behavior by Heidingsfield (1901). Subsequently, the viral etiology of genital lesions was demonstrated by Ciuffo (1907) [6]. Around 40 years later Strauss et al (1949) first isolated the virus by using electron microscopy [7]. It was during this time that all cutaneous and genital warts were associated with the same virus – the human wart virus. Subsequent molecular studies of the bovine papillomavirus helped analyze different viral subtypes, while the characterization of the viral double-stranded circular DNA were first reported by Crawford (1965) - described as HPV [8], and Klug and Finch (1965) – described as the human wart virus of the papilloma-polyoma family of viruses [9]. By 1983 Dr. zur Hausen and his group were the first to demonstrate that HPV was the causative agent for human cervical cancer, incorporating its genes into the host cell DNA [10,11]. The

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largely sexually transmitted HPV was successively found to be the cause of multiple other cancers – anogenital, laryngeal, penile, oropharyngeal, vaginal, vulvar [12–22].

1.1. Transmission of HPV

The main mode of transmission for HPV is intimate skin to skin contact including genital and extragenital (oral cavity or anus) sites. HPV is one of the most common STI. In a study by Sonawane et al, determining prevalence of oral HPV infection across NHANES data, the risk of infection was 11.5% in men and 3.2% in women. The transmission rate for high-risk HPV in men was found to be 12.7% when reported having same-sex oral sex partners, and significantly increased to 22.2% if 2 or more partners were reported [23].

The transmission of virus from women to men was found to be higher than from male to female indicating a higher prevalence of virus in men. Transmission between the female anus and scrotum was also demonstrated through non-penetrative sexual contact [24]. It was found that bisexual, gay men and men who have sex with men had 17 times increased risk of developing anal cancer as compared to heterosexual men, especially those with HIV infection [25]. Partners of oral HPV+ individuals were found to have increased prevalence of oral HPV infection indicating oral-oral transmission. There has been an association between deep mouth kissing and the development of oral HPV infection [26]. Oro-genital contact is the most important for HPV leading to oropharyngeal lesions. It was found that there was an increased incidence ratio of OPC (tonsillar and tongue carcinoma) - 2.7:1 in husbands of women who had invasive cervical cancer [27]. Another study showed that the incidence of OPC, especially in the anatomical sites of tonsils and tongue, in husbands of women with cervical cancer was much higher than those women with cervical intraepithelial neoplasia. Other modes may include autoinoculation, vertical transmission or contact with hands [28]. Autoinoculation is also thought to be a mode of transmission of the virus between the genital and oral sites. It has been observed that women who have been diagnosed with invasive cervical cancer, cervical dysplasia or cervical infection with HPV tend to have a higher prevalence of the virus in the oral samples [29]. Nosocomial transmission of the HPV is also possible which may occur during the use of flexible endoscopes used in otolaryngology may also serve as a source of infection in the oral region [30]. 3% of the probes were found to be positive for HPV DNA after examination and 1.9% were found to be positive in pre-examination samples [31]. Early onset of sexual activity in adolescents, multiple sexual partners, cigarette smoking, concomitant STD serve as a risk factor for HPV infection and its persistence [32].

1.2. Epidemiology

Newell et al in 1975 were one of the earliest to report a 5-6-fold increased risk for oral cancer in women with cervical cancer [33]. By 1982-83, Jenson et al and Syrjänen et al reported the first evidence of HPV involvement in benign oral lesions - oral squamous papillomas and oral squamous cell carcinoma (OSCC) [15,34,35]. This association was further confirmed throughout the late 1980s and 1990s, across multiple studies, demonstrating detection of HPV 16, 11, 18, 6, 2 in OPC, verrucous carcinoma, tonsil carcinoma [35].

In a key systematic review by Kreimer et al (2005), across 60 studies and covering 5,046 head and neck squamous cell carcinoma (HNSCC) specimens, HPV prevalence was found to be significantly higher in OPC than OSCC, particularly the HPV 16 subtype, while fewer HPV+ HNSCC were associated with HPV 18 and other high-risk oncogenic subtypes [36]. Further epidemiologic support of the association of HPV in OPC pathogenesis was established in a case-control study by D'Souza et al. The strength of this association was evidenced across a subgroup of OPC patients and underscoring the connection between high-risk sexual behavior and oral HPV infection, particularly HPV 16 [37].

It is now well established that around 52 - 70% of all OPC in the UK and US are attributable to HPV, particularly HPV 16 infection (85-96% of all HPV+ OPC) [38]. In contrast 3.9% OSCC, including significantly lower incidence of HPV+ carcinomas in the larynx [39,40]. Curiously, tonsillar OPC are suggested to have higher susceptibility to HPV infections as a result of the single layered

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discontinuous epithelial arrangement in the tonsillar crypts, making them more prone to carcinogenesis, compared to OSCCs. HPV- HNSCC can be associated with Epstein-Barr virus (EBV) and polyomaviruses with potential HPV co-infection [41]. It is important to note that there is still some discrepancy in data regarding the association of HPV and different anatomical subsites in HNSCC, possibly attributable to insufficient detection methods by lack of localization distinction [40].

HPV+OPC is one of the most rapidly rising cancers, especially in high income countries [42]. Some of these countries have seen a 3-fold increase in HPV+OPC, over the last 2 decades [43–46]. Interestingly, the incidence of OPC in men, involving the base of the tongue and tonsils in younger men <50 years without any history of alcoholism and smoking, has surpassed that of cervical cancer in women [47,48]. A recent systematic review and meta-analysis showed that globally almost 1 in 3 men over the age of 15 are infected with at least one genital HPV subtype, while 1 in 5 men are infected with more than one or more high-risk HPV subtypes [49]. In a case study involving 240 cases of OPC, patients who were positive for HPV-16 had a history of oral sex and multiple sexual partners while those who were negative for HPV-16 did not have the history of oral sex but had a strong history of smoking and drinking [50]. Additionally, the risk stratification for oral HPV infection in an individual is associated with frequency and number of oral sexual partners, especially within the previous 3 months. Studies have also shown that oral HPV clearance is significantly lesser in males than female [51]. Although smoking and alcohol have been demonstrated as risk factors for HNSCC and a substantial history of consumption of these agents is associated with worse outcomes even in HPV+ OSCC [52]. However, the association between HPV positivity and HNSCC, especially the OPC, demonstrates a distinct disease entity in comparison to HPV- HNSCC. Clinical studies have demonstrated that HPV+ HNSCC have more favorable prognosis and are more susceptible to radiation therapy and anticancer drugs, in contrast to HPV- HNSCC [53,54]. This may be due to the stronger immune response (tumor T-cell infiltration), tumor biology (eg; cell cycle dysregulation and impaired DNA double stranded break repair), among other mechanisms associated with HPV infection driven oncogenesis. Recently there has also been interest to help establish these factors and develop a risk-stratification strategy for HPV+ HNSCC [55–57].

2. HPV Structure

HPV is a small non-enveloped double stranded DNA virus, icosahedral and 50-60 nm in diameter. Papillomaviruses are highly species specific, infecting the epithelium and mucosa across fish to mammals, and have co-evolved with the vertebral host. The *Papillomaviridae* family is organized into 5 genera – Alphapapillomaviruses, Betapapillomaviruses, Gammapapillomaviruses, Mupapillomaviruses and Nupapillomaviruses. Many of the HPVs cause asymptomatic infections in humans and are considered normal epithelium microflora [58]. Although it is the Alphapapillomaviruses that are tropic for genital and oral epithelium and mucosa, while the Betapapillomaviruses, Gammapapillomaviruses are cutaneous HPV subtypes [59].

The mucosal type or Alphapapillomaviruses can be divided into low risk and high risk. Some of the benign conditions associated with these include plantar warts, periungual warts, anogenital warts, recurrent respiratory papillomatosis, conjunctival papillomatosis [60]. Malignant conditions include cervical, vaginal, vulvar, penile, anogenital cancers, squamous cell carcinoma of the tonsils, pharynx, base of the tongue, larynx, etc . More than 450 subtypes of the HPV have been identified [59], out of which the low risk subtypes include 6 and 11 which cause warts of the genitals, anus, mouth or throat, and larynx or respiratory tract (which may lead to respiratory papillomatosis). There are 12 high risk HPV subtypes - 16, 18, 31, 33, 35, 45, 51, 52, 56, 58 and 59, which have the propensity to cause squamous cell carcinomas [1,61], while HPV 16 and 18 are the most commonly carcinoma causing subtypes. Co-evolution with humans has allowed all these viruses to benefit, persist and replicate across diverse mucosal epithelium, anatomical and biological niches, while exploiting host cellular pathways, immune response, to engineering proliferation and differentiation [59].

The HPV genome contains 8000 base pairs containing episomes which contain 8-9 ORFs. There are 3 distinct regions in the HPV genome – early (E) spread across 50% of the genome encoding the

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nonstructural proteins, late (*L*) encoding structural proteins and representing 40% of the genome. The remaining genome is largely represented by the noncoding or long regulatory regions [62,63]. The capsid is composed of 72 pentameric capsomers all around. It is the capsid that contains the two late structural proteins *L1* (360 copies) and L2 (12 copies) [64,65]. The L1 and E1 protein encoding regions are the most conserved in HPV genome, hence HPV taxonomy is based on nucleotide sequence coding for the capsid based *L1* protein. At least a 10% Differences within the *L1* gene sequence is required for distinguishing HPV types and genotypes. While differences of 2-10% between HPV variants are considered subtypes [66,67].

2.1. Host Cell Entry & Infection

Entry of the virus into the host cell occurs when L1 binds to the heparin sulphate proteoglycans which is present in the epithelial basement membrane. There occurs a conformational change in the capsid of the virus which helps in the exposure of L2 which binds to molecules on wound keratinocyte causing conformational change again which causes the L1 to be exposed and be more accessible to attachment. Through micro abrasions, the virus gains access to the basal layer of the epithelium [68,69]. The life cycle of the virus is intra epithelial, it causes no viremia, lysis/ death of the cell. There is no role of inflammatory cytokines and the signals for the recruitment and migration of langerhans cells and dendritic cells is absent. There occurs release of the viral particles from the epithelium away from the immune cells. Once entry of the virus occurs, there occurs replication of the viral DNA as the basal cells progress to the surface epithelium. When the virus is present at the basal layer, the replication occurs at a slower rate while there is amplification of the DNA to a higher number in the superficial layer [70]. This begins the transcription of E6 and E7 which respectively act on tumor protein 53 (p53) and retinoblastoma (Rb). The E5 gene induces mitogen activated protein kinase activity which leads to cellular proliferation. After this E1 and E2 synthesis occurs. The E2 inhibits E6 and E7 transcription which then allows E1 to bind to the origin of replication. This in turn initiates replication as extrachromosomal elements. L1 and L2 are activated by the late promoter. E4 helps in maturation and release of the viral particles from the superficial layer of the epithelium. Although the viral DNA is present in all the layers, it is only in the superficial layer that the virions are present and released from [70].

2.2. HPV & The Oropharynx

Studies have shown that while the discontinuously organized oropharyngeal stratified squamous epithelium, particularly tonsillar crypt epithelial cells, may be ideal for a productive HPV infection, in fact reflect low infection rate and may be non-permissive for productive infection; based on exposure and immune control, yielding high viral titers. While the more stratified squamous epithelium of the oral cavity seems less conducive to HPV infection, in reality, HPV positivity is higher in the oral cavity [71,72]. However, these HPV+ oral cells hardly transform to carcinoma (<3.9%), and while tonsillar HPV infections are rare a significant proportion transform to carcinoma [73]. HPV driven pathogenesis in OPC, like infected epithelium transitioning to dysplastic precursors, still remains little known. HPV+ dysplastic lesions in the oropharynx are rarely observed or found [74].

Observations from the pathologic changes across high-risk HPV infected cells, where at least 20% infections result in dysplastic lesions, can help shed light into the transformation of HPV+OPC. These productive infections display mild to moderate dysplasia, without oncogenic transformation and minimal E6 and E7 expression. While in 3-5% of high-risk HPV+ proliferating cells, E6 and E7 expression is strongly upregulated, leading to moderate to severe dysplasia [75]. Herfs et al suggest that the squamocolumnar junction in non-oropharyngeal HPV infected cells, often positive for cytokeratin7 – CK7 immunostain, are associated with highly susceptibility for infections' transformation to carcinomas. Generally, productive infections in the oropharyngeal regions may be arising from basal squamous epithelial populations [76]. However, in tonsillar specimens, a strong CK7 staining of the tonsillar crypt epithelia and absence in stratified squamous epithelia, also suggest a strong association with high-risk HPV+OPC [77,78].

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Aside from the tonsillar and other oropharyngeal anatomical sites, other potential reservoirs within the oral cavity encompass inflamed gingival pockets, ductal epithelium within salivary glands, cryptal epithelium of the tonsils, the oral cavity's border, and the oropharynx—akin to the border between ectoderm and endoderm, analogous to the transformation zone observed in the uterine cervix [79]. Since gingival pockets have basal cells – the known targets of latent HPV infection it is hypothesized that inflamed gingival pockets could be a possible first site of infection in oral mucosa. A study by Tezal et al also concluded that chronic periodontitis played a significant factor in the natural history of HPV infection in patients with base of tongue cancers [80]. Benign oral lesions such as squamous papilloma is the most common manifestation of oral HPV infection and is associated with HPV 6, 11. It is localized on the nonkeratinized mucosa (lingual belly, soft palate) or keratinized (hard palate) and appears as an exophytic neoformation [81].

3. HPV & Molecular Features of Oncogenesis

Replication of the viral genome and the transcription of proteins is regulated by E1 and E2 proteins [82,83]. E2 protein also helps in the repression of transcription of the oncoproteins E6 and E7. Hence, the loss or deletion of E2 protein results in upregulation of the oncoproteins leading to tumorigenesis [84]. E6 and E7 proteins are key drivers of malignant transformation in high-risk HPV subtypes. They facilitate integration of the viral genome to host DNA, inactivate tumor suppressor proteins, like p53 - associated with 60-80% HNSCC, and Rb which promotes tumor growth [85–87]. E6 and E7, also help in viral replication into the keratinocytes, while promoting cell-cycle progression [88,89]. HPV+OPC is hence very different from HPV- carcinoma, relating to the distinct roles of E6 and E7 and the p53, Rb pathways disruption [90].

An early and frequent genetic alteration in HNSCCs is the cell cycle control pathway. Loss of *CDKN2A* combined with amplification of cyclin D1 causes unscheduled DNA replication, through G1-S checkpoint of cell cycle – leading to DNA damage and p53 activation [91]. The binding of E6 protein to cellular E3 ligase and E6 associating protein leads to the formation of a heterodimer which degrades the p53 via ubiquitin- proteasome pathway [85,86,92]. The E6 protein causes repression of apoptosis and promotes the survival of damaged cells which leads to immortalization of cells. It also upregulates telomerase, maintaining telomere length and preventing senescence in continually proliferating cells [93,94]. Similarly, E7 protein which plays a role in cell growth and replication causes degradation of pRB, likely by inactivating cell cycle regulators like cyclin D1, CDK6 [95,96].

Although E5 protein is weakly oncogenic, it is encoded only by Alphapapillomaviruses, it helps in enhancement of the oncogenic potential of E6 and E7 proteins. E5 also causes downregulation of antigen processing, immunoproteasome function inhibition – downregulating host antiviral responses [97,98]. There is evidence that E6, E7 and E5 oncoproteins also alter multiple other cellular signaling pathways - JAK-STAT activation plays an important role in the carcinogenesis, making the cancer cells more sensitive to the chemotherapeutic drugs [99–101]. E6 is shown to enhance the Wnt/β-Catenin Pathway, possibly as the key mediator of a broad range of cell proliferation genes [102]. PI3K signaling activation by somatic mutation and/or copy number alteration is another key feature of HPV+OPC, early on during carcinogenesis [103,104]. Loss of function mutation of *CYLD* and *PTEN*, and gain of function mutation in FGFR3 – all activating PI3K signaling, are significantly enriched in HPV+OPC [50]. Indeed, the PI3K amplifications have been associated with longer overall survival in these OPC [105].

These oncogenic HPV drive phenotypical changes in epithelial cells, causing a polar shift of infected cells towards tissue invasion, metastatic potential, inhibition of apoptosis and senescence, creation of an immunosuppressive microenvironment, potential therapy resistance – identified as oncogenic epithelial-mesenchymal transition (EMT). E6 and E7 induce the development of spindle shaped mesenchymal-like translation of the otherwise cobblestone-shaped epithelium and induce the expression of Slug, Twist and ZEB 1/2 transcription factors associated with upregulated tumor invasion [106]. E7 also causes actin reorganization and cell adhesion changes [107]. E5 upregulates EGFR and allows evasion of programmed cell death. Loss of the typical apico-basal orientation is driven by detachment of cells from the basal membrane through proteolytic degradation and

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associated angiogenesis - promoting metastasis of HPV+ cancers, by upregulating MET and hepatocyte growth factor receptor [108,109]. This causes chronic stromal inflammation. Although, the virus inhibits the NF-Kb which signals the inflammatory pathway, as the progression of the cancer takes place, there is increased inflammation. There is increased recruitment of Th17 which is mediated by CCL20. It promotes the further growth of the tumor and angiogenesis. Figure 1 summarizes HPV driven oropharyngeal epithelial infection and carcinogenesis processes.

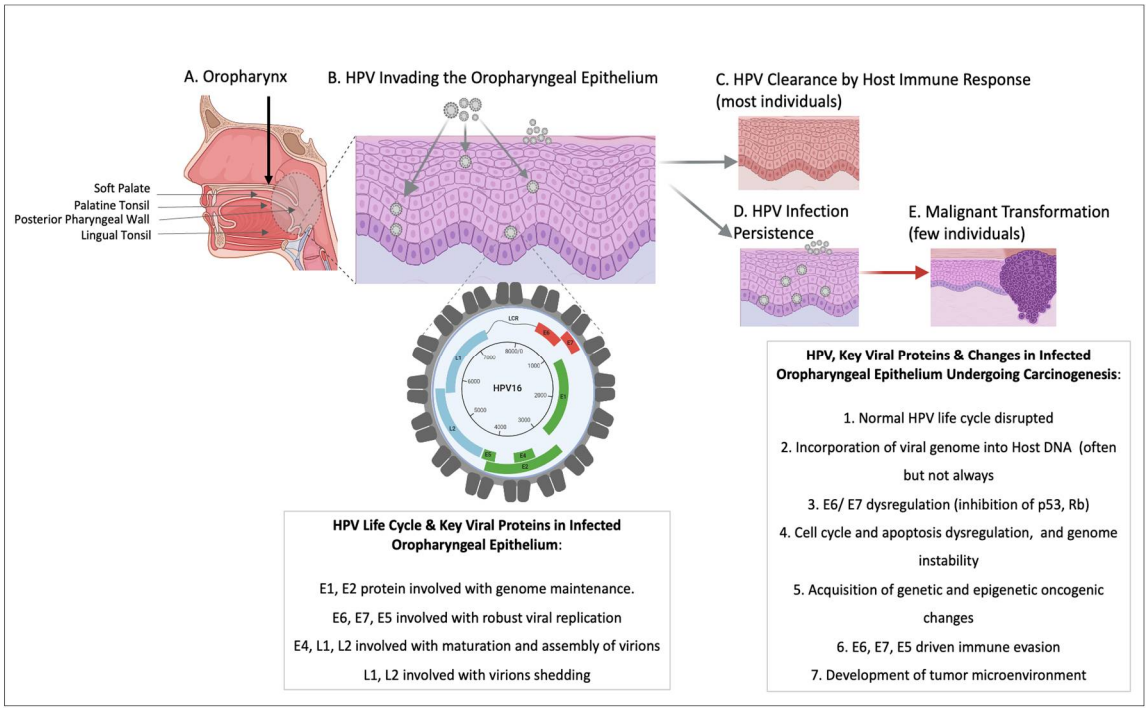

Figure 1. A schematic of the HPV driven oropharyngeal epithelial infection and carcinogenesis. The oropharyngeal anatomy involving the soft palate, palatine tonsil, posterior pharyngeal wall and lingual tonsil may be sites for HPV infection, largely sexual transmission (A). HPV infection of the oropharyngeal epithelium in B describes the life cycle and key viral proteins involved in its genome maintenance, replication, maturation, virion assembly and shedding across the various host squamous stratified epithelial cells layers (viral copy number is higher in the superficial layer and reduces towards the basal epithelial layer). In most individuals, HPV infection clearance occurs naturally without any further consequence (C). In some individuals HPV infection may persist for years without further changes (D). Further, in some individuals persistent E6/E7 driven clonal selection and expansion (among other viral proteins) can drive malignant transformation (E). Figure created using Biorender.com.

4. HPV & Host Immune Response

The well-orchestrated life cycle of HPV mediates multiple host immune response evasion strategies. The co-evolution of the host and HPV, across multiple anatomical sites, has allowed the virus to strategize defenses not only through cell-mediated immune responses but also physically. Owing to the entire life cycle of the HPV occurring within the host epithelial cells particularly the most well-differentiated, superficial layers of the stratified squamous epithelium, and preventing any cell lysis or viremia immediately post infection, the viral machinery and infected cells are situated far away from innate host defenses while it undergoes high levels of viral gene expression and replication [110]. Its virions eventually do shed alongside fully differentiated superficial cells, during desquamation, but this early and robust viral replication benefits from immune non-detection. This strategy also significantly impedes any antibody-mediated humoral or T cell response in infected individuals, against HPV proteins [111,112]. Indeed, another early response to evade the innate

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immune surveillance begins immediately at the time viral entry into the host epithelial cell, hitchhiking its genome via the protection of endosomal vesicles into the nucleus [110].

The viral L2 and other early viral proteins, especially in high-risk oncogenic subtypes, further disrupts the innate immune signaling pathway by repressing the antiviral component of the host cell nuclear bodies, during early stages of infection [113]. In most HPV infections, the cell-mediated immune response does eventually recognize the infected cells and responds to HPV viral proteins - L1 capsid protein, E6, and E7 being the primary antigens recognized, subsequently attracting T cells to infiltrate for infection resolution and clearance [114]. Interestingly, post-infection humoral response against specific HPV types is highly limited, in contrast to HPV vaccination where the humoral response to the vaccine achieves high seroconversion at the levels of infection site, mucus and serum [115]. The persistence of the virus occurs when there are not enough effective immune control mechanisms. There is clearance of the virus in immunocompetent patients, while those who have impaired immunity show a higher prevalence of the infection and subsequent carcinogenesis [116]. As an example, Fanconi anemia patients have very high susceptibility to HPV infections leading to carcinoma, proposed to be associated with the high genetic mutations in cells as a result of HPV oncogene promoted genetic instability in Fanconi anemia cells with already defective DNA repair mechanism [117]. Carcinogenesis through oncogenic HPV is not a process that occurs within a few days, it takes years to occur and the immune system evasion of HPV plays a key role in this process.

Studies have shown that oncogenic HPV E5 and E7 antagonize activating stimulator of interferon genes (STING) protein, thus downregulating the viral detection pathway via Type-1 interferon (IFN) response. E5 protein allows evasions of both innate and adaptive immunity. It disrupts the function and synthesis of major histocompatibility complex (MHC) class I and II proteins, in turn promoting immune evasion by preventing infected cell recognition by the CD8+ cytotoxic T cells [97,118]. Antigen presenting cells such as langerhans cells and dendritic cells play a role in clearing the infection. HPV causes a reduced expression of E-cadherin on the epithelium which is why the localization of langerhans cells is reduced. Toll-like receptors generate an immune response against the virus which releases inflammatory cytokines like interferons, tumor necrosis factor and interleukins [119]. E6 and E7 oncogenes also prevent secretion of CXCL14 chemokines and NF-κB-dependent CCL20, interfering with langherhan and dendritic cell migration to infection sites [120,121]. The virus induces a lot of immune evasion mechanisms. Not only does the virus have the ability to hide itself from recognition by downregulation of the antigens, it sheds from the superficial layer of the epithelium where the presence of immune cells is minimal [111].

Immunosuppressive microenvironments are well established across most tumors, including avoiding recruitment of potent cytotoxic natural killer (NK) cells. There is also impaired tumor infiltrating T cell recognition of antigens, cytokine secretion and activation of CD8+ cytotoxic T lymphocytes (CTLs), a T cell subset efficient in anti-tumor responses. It has been observed that HPV-positive HNSCC and OPC had a significantly higher tumor infiltrating $CD4^+$, $CD8^+$, and $CD3^+$ subsets and chemokine response [122,123]. HPV positive OPC are one of the most highly immune-cell infiltrated tumors that includes - $CD3^+$ T cells, $CD8^+$ T cells, Treg cells, B cells, and plasma cells, in comparison to HPV- OPC [124]. Additionally, ~64% of HPV+OPC have been shown to have HPV 16 specific infiltrating T cells as well [125]. Some studies have recently shown that tissue infiltrating macrophages like the M1 (CD68+) macrophage in HPV+OPC also add to anti-tumor activity by supporting CD4+ T cell differentiation, activation of CD8+ T cells. Multiple studies have shown that tumors with high CTL infiltration, also had high chemokine signature and were associated with improved patient prognosis and overall survival [126].

However, despite the high tumor microenvironment infiltration HPV+OPC still exhibit continuous immune evasion and disease relapse by benefitting the immune checkpoint mechanisms. One such checkpoint – programmed cell-death 1 (PD-1) expressed on activated T and B cells limits T cell function and is seen to be highly expressed in some HPV+OPC [127]. These tumors are now being targeted by immune checkpoint inhibitors across some key clinical trials and demonstrating

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improved response rate [128–131], paving the way for further assessment of various immune-checkpoint inhibitors for treatment.

5. Clinical Management

5.1. Standard Management of HPV

Individuals who have developed HPV symptomatic infections may sometimes require treatment. Unlike other viral infections that respond to drug therapy, there are no active ingredients currently available to eliminate HPV infections or regress the clinical lesions. Antiviral drugs like acyclovir and ribavirin have shown no success in eliminating oral HPV infections, mirroring the lack of effective treatment for their genital counterparts [81]. The commonly available treatments available for papillomas, condylomas, verrucas, and Heck's diseases are cryotherapy, electrosurgery, surgical removal, laser therapy, and trichloroacetic acid [79]. Lordyua et al. demonstrated that three applications (each lasting 30–60 seconds) of trichloroacetic acid led to atraumatic resolution of such oral lesions within 45 days. Additionally, Mendoz-Flores et al. successfully treated Heck's lesions by applying topical 5% imiquimod cream every night for two weeks [132,133].

5.2. Management of OPC

Approximately 30 - 40% of all OPC patients present at an early-stage, and are typically treated with curative intent, employing either single-modality treatments such as radiotherapy with or without chemotherapy, or surgery alone. The decision between the two modalities is generally influenced by an evaluation of functional, cosmetic outcomes and competing morbidities, as both options yield comparable rates of local control and survival [134]. Up to 50% of fatalities in HNSCC are attributed to locoregionally recurrent disease as the exclusive site of failure [135]. In cases where surgery serves as the primary treatment, adjuvant therapy is traditionally considered if positive or close margins, bone involvement, or pathologically positive lymph nodes are present. Risk stratification is key to designate patients into management options to achieve improved outcomes. Studies have shown that primary surgery without adjuvant therapy (chemoradiotherapy with concurrent cisplatin and post-operative radiotherapy) increases risk for recurrence [136]. Ongoing and recently concluded clinical trials exploring the effectiveness of de-escalated adjuvant therapy or reduced-dose radiotherapy are finding favorable outcomes in the target group (eg; PATHOS - NCT02215265, MINT - NCT03621696, NCT04178174, Quarterback trials - NCT01706939, ECOG3311, DELPHI - NCT03396718). Up to 25% patients undergo disease relapse within 2 years of initial diagnosis. Effective follow up and monitoring through examination every 1-3 months in the first year, 2-6 months in the second year and 4-8 months until 5 years is recommended by the National comprehensive Cancer Network [137].

The utilization of immunotherapy in HPV+ cancers has been explored for over 20 years. In 2016, Pembrolizumab and nivolumab, two anti-PD-1 antibodies was approved for use in platinum-refractory metastatic HNSCC, while the former was approved as first-line monotherapy in PD-L1+ unresectable or metastatic HNSCC – both studies including HPV+ carcinomas [128,130,131]. Further studies branching from these three clinical trials have explored the efficacy of PD-L1 blockade, showing improvement in overall survival, objective response rates [138]. These landmark studies have allowed the introduction of the PD-1 inhibitors as frontline and second-line therapy for recurrent/metastatic (R/M) HNSCC, per current ASCO guidelines [139]. Several ongoing clinical trials are also investigating immune checkpoint inhibitors in a curative setting, alternative immune checkpoint inhibitors, immunotherapy vaccines, and chimeric antigen receptor (CAR) T-cell therapy against HPV+OPC with standard of care therapy (some eg; Keynote-689 - NCT03765918, IMSTAR-HN - NCT03700905, RTOG 1216-NCT01810913, NCT04080804, NCT03690986, NCT04847466, NCT05639972, NCT04290546).

Recently, some clinical trials have also examined targeted therapy – anti-EGFR monoclonal antibody (Cetuximab), in combination with other treatment modalities, an alternative to cisplatin (De-ESCALaTE HPV - ISRCTN33522080, NRG Oncology RTOG 1016 - NCT01302834). However, the

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cetuximab treatment arm was associated with no reduction in treatment associated morbidity or toxicity, lower overall survival and high recurrence rate during the 2-year timeline, in comparison to the cisplatin arm. Genomic studies have shown that the difference in EGFR expression in HPV+ tumors compared to HPV- OPC may demonstrate the lack of efficacy with anti-EGFR monoclonal antibodies [140].

E6/E7 based therapeutic vaccines, in combination with immunomodulatory agents are also currently being explored in trials with HPV+OPC patients; eg: HARE-40 - NCT03418480. In a first, a human trial - NCT04180215, the E6/E7-targeting single vector therapy and two-vector therapy is being examined across metastatic or recurrent HPV+OPC patients, otherwise eligible to receive pembrolizumab as standard care.

6. Prevention

Currently, three prophylactic vaccines have been approved for HPV infections. Cervarix (*GlaxoSmithKline*), Gardasil-4 (now discontinued in the US), and Gardasil-9 (*Merck Sharp* and *Dohme*). These vaccines consist of virus-like particles (VLPs) derived from the major capsid protein (L1) of HPV. While these vaccines exhibit high immunogenicity, it's essential to note that their effectiveness is primarily limited to the HPV types specifically included in the vaccines. These L1 proteins from which these VLPs are derived, lack conservation across various HPV types, resulting in minimal cross-protection against non-vaccine HPV types [141–143].

Cervarix, a bivalent vaccine, predominantly protects against HPV16 and HPV18, is approved for females aged 9 to 25, while Gardasil-4 - a tetravalent vaccine, protects against HPV6, HPV11, HPV16, and HPV18. Gardasil-9, a second-generation vaccine, provides protection against the above-mentioned four high-risk HPV types and additionally targets other high-risk HPV types - HPV31, HPV33, HPV45, HPV52, and HPV58. Gardasil-4 and Gardasil-9 are approved for administration to everyone aged 9 to 26, and per clinician's consideration vaccines can be administered at 27 - 45 years of age as well [144]. Despite these vaccines being available for over a decade, vaccination campaigns have primarily focused on preventing cervical, vaginal, anal, penile cancers, and genital warts. Limited information exists regarding the efficacy in preventing HPV-related HNSCC, especially in men. However, recent studies suggest that these vaccines may confer protection against oral HPV infections [145].

Vaccination with Gardasil-4 or Cervarix has been shown to induce anti-HPV16 and HPV18 IgG antibodies in the oral cavity. Another study observed that individuals immunized with Gardasil-4 produced anti-HPV IgG antibodies in saliva, which, albeit at low titers, neutralized pseudoviruses representing HPV6, HPV16, and HPV18 in vitro [145,146]. The detection of neutralizing anti-HPV antibodies in saliva post intramuscular immunization indicates a potential prevention of oral infections with these HPV types and, consequently, a subset of HPV + HNSCC. However, it is crucial to emphasize that certain findings mentioned earlier [145,147], were solely based on the detection of antibody titers in the saliva of individuals vaccinated with the HPV vaccine and/or in vitro neutralization assays utilizing saliva from these individuals. In a very recent retrospective study, from the TriNetX United States Collaborative Network, males (n = 760,540) vaccinated for HPV were at decreased odds for HPV-related cancers, primarily driven by a significant reduction in HNSCC [148]. These results provide early evidence of the significant efficacy of HPV vaccine in preventing HPV driven cancers. Figure 2 summarizes key milestones in the association of HPV and OPC.

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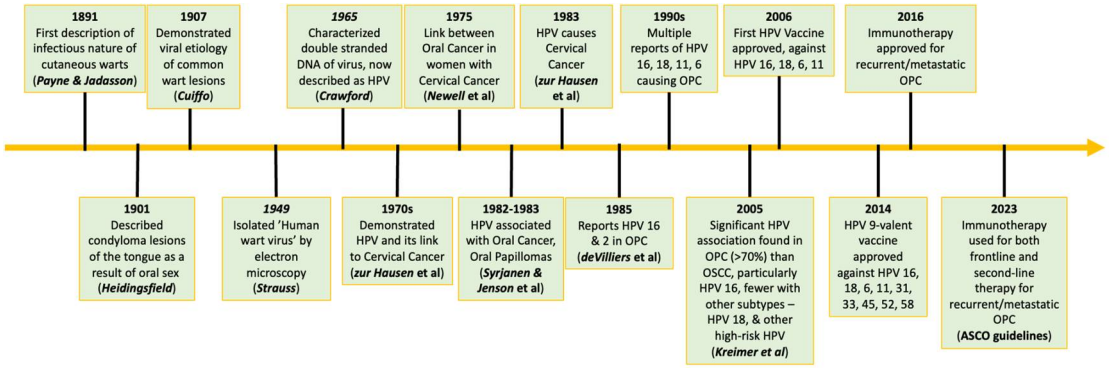

Figure 2. A timeline of key milestones in the journey of HPV and OPC.

7. Diagnosis

Patients with OPC routinely present with a sore throat or as a neck mass, dysphagia, globus sensation, otalgia, odynophagia or a visual mass and nodal metastases [149]. Initial diagnosis of suspicious lesions requires tumor visualization either endoscopically, a rhinoscope or ultrasonography, followed by fine-needle biopsy sampling of the lesion [150]. The presence of HPV DNA in biopsied tissue is routinely determined by PCR techniques, unrelated to oncogenesis. Testing for HPV+OPC is recommended through detection of E6/E7 oncogenes via RT-PCR, p16 and HPV DNA detection in situ hybridization (ISH) [73,151]. However, these methods are labor, cost and resource intensive. P16 immunohistochemistry in biopsied using formalin-fixed paraffin-embedded tissue specimen has increasingly been used as a recommended stand-alone prognostic, surrogate, marker for detection of high-risk HPV, as a result of the overexpression of p16, a cell-cycle protein, in the carcinogenesis process of HPV+OPC, per the 8th edition of American joint commission on Cancer (AJCC) – to TNM stage OPC [128]. Although recent evidence suggests p16 may not always be consistent with HPV+OPC [152]. Viral detection spanning across various detection methods have been found to either over- or underestimate the number of patients affected with HPV+OPC. Smith et al suggest that testing for a combination of markers – HPV, p16 and p53 may improve the prognostic accuracy of OPC [153]. As a result of moderate specificity associated with p16 testing, diagnosis of HPV+OPC is recommended in combination with HPV DNA PCR, for optimal accuracy. Additionally, both MRI and PET-CT imaging are recommended for primary tumor staging, determination of tumor invasion degree, extent of metastasis or nodal spread [154]. In the United States, [18F] fluoro-2-deoxy-D-glucose (18F-FDG) PET–CT is the primary modality employed to evaluate tumor extent and the presence of metastases, with MRI potentially used to assess local invasion extent [53]. Clinical prognostication generally relies on factors such as tumor diameter, nodal status, positive surgical margins, and grade of differentiation (well, moderate, or poorly differentiated). This includes assessing the invasive front's grade, considering factors like keratinization, pleomorphism, mitotic rate, invasion pattern, and patient response [155].

7.1. Screening

The incidence of OPC in middle-aged and older adult men is projected to double over the next decade, despite current prophylactic vaccination efforts [156]. These shifts in HPV driven oncogenesis of the head and neck highlight the need for parallel cervical cytology screening in the head and neck area. However, these measures have been argued against by the need for further research in the OPC area, low incidence rate in comparison to other HPV driven carcinomas, available therapy and favorable outcomes, and prevention strategies through HPV vaccination. Other deterrents to screening include the need for a robust understanding and strategy for identifying precancerous lesion, efficient biomarker and diagnostic technology to detect lesions, particularly minimally invasively, and the absence of triaging and subsequent clinical management for patients with precancerous lesions or early-stage OPC [156].

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Oral cytology, similar to cervical cytology screening methods, has been garnering significant importance in recent times [157,158]. In a study by Broglie et al, brush cytology was able to detect HPV positivity in 66% patients, but was able to identify dysplastic cells in 88% patients [159]. Castillo et al performed liquid -based brush cytology for detection of HPV in patient suspicious of OPC, with 88% sensitivity of the cytology assessment pre-treatment, 71% post-treatment, while 91% accuracy of HPV-DNA assessment, across 75 patients [160]. In the OHMAR study by Benevolo et al, testing of oropharyngeal cytobrush samples and oral rinse and gargle specimens were testing by cytological evaluation and HPV genotyping, respectively, in a group of men who have sex with men at 6-months intervals. The study found that infection of high-risk HPVs, including HPV 16 did not increase the risk for cytologic abnormalities [161]. While these and other studies highlight the growing value of oral cytology, further research is necessary to evaluate the efficacy of this screening methodology in the same light as HPV driven cervical cytology, including the inability to access the target anatomical sites region like the tonsils or base of the tongue [162].

8. Conclusion

HPV have been long associated and co-evolved with humans, allowing adaptation to multiple epithelial anatomical niches, establishing robust infection, and in some cases furthering the infection state into carcinogenesis. HPV driven oncogenesis in the oropharynx presents a complex and unique molecular landscape, tumor microenvironment and immune response, in comparison to HPV-HNSCC. Recent epidemiology data has well documented the rapidly rising rates of HPV+OPC, paralleled with rising infection rates in high income countries. Prophylactic vaccination campaigns have also primarily focused on preventing cervical, vaginal, anal, penile cancers, and genital warts, although recent studies suggest that these vaccines may confer protection against oral HPV infections as well. Despite these successes, control in the rising HPV+ OPC, particularly among men, is not expected immediately, especially unless these campaigns robustly target vaccinations in men. While vaccinations, routine screening and early detection have led to milestone achievements in HPV+ cervical cancer, the lack of precursor lesions in OPC remains perplexing, indicating there remain key differences across local tissue ecology where HPV infection occurs and transition to malignancy. Recent therapeutic strategies from completed and ongoing clinical trials have improved survival outcomes in HPV+OPC, though significantly more trials are clearly needed to significantly reduce disease and therapy associated morbidity, and mortality. Rapid advances in the fields of multi-omics, epigenetics and imaging technologies are continuing to improve our understanding of oral and oropharyngeal HPV infection and associated oncogenesis, towards improving screening, early detection, triaging, risk stratification of patients for better outcome clinical management, and development of new therapeutic opportunities.

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