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Original contribution

Small cell neuroendocrine carcinomas of the lung do not harbor high-risk human papillomavirus☆,☆☆

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Summary High-risk subtypes of the human papillomavirus (HPV) are known to drive the pathogenesis of cervical, anogenital, and oropharyngeal squamous cell carcinomas. Recent reports have shown that HPV is also associated with small cell neuroendocrine carcinomas of the cervix and oropharynx. Little is known about HPV as a driver of neuroendocrine tumors at other sites, in particular, small cell lung cancer (SCLC). The aim of this study was to evaluate SCLC for the presence of high-risk HPV to further elucidate the role of HPV in SCLC. Archived formalin-fixed, paraffin-embedded surgical resection specimens from 20 primary SCLC from 19 patients were identified from 2004 to 2013. Two cervical small cell carcinomas were included as controls. Small cell neuroendocrine phenotype was confirmed by review of morphology and prior immunohistochemistry staining. Immunohistochemistry for p16 (INK4a) expression was performed in all cases. DNA was extracted from formalin-fixed, paraffin-embedded specimens and run on the Roche Linear Array HPV Genotyping test and a real-time polymerase chain reaction HPV assay. Pathologic tumor stage was collected from surgical pathology reports. High-risk HPV genotypes were not detected in any of the 20 SCLC specimens, whereas p16 was up-regulated in 14 (70%) of 20. p16 up-regulation can be used as an indicator of disruption of the Rb pathway either by integration of the HPV E7 oncoprotein or other mechanisms. In conclusion, our findings indicate that, unlike some other small cell neuroendocrine carcinomas, the pathogenesis of SCLC does not appear to be associated with high-risk HPV infection, a potentially very useful characteristic when determining primary from metastatic tumors.

1. Introduction

Small cell lung cancer (SCLC) is the third most common subtype of lung cancer following adenocarcinoma (ADC) and squamous cell carcinoma (SqCC) with an expected 30 000 new diagnoses in the United States in 2014 [1]. Small cell lung cancer is aggressive with a 5-year survival rate of only 5% [2,3]. Because of the frequent advanced stage at diagnosis and the chemosensitivity of these tumors, the availability of surgically resected cases is limited; thus, systematic

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molecular analysis into the pathogenesis of this tumor type has been challenging because of lack of suitable samples.

In recent years, there has been an expansion in the number and types of cancers that are related to high-risk human papillomavirus (HR-HPV) pathogenesis. This association has been well described in cervical and oropharyngeal squamous cell carcinoma, but it has now been demonstrated that small cell neuroendocrine carcinomas of the cervix [4,5], oropharynx [6-8], and anal canal [9] are also human papillomavirus (HPV) related. Little is known about HPV as a driver of neuroendocrine tumors at other sites, in particular, SCLC. Previous studies have shown that the prevalence of HPV-positive lung non–small cell carcinomas is inconsistent worldwide with reports of more than 50% in China and Japan, 28% in Latin America, 2% to 20% in Western Europe, and 1.5% to 20% in North America [10,11]. The few studies that have included a small number of SCLC report a wide range of HPV positivity (0%-100%) by polymerase chain reaction (PCR) and in situ hybridization (ISH) [12-14]. The aim of this study was to determine the HPV status in a series of SCLC to further elucidate the role of HPV in SCLC.

2. Materials and methods

2.1. Case selection

Twenty cases selected from 19 patients with primary SCLC and 2 cases of cervical small cell carcinoma (CSC), included as control tissues, were identified from the Dartmouth-Hitchcock Medical Center Pathology Archives from 2004 to 2013 subsequent to internal institutional review board approval. Eighteen of the cases were contiguous resections, and 2 were biopsies with sufficient tissue for isolation of DNA from formalin-fixed, paraffin-embedded (FFPE) tissue for HPV genotyping analysis. Small cell neuroendocrine phenotype was confirmed by review of morphology and prior immunohistochemistry (IHC) staining. Patient demographics and tumor stage were gathered from the surgical pathology reports.

2.2. Immunohistochemistry

Immunohistochemistry for p16 (INK4a) (prediluted mouse monoclonal anti–human p16 [INK4a] clone E6H4; CINtec, Ventana Medical Systems, Inc, Tucson, AZ) was performed using Bond Epitope Retrieval solution 1 and the Leica Bond Polymer Refine Detection Kit with DAB chromogen and hematoxylin counterstain on the Leica Bond Rx instrument (Leica Microsystems Inc, Buffalo Grove, IL). Strong and diffuse nuclear and cytoplasmic staining in 70% or more of tumor cells was considered positive [6].

2.3. DNA extraction

DNA was extracted from 2 to 5 sections (4 μm) from each of the 20 FFPE tissue blocks using the Gentra Puregene Kit (Gentra Systems, Inc, Minneapolis, MN) according to the manufacturer's instructions after deparaffinization with xylene. Hematoxylin and eosin–stained slides were reviewed to determine the area of tumor for extraction. Extracted DNA was rehydrated with 50 μL of hydration solution and quantified with the NanoDrop 1000 (Thermo Fisher Scientific Inc, Wilmington, DE).

2.4. HPV linear array genotyping

Human papillomavirus genotyping was performed on the extracted FFPE tissue as previously described [15]. Briefly, the Roche Linear Array HPV Genotyping test kit (Roche Molecular Diagnostics, Branchburg, NJ) uses amplified DNA that hybridizes to test strips for detection of 37 HPV DNA genotypes (6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73 [MM9], 81, 82 [MM4], 83 [MM7], 84 [MM8], IS39, and CP6108). The HPV genotyping calls were made according to the recommendation of the manufacturer using the detectable hybridization bands.

Human papillomavirus genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 were considered high risk, whereas HPV genotypes 6, 11, 26, 40, 42, 53, 55, 61, 62, 69, 70, 72, 73, 81, 82, 83, and 84 were considered low risk. *β-Globin* is used as an internal control. This assay is currently used in our clinical laboratory for genotyping of head and neck cancers [15].

2.5. Cobas HPV genotyping

An alternative HPV testing method was also used to confirm the findings of the Linear Array test. The cobas HPV test (Roche Molecular Diagnostics) is an FDA-approved, real-time PCR–based qualitative in vitro test for the detection of HPV in liquid cytology specimens. This test specifically identifies 14 HR-HPV subtypes, including 16 and 18, in a single analysis along with *β-globin* as an internal control. The genotype results are reported as negative, positive for HPV 16, positive for HPV 18, or positive for pooled "other" HR-HPV subtypes. We modified the assay for FFPE samples using 25 μL of DNA master mix (supplied in the cobas 4800 HPV Amplification/Detection kit) and 25 μL of undiluted DNA extracted from lung FFPE tissue. Human papillomavirus PCR was then processed on the cobas 4800 instrument using the HPV PCR-only assay according to manufacturer's recommendations in accordance with our standard laboratory operations. Two kit controls (positive and negative) and 4 external controls were used (HPV 16, 18, 68, (AcroMetrix, Life Technologies, Grand Island, NY) and a previously run non-lung FFPE positive for HPV 16 by Linear Array).

3. Results

3.1. Clinicopathological findings

Twenty cases of SCLC were identified from the Department of Pathology Archives from 2004 to 2013 that had an adequate