Head and Neck Cancer is the sixth most common carcinoma worldwide classically associated with tobacco and alcohol. (1) However, over the past decades the etiology of head and neck cancers is now associated with viruses, in fact worldwide up to 15% of tumors are attributable to viruses. (2,3) The two viruses this talk focuses on include the Human Papilloma Virus (HPV) and the Epstein Barr Virus. Currently, HPV is implicated in an increased prevalence head and neck cancer (4,5, 6) and the presence of Epstein Barr Virus (EBV) appears ubiquitous with up to 95% of adults affected. (7) EBV was one of the first viruses to be associated with carcinogenesis and can affect the lymph tissue including B and T cells as well as the lymphoreticular tissues within the fossa of Rosenmuller. (8) This talk focuses on updates regarding HPV and EBV related head and neck cancers, reviewing the background of viral tumorogenesis, what HPV and EBV are and mechanisms these virus undergo that are believed to cause tumors. In addition, this talk reviews what p16 positivity means, and the radiographic characteristics associated with HPV and EBV related head and neck tumors.

The causative carcinogenic nature of viruses cannot be understated because worldwide, HPV is associated with 600,000 cases of the cervix, oropharynx, recurrent papillomatosis of the lungs, genitoanal carcinomas and warts. (9,10,11) In men and woman on a global scale HPV infections results in up to 50% of infection related cancers in females and 5% in males. (12) Based on SEER data, Surveillance Epidemiology and End Result Registries studies, oropharyngeal cancers had HPV detected in 16.3% of tumors from 1984-1989 and markedly increased to 71.7% from 2000-2004. (9,10,11)

So what is HPV? Human Papilloma Virus (HPV) is a double stranded DNA (dsDNA – deoxyribonucleic acid) non-encapsulated icosahedral virus of the Papillomaviridae family with approximately 8000 base pairs (bp) encoded for 10 proteins. One strand of the DNA is transcribed into mRNA (microRibonucleic Acid). The other strand has a 4000 bp encoding proteins for viral replication and cell transformation, a 3000 bp encoding the structural viral proteins, and a 1000 bp noncoding region containing the viral DNA origin for DNA replication and regulatory elements for transcription. (13) In HPV there are eight genes, six encode for early proteins (E1, E2, E3, E4, E5, E6 and E7) that allow for DNA replication and production of new virus particles in the cell. Of these E1 and E2 encode for regulatory proteins and E5, E6 and E7 encode for three oncoproteins. The late genes encode for late proteins (L1 and L2) for the viral protein shell or the capsid proteins. (14.)

There are over 170 subtypes of HPV which are classified based on the L1 protein, the capsid protein found in the outer shell. The HPV virus may preferentially involve the mucosa of the upper aerodigestive tract, genital region or skin. (15,16,17,18) Although high-risk HPV types include 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 66, types 16 and 18 are responsible for roughly 70% of cervical and greater than 90% of noncervical cancers (e.g., vaginal, vulvar, penile, anal) and oropharyngeal cancers. (19,20,21)

Research suggests that the HPV virus predates humans and evolved over 200,000 years, the phylogenetics for HPV-16 and HPV-18, follow a topology of the spread of Africans, Caucasians and
East Asians. (22) In fact papillomaviruses are found in non-human primates that appear genetically similar to the alpha-HPVs and can also induce epithelial neoplasias. (23,24) The HPV strains of papillomaviruses are found in mammals, birds and snakes. (25) Interestingly, the mythical “Jackalope” may have likely represented a rabbit with HPV infection resulting in growths that cornify and resemble horns. (26) These supposed “Jackalopes” where in fact researched in 1945, when Dr. Rous and Dr. Shope first identified papillomavirus from the cornified warty growths on cottontail rabbits, demonstrating the virus’s transmissible nature and carcinogenic potential. (27, 28)

The first description of diseases caused by HPV date back to Hippocrates (460-370 BC) who described condyloma from ancient Greek meaning round tumor and acuminata from Latin for sharp points. A Byzantine physician Aetius of Amida also described warts in the anogenital region in the 6th century BC (29, 30) It was assumed genital warts were associated with promiscuous sexual behavior. (26, 30) However, it was not until experiments by Ciuffo in Italy, in 1907 that warts ability to cause infections was determined. (31) Because the warts were in the genital region it was presumed this may be a sexually infectious transmitted disease. Currently, it is suggested that 75% of all sexually active people will be infected with HPV at some point in their lifetime. (32)

How was the association of the HPV virus and infectious sexual transmission made? In 1842, Rigoni-Stern researching female death certificates in Verona from 1760-1839 noted woman who were married, widowed or prostitutes had a high frequency of cervical cancer, and that nuns or virgins did not. (33) It was not until much later that Harold zur Hausen, in 1972, started to determine the relationship of HPV to cervical cancer, and in 1982 the first reports of HPV sequences in human tumors was published. (34) In 1983, using Southern blot hybridization with HPV 11 DNA it was possible to identify the HPV in cervical biopsies. (35) In this same time frame also occurred the characterization of new DNA of HPV from cervical cancer biopsies and from the HeLa cell cancer line labeled as HPV-16. (36) In 2008, Harold zur Hausen was awarded the Noble Prize for demonstrating HPV’s causative role in cervical carcinoma. (18)

So why does HPV occur in the head and neck and how does it cause cancer? Remember the HPV virus can be sexually transmitted and the subtype HPV-16 is the one associated with up to 86.7% of all HPV positive tumors. If orally transmitted the virus may locate in the palatine tonsil where there are crypts. The epithelium overlying the crypts in the palatine tonsil is a unique squamous epithelium containing regions of stratified squamous nonkeratinizing epithelium and reticulated sponge like epithelium, as well as intraepithelial passages filled with non-epithelial cells, and these may be the places allowing viral access. When the high-risk HPV viruses DNA is incorporated into the cell nucleus DNA this starts the production of the E6 and E7 oncoproteins that promote both viral replication and cell cycle progression. The E6 tumor protein binds the p53 tumor suppressor protein and via ubiquitin breaks it down. The E7 oncoprotein binds to the cullin 2-ubiquitan-ligase complex to the retinoblastoma (Rb) suppressor protein. The E7 oncoprotein then destabilizes the Rb tumor suppressor protein and blocks cyclin dependent kinase inhibitors and other proteins encouraging cell cycle progression, with a down regulation of the tumor suppressor pRB by the E7 oncoprotein (13)

It is important to remember the presence of HR-HPV DNA itself may not be adequate to delineate if the head and neck cancer came from an HPV infection, as the HPV may be present and biologically inactive. Therefore additional surrogate markers are used. The presence of the p16 biomarker is a strong indicator of an HPV+ tumor and disease progression. When the p16 biomarker is present; the tumors often have a more favorable course and p16 biomarker may increase in HPV positive Squamous cell carcinomas. The p16 increases because HPV DNA in the cell nucleus is transcribed and makes oncoproteins E6 and E7. E7 inactivates the retinoblastoma protein (pRB); this causes a loss and decrease in p53 expression and an increase in the gene production of the p16 tumor suppressor protein. The p16 protein is also called cyclin-dependent kinase inhibitor 2A, multiple tumor suppressors 1, and is encoded by the CDKN2A gene. Specifically p16 protein decelerates the cell
The p16 protein is an important tumor suppressor in preventing cancers. The production of p16 may occur with squamous cell carcinoma, melanoma, cervical and esophageal cancers, lung adenocarcinomas, small cell lung carcinoma, invasive ductal carcinoma of the breast, melanoma and adenoid cystic carcinoma. When present in head and neck squamous cell carcinoma it is a highly sensitive surrogate marker for the presence of HPV, suggesting the tumor is likely HPV positive. Because p16 expression can also occur in lymphoepithelial cysts or branchial cleft cysts there are pitfalls in its usage as a biomarker. (37,38,39,40)

So what does p16 positivity mean? It is important to remember that immunohistochemical identification of p16 positivity is a surrogate marker for HPV and the activity of the viral E6 and E7 oncoproteins. The p16 is a tumor suppressor gene that inhibits cyclin-dependant kinase 4A. When the cell is infected by HPV, the E7 protein binds to the retinoblastoma protein (pRB), when this occurs the transcriptional activator E2F becomes active and stops the negative feedback of free pRB on p16, and there is an increased and over expression of p16. Ideally, utilization of the surrogate marker of p16 overexpression has a higher correlation if noted in >70% of staining of tumor cells. (41)

The reason this is important is that patients with p16 positivity and increased expression have better outcomes. (42) Patients with HPV+ squamous cell carcinomas tend to be men of a higher socioeconomic status, without alcohol or tobacco abuse, often with the tumor in the oropharynx. These tumors may present at an advanced stage with cystic lymph nodes, however despite more advanced stages these tumors may have a better prognosis, all though there are small subsets which may not. The HPV+ tumors are also occurring in younger patients, and have also been reported in the pediatric population. (43) Although the majority of HPV+ have a good prognosis, there is a subset of highly aggressive tumors, which may show clustering of lymph nodes, more central tumor necrosis and extra capsular spread. This subset of HPV+ patients with aggressive tumors may have metastatic foci and a poorer prognosis. The histopathological features in this smaller subset of more aggressive HPV+ head and neck squamous cell carcinomas demonstrated a small cell component and a lower expression of the NOTCH1 gene. (44) Currently, there are vaccinations targeting the major and minor viral capsid proteins L1 and L2 of HPV. The vaccinations of the L1 virus particles cause a high number of serum neutralizing antibodies namely immunoglobulin G, IgG. (37)

The Epstein-Barr Virus (EBV) was discovered when Denis Burkitt noted cancers in the jaws of African children were in similar locations to that of malaria and thought mosquitoes might be causing the spread of the tumor via a virus. Because of this Tony Epstein and Yvonne Barr examined the jaw tumor samples for viruses, and in 1964 they found herpes like particles via electron microscopy in the tumor cells, discovering the virus. (45) EBV was determined to be the cause of infectious mononucleosis in 1968. (46) Subsequently, in 1970 EBV was found in undifferentiated nasopharyngeal cancer. (47) Therefore, the EBV virus was one of the first human viruses to be associated with carcinogenesis.

The Epstein-Barr virus is an enveloped virus with DNA cord surrounded by a protein capsid and is a member of the Herpesvirus family. EBV infection in lymphocytes or B-cells is via the viral envelope glycoprotein, gp350/220 that binds to the host cell where the viral envelope fuses with the host cell membrane. In humans, EBV is believed to initiate infection in the oropharyngeal epithelial cells susceptible to viral replication, including the epithelial cells of the Waldeyer’s tonsillar ring and may originate in the fossa of Rosenmuller where there is a lot of lymphoreticular tissue or in the nasopharynx roof. The EBV virus is omnipresent with up to 90% of the adult population being infected. Transmission of EBV can occur via saliva or through objects that have EBV infected saliva on them such as toys or utensils. (47, 50)

Although EBV is associated with nasopharyngeal carcinoma (NPC) in adults, it is important to remember EBV related NPC disease can also occur in children (48), with greater frequencies in African countries. (49) Currently, clinical examinations and imaging studies are utilized for early diagnosis;
particular care should be paid if there is a unilateral serous otitis media or cervical lymphadenopathy. The majority of NPC cases originate in the fossa of Rosenmuller, and roughly 70% will initially present with a neck mass, and 60-96% may have lymphadenopathy. Patients who are immunosuppressed may be at higher risk for NPC. After treatment, CT and MRI may be utilized to assess for the detection of residual or recurrence.

References


