Human Papilloma Virus and Laryngeal Squamous Cell Carcinoma: Prevalence and description of a novel HPV+ laryngeal squamous cell carcinoma cell line.

Introduction

Head and neck squamous cell carcinoma (HNSCC) has historically been associated with heavy tobacco and alcohol abuse. Recently, high-risk human papilloma virus (HPV) (HPV 16 and HPV18) has been associated as a potential mediator in the pathogenesis of HNSCC.1 The linkage between HPV and laryngeal SCC (LSCC) remains unclear. The need to further study HPV-related LSCC requires the combination of large epidemiologic studies and in-vitro molecular pathogenesis studies.2 The development of cell-lines enables researchers to better understand the cellular and molecular mechanisms of disease. The identification and passage of an HPV-positive LSCC cell line in the literature has not been previously described. In the present study, we describe a novel HPV-positive cell line and examine the prevalence of HPV-positive LSCC in a select cohort.

Materials and Methods

Procurement and Digestion of specimen: Research consent was obtained prior to specimen collection. Tumor specimens were taken directly from the operating room and minced into fine pieces and digested with Collagenase-Hyaluronidase enzyme. The cells were then passaged 20 times before they were deemed a stable cell culture and subsequently titled UM-SCC-105.

Immunostaining, in situ Hybridization and Scoring

Immunohistochemical (IHC) and in situ hybridization (ISH) were performed from representative archived paffin-embedded tumor tissue blocks. Staining for p16INKA was performed per protocol supplied by the kit (CINtec p16 Assay). Histology slide MM Laboratories, Westborough MA. ISH using the INForm HPV/IISH assay (Ventana, Tucson AZ), which consists of a cocktail directed against 12 high-risk HPV genotypes (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 66, 68, and 73). The HPV type and its competitor were distinguished by mass when analyzed on the MALDI-TOF mass spectrometer.

Tissue Microarray

Three previously constructed laryngeal SCC tissue microarrays (TMA) with 156 patient biopsies or tumor specimens, collected from 1985–2000, were used to determine HPV prevalence:149 specimens were available for IHC and 91 were available for ISH. The TMA was tested and scored for p16-ISH and HPV-ISH as previously described.

Patient Characteristics and Cell Line Analysis

- 51 year-old male with T4aN0 SCC of the true vocal cord
  - Lifetime non-smoker, non-drinker
  - Flexible and direct laryngoscopy revealed a mass occupying the entire laryngeal inlet; right arytenoid and false cord
  - Failed neoadjuvant TPF (carboplatin, docetaxel, and continuous 5-FU infusion) requiring total laryngectomy with bilateral SND
  - Pathologic analysis confirmed a 4.6 x 3.1 cm exophytic and ulcerated tumor based primarily in the right glottis; p16 and HPV in-situ positive (Figure 2)

Results

UM-SCC-105 is the first HPV-positive LSCC cell line reported in the literature and represents an opportunity to further study the molecular mechanisms of HPV and tumorigenesis in LSCC in vitro.

Discussion

Analysis of our LSCC historical cohort determined that 38% of patients samples were p16+, 3.3% were ISH-positive for HPV, while only 2.4% were positive for both IHC and ISH. Assuming that p16+ available for PCR Mass Spec analysis demonstrated 29% of p16+ samples were HPV+.

In this historical cohort, the estimated prevalence of HPV+ LSCC in p16+ tumors is 11%.

A meta-analysis studying HPV associated head and neck cancers identified 24% of all LSCC studied harbored HPV; however the reported prevalence of HPV+ SCC varies based on testing method.4 The incidence of HPV has also shown a dramatic increase over the last 10-years and changing reports of prevalence over the years may represent the etiology of this variation.5,6 The reported prevalence of HPV-positive LSCC reported in the literature is higher than identified in our study. This likely represents a historical bias of the samples analyzed given the recent rise in the incidence of HPV associated HNSCC. In addition, our prevalence analysis was based on PCR-Mass array data in p16+ tumors only.

Conclusion

In this historical cohort, the estimated prevalence of HPV in LSCC is 11%. Given the HPV-induced HNSCC cancer epidemic, greater understanding for HPV-molecular pathogenesis in LSCC is warranted. UM-SCC-105 is the first HPV-18 positive LSCC cell line described in the literature, and it will aid in future research to better understand the pathogenesis of HPV in LSCC.

References


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