


Role of HPV in Oropharynx Cancer

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Faculty Disclosure

- I have no conflict of interest to disclose

Head & Neck Squamous Cell Carcinoma (HNSCC) Risk Factors

- Tobacco and alcohol use
 - Account for disease pathogenesis in about 80% of the cases
- Numerous lines of epidemiologic and molecular pathology evidence suggest that high-risk Human Papillomaviruses (HPV), especially type 16, are etiologically related to a subset of oropharyngeal squamous cell cancers (OSCC)

Epidemiology of oropharyngeal squamous cell cancer (OSCC)

- According to SEER database, the incidence of oropharyngeal cancers increased up to 3.9% per year, from 1973-2001, among white men and women ages 20-44 years, whereas the incidence of other head and neck sites decreased
- Herpes simplex-2 seroprevalence (a marker for high-risk sexual behaviors) increased by 30% between the periods 1976-1980 and 1988-1994

Oropharyngeal cancer and sexual behavior

- Certain sexual habits increase the risk of HNSCC (oral sex, oral-anal contact)
- Among men, young age at first intercourse, number of sexual partners and a history of genital warts significantly increase risk of HNSCC
- Among women, high number of sexual partners is associated with increased risk

Oropharyngeal cancer and sexual behavior

Individuals at increased risk of developing HPV-associated OSCC:

- history of HPV-associated anogenital cancers
- husbands of women with in situ and invasive cervical cancer
- HIV-infected men

Oropharyngeal cancer and sexual behavior

- A recent hospital-based case-control study of 100 newly-diagnosed patients with oropharyngeal cancer and 200 control patients without cancer showed that a high (i.e. 26 or more) lifetime number of vaginal-sex partners and 6 or more lifetime oral-sex partners were associated with subsequent development of oropharyngeal cancer (odds ratios 3.1 and 3.4, respectively)

D'Souza G et al. N Engl J Med 356:1944-56, 2007

Markers of HPV infection and increased risk of OSCC

- In a case control study from Sweden, oral infection by high-risk HPV types (evaluated by nested polymerase chain reaction) was shown to significantly increase risk for oropharyngeal cancer (odds ratio, 230; 95% CI, 44 to 1,200) after adjustment for alcohol and tobacco
- A nested case-control study from Norway showed that HPV16-seropositive individuals had a >14-fold increase in risk of subsequent oropharyngeal cancer compared with seronegative

Markers of HPV infection and increased risk of OSCC

- Oropharyngeal cancer was linked to oral HPV infection and HPV-16 L1 seropositivity among patients with or without history of heavy tobacco and alcohol use in the study by D'Souza et al
- Studies that do not show an association between sexual practices, oral HPV infection and head-and-neck cancer include study populations in which less than 25% of the cases have HPV DNA detected, and thus the risk association might be attenuated. Studies that reduced etiologic heterogeneity by limited enrollment to patients with oropharynx cancer showed a clear association between sexual behaviors, oral HPV infection and HNSCC

D'Souza G et al. N Engl J Med 356:1944-56, 2007

Human Papillomavirus

- Accepted etiologic agent for cervical carcinoma
- First reported association with head & neck cancer in 1985
- However, in contrast to cervical cancer, establishing the link between high-risk HPV infection and the development of head and neck cancer is far more difficult

Proving causal association

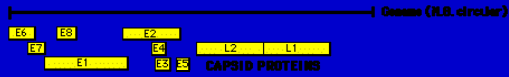
- Epidemiology
- Molecular pathology
- Experimental Evidence
- Animal Studies

Carbone et al: "Modern Criteria to Establish Human Cancer Etiology"
Clin Can Res, 2004

Proving causal association

- HPV DNA presence in tumors per se does not prove causal association
- This is possible when molecular techniques show that the virus is required for malignant transformation of tumor cells

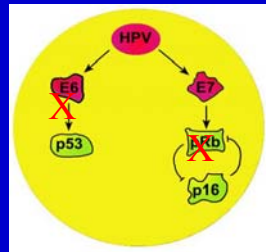
HPV PROTEINS



- E1: Viral replication; maintains episome
- E2: Transcriptional regulation, co-factor for viral replication
- E4: Disrupts cytokeratins
- E5: Interacts with growth factor receptors
- E6: Transforming protein; p53 degradation
- E7: Transforming protein; Rb binding
- Genes L1 and L2 are coding for major and minor capsid proteins respectively

HPV-induced Carcinogenesis

- Two main viral proteins : E6, E7
- Affect cellular p53 and pRb pathways



HUMAN PAPILLOMAVIRUSES AND CERVICAL CANCER

- Cervical cancer is caused by infection with high-risk HPV types
- E6 and E7 are the major HPV oncogenes
- The E6 and E7 genes are expressed in cervical cancer cells
- Continued expression of E6 and E7 is required to maintain the proliferative state of cervical cancer cells

E6 and E7 REPRESSION SYSTEMS IN HeLa CERVICAL CARCINOMA CELLS

- E2 protein represses transcription of the E6 and E7 genes
- This results in reactivation of the p53 and Rb tumor suppressor pathways and cell growth arrest
- The growth-arrested cells rapidly become senescent
- Antisense-mediated repression of HPV oncogene expression in cervical cancer cell lines typically results in several-fold inhibition of proliferation

Goodwin EC, Dimaio D: Repression of human papillomavirus oncogenes in HeLa cervical carcinoma cells causes the orderly reactivation of dormant tumor suppressor pathways PNAS, 2000

Psyrri et al: Role of the retinoblastoma pathway in senescence triggered by repression of the human papillomavirus E7 protein in cervical carcinoma cells Cancer Res, 2004

Providing experimental evidence for a causal association between HPV and OSCC

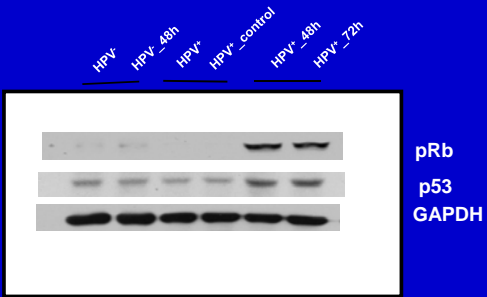
- We used a retrovirus-based E6/E7 repression system to study the biochemical and phenotypical consequences of removing E6 and E7 in HPV16+ oropharyngeal cancer cell lines*

**Rampias et al. JNCI, March 2009*

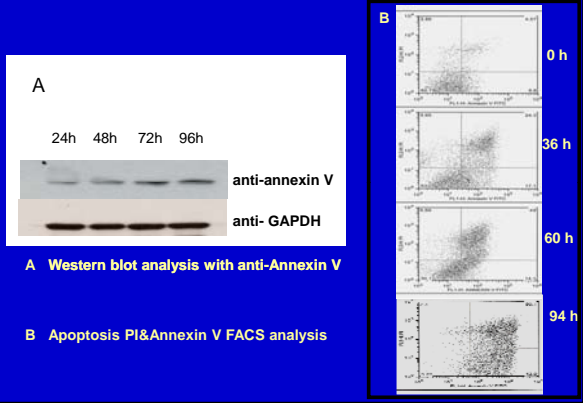
EXPERIMENTAL DESIGN

- Construction of retrovirus vectors encoding for short hairpin RNAs targeting the E6 or E7 genes
- Infection of 93VU147T, UPCI:SCC090 (bearing integrated HPV16 DNA) and 92VU040T (HPV negative) oropharyngeal cancer cell lines with the retrovirus vectors
- Measurement of markers of senescence and apoptosis and biochemical analysis of p53 and Rb tumor suppressor pathways

p53 and pRb restoration with E6/E7 shRNA



shRNA INDUCED APOPTOTIC CELL DEATH

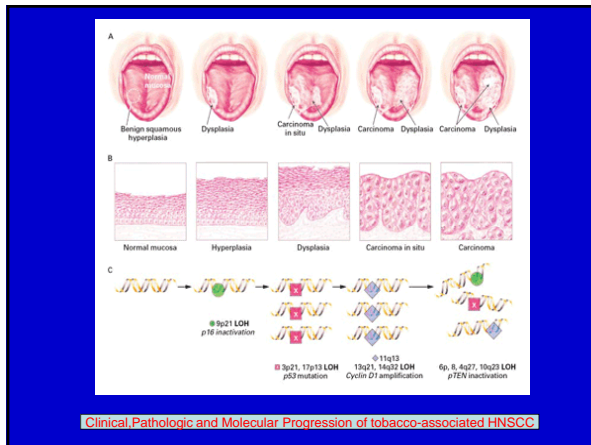


CONCLUSIONS

- Continued expression of E6 and E7 is required to maintain the proliferative state of HPV-associated oropharyngeal squamous cancer cells
- Repression of E6 and E7 oncogenes results in reactivation of the p53 and Rb tumor suppressor pathways and in apoptosis
- Anti-HPV strategies may be effective in the treatment of HPV-associated head and neck cancers

When to suspect HPV-associated HNSCC

- Location: Lingual and palatine tonsil
- Patients are nonsmokers, nondrinkers
- Basaloid or poorly differentiated histology
- Young patients
- Immunocompromised patients
- Patient with Fanconi anemia



P16

- The p16 protein exerts a tumor suppressor function by binding to the cyclin D1 CDK4/CDK6 complex preventing phosphorylation of the retinoblastoma protein
- Thus, p16 is a cyclin-dependent kinase (CDK) inhibitor which, by inhibition of Rb phosphorylation, preserves the integrity of the G1/S checkpoint and also regulates the transcriptional program involved in cell proliferation

P16 and HNSCC

- According to the molecular progression model for HNSCC, loss of 9p21-22 is the most frequent event and is also present in the earliest definable lesions, including dysplasia and carcinoma *in situ*.
- The p16 (CDKN2) protein resides within this region. Thus, it has been hypothesized that p16 is the candidate tumor suppressor gene within this critically deleted area

P16 and HNSCC

- Although point mutation of p16 gene in HNSCC is rare, alternative mechanisms of abrogation of p16 function such as homozygous deletions and methylation of the 5' CpG promoter region of p16 have frequently been identified, suggesting that functional inactivation of p16 is a common event
- Studies have also suggested that loss of p16 function may be associated with tobacco use

P16 and HPV-induced cancers

- Overexpression of p16 has been repeatedly reported in HPV-associated cancers. In one study in cervical and genital lesions, levels of p16 protein expression were associated with HPV oncogenic potential
- Rb acts as a negative regulator of p16 expression at the transcriptional level; it has been demonstrated that p16 and Rb inactivation are almost always mutually exclusive

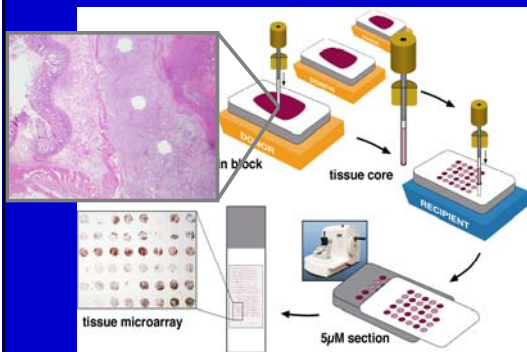
Objectives

1. We hypothesized that p16 expression in OSCC defines a subgroup of HPV-induced tumors with favorable prognosis
2. To determine if HPV/p16 classification can prognosticate local recurrence and overall survival

Methods

- A cohort of 107 patients with oropharyngeal SCC treated at Yale University from 1980-1999 was assembled
- HPV 16 DNA viral load (the subtype implicated in >95% of HPV+ oropharyngeal cases) was determined using real-time quantitative PCR with primers specific for E6 target gene
- Expression of p53, p16 and retinoblastoma (Rb) proteins was determined using quantitative fluorescent immunohistochemistry (IHC) on a tissue array composed of the cohort of oropharyngeal cancers. For p16, conventional IHC was also performed

Tissue Microarrays



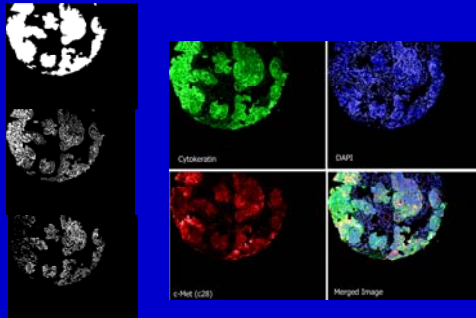
Dollé, Filhant and Rimm (2002) Principles and Practice of Oncology: Technology Update

AQUA (Automated Quantitative Analysis System)

- Molecular (not morphologic) quantification
- A combination of two algorithms that:
 - DEFINES A REGION OF INTEREST (Tumor) by Binary Masking
 - DEFINES COMPARTMENTALIZATION
 - GENERATES QUANTITATIVE ANALYSIS OF TARGET PROTEIN EXPRESSION

Camp RL, Chung GC, Rimm DL. "Automated subcellular localization and quantification of protein expression in tissue microarrays" Nature Medicine 8(11), November 2002.

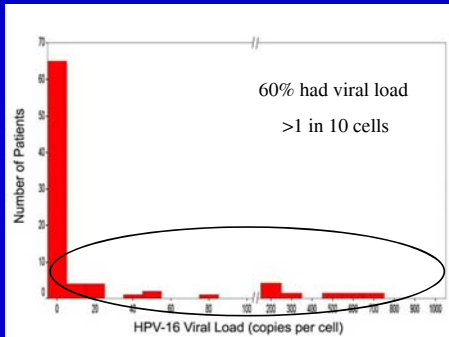
AQUA



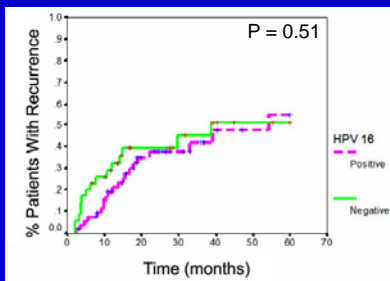
Results: Patient Demographics

	<u>N = 107</u>
	n (%)
<u>Tumor Type</u>	
Primary	91 (85)
Recurrent	13 (12)
<u>TNM Stage</u>	
II	14 (13)
III	33 (31)
IV	60 (56)
<u>Alcohol Use</u>	
Social or none	18 (17)
<u>Tobacco Use</u>	
Remote or none	11 (10)

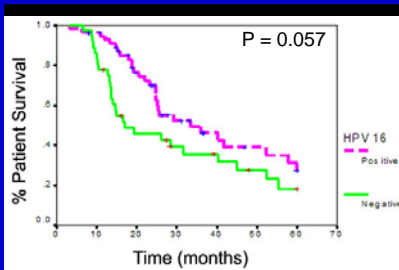
HPV-16 Viral Load



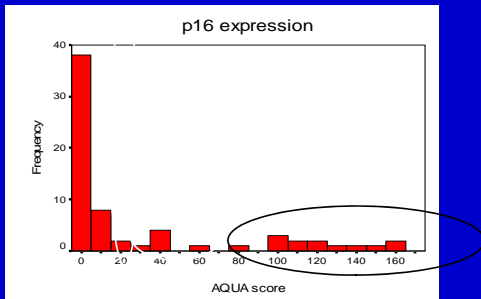
Local Recurrence



Overall Survival



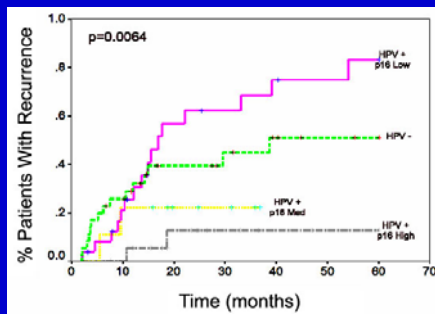
Protein Expression: p16



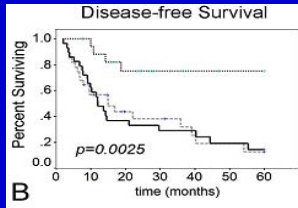
Based on HPV positivity and p16 protein expression tumors were classified into 3 categories

- Class I: HPV negative, p16 low
- Class II: HPV positive, p16 low
- Class III: HPV positive, p16 high

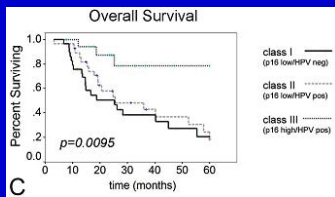
Local Recurrence: by p16/HPV



Disease-free survival by HPV class



Overall Survival by HPV class



Clinicopathological Correlations

- HPV negative /p16 low (class I)
 - Worse prognosis
 - Less likely to respond to initial therapy
 - More likely to report tobacco usage
 - Higher T stage

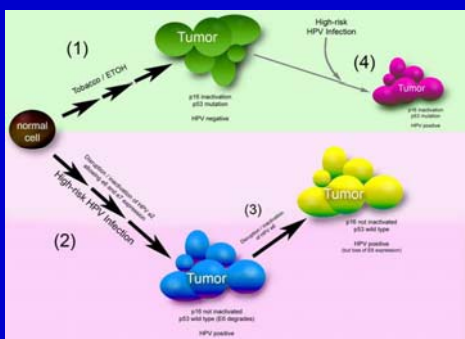
Clinicopathological Correlations

- HPV + / p16 Low (class II)
 - Marked increase in local recurrence
 - Lower TNM stage

Clinicopathological Correlations

- HPV + / p16 High (class III)
 - Improved survival / lower local recurrence
 - Lower T stage / Higher N stage
 - More likely to report no tobacco usage
 - All primary tumor type
 - Poorly differentiated histology

Proposed Model of HPV-associated Oropharyngeal Cancer



Conclusions

- HPV status in combination with p16 expression is useful in classifying oropharyngeal squamous cell carcinomas into biological distinct subgroups
- These subgroups have distinct molecular phenotypes
- HPV positive tumors with high p16 expression have improved prognosis for survival and local recurrence
- Clinical trials in patients with OSCC should stratify by p16 expression/HPV DNA status or at least include them as a prognostic variable

Weinberger, PW, Z. Yu, C.Sasaki, D. Rimm and A. Psyrri: et al: JCO Feb 2006

Making the diagnosis of HPV-associated OSCC

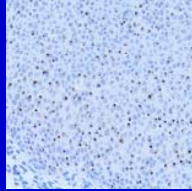
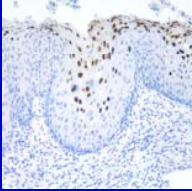
- p16 immunohistochemistry (IHC)
- HPV *in situ* hybridization
- HPV16 DNA in plasma
- HPV16 E6 and E7 antibodies in serum

IHC for p16

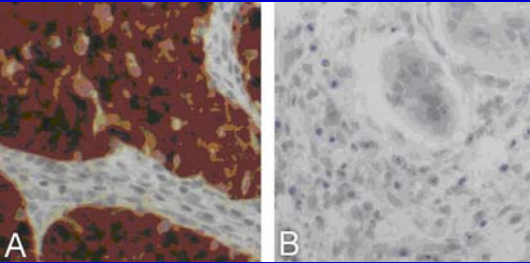
- Surrogate marker for HPV-associated tumor
- IHC for p16 can be used to determine whether biologically relevant HPV is present in tumors

HPV ISH

- Episomal HPV DNA is evident by the complete nuclear staining pattern
- Integrated HPV virus is denoted by the single punctate staining pattern



P16 IHC



Prognosis of HPV-associated OSCC

- HPV-associated OSCC have improved prognosis compared to stage-matched HPV-negative tumors in the majority of studies
- HPV-positivity confers a 60% to 80% reduction in risk of death from cancer relative to comparably treated HPV-negative tumors
- Studies that fail to show a better prognosis may be associated with the molecular heterogeneity of the HPV+ group
- Knowledge of the p16 protein status may clarify delineation of the proportion of HPV-induced oropharyngeal cancers

Treatment of HPV-associated OSCC

- Similar to stage-matched non-HPV-associated ones
- Organ-preservation strategies may be more successful in HPV-associated OSCC than in HPV negative cancers*
- ECOG conducted a trial of taxane-based induction and chemoradiation therapy for organ preservation, and outcome has been compared for HPV positive and HPV negative cases
- HPV+ (by ISH and multiplex PCR) patients had superior response rates after IC and CRT
- After a median follow-up of 39.1 months, HPV+ patients had a risk of progression 72% lower and a risk of death 79% lower than patients with HPV negative tumours after adjustment

*Fakhry C J Natl Cancer Inst 100:261-9, 2008

HPV-targeted strategies

- Prophylactic Vaccines
- Therapeutic vaccines
- Antisense strategies

Prophylactic vaccines

- Recombinant L1 protein displaying neutralizing epitopes assembles into virus-like particles (VLPs) that resemble authentic virions but are non-infectious
- Two vaccines targeting high-risk types 16 and 18 are available
- These vaccines have been licensed in over 80 countries and have received FDA license for prevention of CIN lesions and eventually cervical cancer

Prophylactic vaccines

- These vaccines appear promising in reducing the incidence of HPV-associated oropharyngeal cancers considering the presence of HPV16 DNA in the vast majority of HPV16+ OSCC
- Animals immunized against HPV16 showed a reduction in the development of HPV16+ oral lesions
- The impact of these vaccines on the incidence of persistent oral HPV infection in humans is unknown

Prophylactic vaccines

- The National Cancer Institute (NCI) is considering of introducing an oral HPV component to its follow-up study of women in Costa Rica who participated in a study of a prevention vaccine
- The aim of this oral component will be to compare the prevalence of oral HPV infection in women who received the vaccine compared with those who did not

Therapeutic Vaccines

- Various forms of vaccines—such as vector-based vaccines, tumor-based vaccines, DNA-based vaccines, and protein/peptide-based vaccines—have been tested in experimental systems targeting HPV-16 E6 and/or E7 proteins
- Although therapeutic vaccination strategies can generate a cytolytic CD8+ T cell response, they have not been successful against CIN and cervical carcinoma in humans

Therapeutic Vaccines

- A phase I clinical trial using an HPV16-specific therapeutic vaccine as adjuvant therapy aiming to generate cytotoxic T cell response to the HPV16 oncoproteins is closed to accrual and data are being analyzed (Johns Hopkins Hospital, Baltimore, Maryland)
- After 2 years of follow-up all 18 patients are alive and well

Conclusions

- The incidence of HPV-associated HNSCC has increased over the past 3 decades probably due to sexual habits associated with viral transmission
- HPV- association should be suspected in young nonsmokers, nondrinkers bearing basaloid tumors
- HPV-associated HNSCC have a better prognosis and respond better to chemoradiation
- P16 protein expression, as determined by IHC, is a surrogate marker for HPV-associated tumor

Conclusions

- An improved mechanistic understanding of the virologic basis for head and neck cancers will help to prevent and treat these diseases
- The currently available prophylactic HPV vaccines might be particularly effective in preventing HPV-associated HNSCC considering the presence of HPV16 DNA in the vast majority of HPV-associated HNSCC
- Therapeutic HPV vaccines are currently being tested in clinical trials