HUMAN PAPILLOMAVIRUS (HPV) – RELATED CARCINOMAS OF THE UPPER AERODIGESTIVE TRACT

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* The most common site of HPV-related carcinomas of the UADT is the Oropharynx and particularly the tonsils and base of tongue.

* HPV + tumors are distinct clinically and morphologically. Microscopically they are nonkeratinizing squamous cell carcinomas with basal cell features and have a characteristic immunohistochemical profile.

* An anti-HPV vaccine has recently been made available for prevention of cervical cancer. The impact of wide use of the vaccine on the prevalence of HPV related carcinomas of the UADT is currently not known but likely beneficial.

Background

The role of human Papillomavirus (HPV) as a prerequisite for the development of cancer of the uterine cervix has been established for many years. The prevalence of high risk (oncogenic) HPV infection in cervical cancer tissue is estimated to be in the range of 90-99%. According to the WHO, about 500,000 new cases of cervical cancer occur globally each year. The majority are identified in developing countries [26,37,39].

More recently epidemiologic as well as clinical and molecular evidence have implicated HPV particularly type 16 in the causation of some upper aerodigestive tract (UADT) carcinomas, particularly in the oropharynx and notably the tonsils and base of tongue [7-9,11,13,16,20,24,25,36].

The virus

Human papillomaviruses belong to the family of DNA Papovaviridae. They are small non-enveloped icosahedral viruses with an 8 Kbase-long double-stranded circular DNA genome. They include more than a 100 different strains or genotypes. More than 30 of
which are sexually transmitted, infecting the genital areas of men and women. Some of these viruses can cause premalignant lesions and carcinomas in the affected areas, and are called “high-risk” types. Others called “low-risk” types may cause mild cytologic abnormalities and genital warts as well as laryngeal papillomatosis and oral condyloma acumínata. The most common high-risk types are types 16 and 18, while the most common of the low risk ones are types 6 and 11.

According to the CDC epidemiologic studies, 75% of the 15-75 year-old populations acquire genital HPV infection at some point in their lives. By the age of 50 at least 80% of women will have acquired genital HPV infection [26,37,39]. Human papillomavirus genome is made up of 7 early (E) genes and 2 late (L) genes that encode the early proteins E1-E7 and late proteins L1-L2. The E proteins are nonstructural and are involved in replication and transcription of the genome, while the L proteins are the structural capsid proteins of the intact virion [3,35,39].

High-risk HPV infection

It is has been known that persistent infection with high risk HPV particularly types 16 and 18 are necessary cause of high grade cervical dysplasia and cancer. However, cervical cancer is a rare complication of HPV infection. The majority of Infections in young women, (about 80%) resolve spontaneously without even giving rise to dysplastic lesions. Development of cancer requires multiple additive events both genetic and epigenetic, in addition to the persistent high risk HPV infection. On the other hand, cases that resolve may develop into a productive viral infection with new virions assembly and shedding from the fully differentiated squamous epithelial cells [3,35,39]. High grad dysplasia and cervical carcinoma often show integration of the viral genome into the host cell with a resultant deregulation and uncontrolled expression of E6 and E7 viral oncogenes. Molecular evidence in cervical as well as oropharyngeal carcinomas show that the HPV oncogenes E6 and E7 act through inactivation of p53 and retinoblastoma (Rb) tumor suppressor genes, inducing cell cycle deregulation and genomic instability. In addition E6 can directly activate telomerase and E7 induces abnormal centrosome duplication [3,22,35,39].

Clinical as well as in vitro studies suggest a model for carcinogenesis in which immortalization of the epithelial cells combined with genomic instability and accumulating genetic alterations lead to overt malignancy [3,35,39]. A familial predisposition for the development of cervical cancer has been suggested in a study in which the Swedish Family-Cancer Database was used to analyze a large number of invasive and in-situ cervical cancers in mothers and daughters [15]. It is suggested that impaired immunity may be a factor

HPV-related Carcinomas of the Head and Neck

During the last few decades there has been an increase in incidence of oropharyngeal carcinoma in young patients under 45 years of age. Using the SEER data base a statistically significant increase in incidence of carcinomas of the tonsils and base of tongue was documented during the period 1973 – 2001 in U.S. population 20-44 years of age. No similar increase occurred in other oral sites outside the oropharynx. Many of
these patients have little or no exposure to known risk factors such as smoking or excessive drinking [12,31].

About 20 years ago high risk HPV was identified in squamous cell carcinoma of the head and neck [20]. A multitude of studies using a variety of techniques including in situ hybridization (ISH), immunohistochemistry, and polymerase chain reaction (PCR) have since been able to demonstrate the presence of HPV genome in some UADT carcinomas particularly of the tonsils and base of tongue where 18-90% of the tumors are HPV +. HPV DNA has also been identified in some laryngeal and sinonasal carcinomas (12-20%). The virus is very rarely identified in squamous cell carcinoma of the oral cavity [7-9,11,13,24,25,36].

### HPV-Related Oropharyngeal Squamous Cell Carcinoma

#### Demographic and Clinical features

As mentioned above the prevalence of HPV DNA in oropharyngeal carcinoma has varied in different studies form 18 to 90%. In a review of 235 cases of oropharyngeal carcinomas, in all age groups, at our institution we found that 36% of tonsillar and 32% of base of tongue carcinomas were HPV related. Alternatively, 91% of tonsillar carcinomas in young patients, 40 years of age or younger were HPV type 16 positive. The male to female ratio for all age groups combined was 4:1 [8,9].

In another a large study of 1670 patients who had oral or oropharyngeal carcinomas and 1732 healthy volunteers, from nine countries, the International Agency for Cancer found that 18.3% of oropharyngeal carcinomas were HPV 16 positive. Patients with HPV positive tumors were three times as likely to report having had oral sex as those with HPV negative tumors. Patients with HPV positive tumors were also more likely to have had multiple sex partners. HPV is less frequently detected in cancer biopsies from patients who are tobacco smokers or paan chewers [12,30,31].

An analysis of the Swedish cancer registry data (1958-1996) showed that husbands of women with cervical cancer had significantly increased risk of developing tonsillar carcinoma [31].

Early asymptomatic carcinomas usually develop in the crypts of the palatine and lingual tonsils without apparent clinical manifestations. Because of their deep location neither clinical examination nor cytologic tests, analogous to the Pap smears used for cervical lesions, are useful in early detection. These small, occult tumors are not uncommonly associated with extensive cervical lymph node metastasis. In the more advanced primary tumors, patients may complain of sore throat, dysphagia, otalgia, and sensation of a foreign body in the throat.

#### Pathologic features

HPV-related oropharyngeal carcinomas are not only distinct clinically but also microscopically and molecularly. The tumors are characterized by nonkeratinizing, basaloid cell morphology. Microscopically the neoplastic cells are generally monomorphic, oval or spindle shaped, with hyperchromatic basophilic nuclei, inconspicuous cytoplasm and indistinct cell borders. They form cords, sheets and nests with sharply defined borders. Palisading of the peripheral cells may be present. Excessive mitosis and apoptosis are observed as well as comedo type necrosis. Keratinization and
keratin pearl formation is generally absent although some trend towards cell maturation may occasional be present in focal areas. In lymph node metatasis tumor masses commonly show extensive central necrosis leading to a characteristic cystic change. The lining epithelium of the cystic structures may be so scant and bland appearing that diagnosis of a benign cyst may be erroneously made, particularly in cases in which the primary tumors are occult [8,9,36].

**Immunohistochemistry:**
A characteristic and distinct immunophenotype is exhibited by oropharyngeal HPV-related nonkeratinizing carcinoma. These tumors are distinguished by a strong and diffuse staining for p16INK4a (p16) antibodies, a negative or weak reactivity to p53 protein and higher Ki67 staining scores, as compared to the keratinizing type carcinomas of the same site [8,9].

Overexpression of p16 has also been extensively documented in HPV-related carcinomas of the uterine cervix and ano-rectal tract and is considered to be a surrogate marker for HPV+ carcinomas. p16 is a cell cycle protein which acts as cyclin-dependant kinase (CDK) inhibitor which is involved in tumor suppression by the retinoblastoma pathway [2,6,10,21,28]. It is believed that deregulation of pRb by HPV E7 oncoprotein results in a paradoxical overexpression of p16 by feed back control. p16 immunoreactivity in keratinizing squamous cell carcinoma is usually either absent or weak [7-9].

The lack of correlation between NKCa and p53 reactivity contrasts with well documented p53 mutations identified in the majority of conventional keratinizing SCCa of the upper aerodigestive tract. In the case of HPV related carcinomas interference with p53 function is achieved by viral E6 protein which targets p53 resulting in its ubiquitination and degradation [32,35,39]. The high mitotic activity observed in NKCa is reflected in high labeling scores for the cell cycle specific protein Ki67 [7-9].

The exact mechanism by which HPV related carcinomas acquire non keratinizing basaloïd histomorphology is not known. However, several of the viral oncogenes that are expressed in the tumor cells are known to interfere with cell cycle regulatory mechanisms resulting in cell immortalization with uncoupling of proliferation and differentiation. Interestingly HPV positive tumors of the larynx and sinonasal tract show identical histologic and immunophenotypic features like the HPV-related oropharyngeal carcinomas [7-9].

**Detection**
Currently no cytologic tests, analogous to cervical Pap smears, are used for early detection of HPV related oropharyngeal dysplasia and carcinoma. Unfortunately such techniques may not be useful because the majority of oropharyngeal lesions start at the bottom of the crypts of the palatine and lingual tonsils, and thus inaccessible to routine cytologic smears.

Advanced disease is symptomatic and manifested, clinically and radiographically, by tumor mass at the primary site as well as enlarged neck lymph nodes. Occasionally small primary tumors that are undetectable on routine clinical examination are associated with significant neck node metastasis (occult primary). We have recently shown that more than 90% of HPV positive neck metastasis arise from the oropharynx, mainly the tonsils and base of tongue, while less than 10% of those metastasis originated outside the oropharynx including the oral cavity proper, larynx and hypopharynx . HPV related
metastatic carcinomas were identified in FNA biopsies as well as in surgical specimens by morphologic criteria and ISH for high risk HPV [40-42].

**Treatment and prognosis**

Accumulating body of evidence in the American as well as the international literature confirms that HPV positive carcinomas of the tonsils and base of tongue have statistically significant better prognosis, regarding disease free and overall survival, than HPV negative tumors. The favorable outcome for patients with HPV positive tumors is independent of TNM stage, nodal status, age or gender. It is suggested that the favorable outcome is attributable to increased sensitivity toward radiotherapy [5,18,19,23,27,38]. Early experimental evidence show that the broad spectrum anti DNA virus agent Cidofovir can inhibit proliferation and induce apoptosis in HPV transformed cultured cells. It also enhanced their radiosensitivity. Cidofovir is also used clinically, with some success, in treatment of laryngeal papillomatosis, when injected intralesionally [1,17,33].

**Prevention:**

Because HPV infection of the oropharynx is believed to be sexually transmitted, the practice of protective sexual behavior is likely to have preventive effects. Abstinence, monogamy, limiting the number of sexual partners has all been advocated for prevention of STD. Unfortunately HPV infection can occur in the genital areas that are covered, as well as areas not covered, by a latex condom. According to NCI the efficacy of the use of condoms in prevention of HPV infection is not known, although condom use has been associated with a lower rate of cervical cancer.

**The HPV Vaccine**

In June 8, 2006, the Food and Drug Administration (FDA) licensed the first anti HPV vaccine. The quadrivalent vaccine, Gardasil, immunizes against HPV types (6,11,16,18). It is made from non-infectious viral-like particles (VLP) [4,14,34]. On June 29, 2006, the Advisory Committee on Immunization Practice (ACIP) voted to recommend this vaccine in females, ages 9-26. The vaccine has been tested in over 11,000 females of that age group in many countries around the world including the USA. These studies demonstrated 100% efficacy in preventing cervical precancers, and nearly 100% efficacy in preventing vulvar and vaginal precancers, as well as genital warts, caused by the targeted HPV types. These studies also found that the vaccine is safe and cause no side effects [14,29,34].

The Impact of wide use of anti HPV vaccines on HPV-related oropharyngeal carcinoma is currently not known. However is reasonable to conclude that a direct or indirect benefit may be achieved.

**Summary**

- Epidemiologic, clinical, morphologic and molecular evidence show that high risk HPV particularly type 16 - like in the case of cervical cancer- is a prerequisite for some carcinomas of the upper aerodigestive tract (UADT).
- Sexual transmission is an important mode of infection.
- The most common site of HPV-related carcinomas of the UADT is the Oropharynx and particularly the tonsils and base of tongue, where they constitute one third of carcinomas in that location.
- HPV + tumors are distinct clinically and pathologically. They are more common in young patients (<40 years) with a male to female ratio of 4:1. They usually present as a small or occult primary with advanced neck disease. Tobacco use and excessive drinking are not necessary risk factors.
- Microscopically they are nonkeratinizing squamous cell carcinomas with basal cell features, excessive mitosis and comedo type necrosis.
- The tumors have a distinct immunohistochemical profile characterized by strong and diffuse p16 reactivity, low or negative p53 staining and high Ki67 labeling scores.
- HPV + Carcinomas are more radio-sensitive and have better prognosis than the classical keratinizing SCC of the UADT.
- An anti-HPV vaccine has recently been made available for prevention of cervical cancer. The impact of wide use of the vaccine on the prevalence of HPV related carcinomas of the UADT is currently not known but likely beneficial.

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