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ORIGINAL ARTICLE

No Detection of Episomal or Integrated High-Risk Human Papillomavirus in Nonsmall Cell Lung Carcinomas among Korean Population

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Abstract
Objectives: High-risk human papillomavirus (hrHPV) is known to be a representative cancer-causing agent in the genital and head and neck regions. Many studies have detected hrHPV DNA in nonsmall cell lung carcinoma. However, hrHPV–etiologic correlation in nonsmall cell lung carcinoma remains unclear. This study is designed to determine the prevalence of episomal or integrated hrHPV DNA in nonsmall cell lung carcinoma among the Korean population.
Methods: Surgically resected nonsmall cell lung carcinoma tissues, including 134 cases of squamous cell carcinoma (SqCC) and 99 cases of adenocarcinoma (ADC), were examined. *In situ* hybridization (ISH) for detecting episomal or integrated hrHPV DNA was performed using the INFORM HPV III Family 16 Probe (B) in the Ventana-validated assay. Anyplex II HPV28 detection kit based on real-time polymerase chain reaction was used for HPV DNA detection and genotyping.
Results: All members of the study population were of Korean ethnicity. Episomal or integrated hrHPV DNA ISH analysis result was negative in all 233 cases. One SqCC of 89 samples (42 SqCCs and 47 ADCs) was positive for an hrHPV genotype by Anyplex II HPV28 detection kit.
Conclusion: Our finding did not demonstrate hrHPV–etiologic correlation in primary lung SqCC and ADC in the Korean population.

1. Introduction

Human papillomavirus (HPV) is a double-stranded DNA virus and is a representative virus known to be an etiologic agent in both benign and malignant tumors. There are more than 100 different types of HPV, of which cancer-causing types are called "high-risk HPV (hrHPV)." The cancer-causing roles of hrHPV in uterine cervical carcinoma and oropharyngeal carcinoma are well established [1–3]. Epidemiologic research, clinical research,

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and basic science have continued to clarify their relationship in head and neck cancer, anal cancer, esophageal cancer, and lung cancer [4–6]. Based on a series of epidemiologic changes, a viral etiology has been theorized as playing a role in lung cancer initiation or evolution. Many studies have detected HPV DNA in lung cancer and the possible correlation between lung cancer and HPV has been suggested [6–8]. However, inconsistent data regarding HPV DNA detection in lung cancer have been observed between studies. We considered that the heterogeneity of HPV DNA prevalence in lung cancer might be due to various HPV DNA detection methods as well as due to variations in geographical study origin and histological types of cancer. Usually, to detect HPV DNA, polymerase chain reaction (PCR) in DNA samples purified from tissue specimens and *in situ* hybridization (ISH) signal amplification in target tissues are used. A strong advantage of ISH is the direct visualization of HPV in either the episomal form or an integrated pattern within the nuclei of tumor cells in tissues.

The aim of this study is to determine the prevalence of episomal or integrated hrHPV DNA in malignant cells of primary lung squamous cell carcinoma (SqCC) and adenocarcinoma (ADC) among Korean population, using the INFORM HPV III Family 16 Probe (B) in the Ventana-validated assay.

2. Materials and methods

2.1. Patients and tissue samples

We obtained 233 formalin-fixed, paraffin-embedded (FFPE) lung samples from 233 patients who underwent surgical resection at Chungnam National University Hospital, Daejeon, South Korea, between 1995 and 2010. The tissues consisted of SqCC ($n = 134$) and 99 ADC ($n = 99$) samples. All samples were isolated from lobectomy or pneumonectomy specimens. Clinical records were reviewed. Of these, none of the 233 cases had any known history of head and neck primary cancer or uterine cervical cancer. None of the patients had received preoperative chemotherapy or radiotherapy. The lung cancer stages were determined according to the American Joint Committee on Cancer Staging System, Seventh Edition. Clinical data were provided by the National Biobank of Korea, Chungnam National University Hospital, Daejeon, South Korea. This study was approved by the Institutional Review Board of Chungnam National University Hospital.

2.2. Tissue microarray construction

All cases were histologically reviewed by two pathologists (K.-H.K. and Y.-M.L.), and the two most representative areas of viable carcinoma tissue were selected and marked on the hematoxylin and eosin-stained slides. To create a tissue microarray (TMA), tissue columns (diameter, 3.0 mm) were punched from the original paraffin blocks and inserted into new recipient paraffin blocks (each containing 30 holes for tissue columns). Arrays were constructed using two 3-mm diameter cores per tumor.

2.3. High-risk HPV DNA ISH

All TMA blocks and 20 whole-FFPE SqCC blocks were used for hrHPV DNA detection. ISH was performed using the Ventana INFORM HPV III Family 16 probe (B) kit (Catalog Number 800-4295; Ventana Medical System, Inc., Tucson, AZ, USA) according to the manufacturer's recommendations on the BenchMark, Automated Slide Staining System (Ventana Medical System, Inc.). The INFORM HPV III Family 16 probe (B) kit is composed of oligonucleotides designed to hybridize with hrHPV genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 66. The positive hrHPV signal demonstrated either episomal or integrated HPV. The episomal pattern appears as a large, homogeneous, globular navy-blue precipitate and the integrative pattern as a discrete, stippled navy blue nuclear pattern within the nuclei of neoplastic cells. Five hrHPV-positive cases of laryngeal SqCC were used as positive controls. Two pathologists independently reviewed the hrHPV ISH slides and a consensus was obtained after discussion.

2.4. Detection and genotyping of HPV DNA

DNA was extracted from the whole-FFPE samples of 42 SqCCs and 47 ADCs. FFPE blocks were sectioned in six 7-μm sections. Total DNA was extracted using the G-DEX IIc Genomic DNA Extraction Kit according to manufacturer's instructions (iNtRON, Gyeonggi-do, South Korea).

The Anyplex II HPV28 Detection kit (Seegene, Seoul, South Korea) identifies simultaneously 19 hrHPVs (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 69, 73, and 82) and nine low-risk HPVs (6, 11, 40, 42, 43, 44, 54, 61, and 70). Real-time PCRs were performed on a CFX96 Real-Time PCR System (Bio-Rad Laboratories, Hercules, CA, USA) with 20-mL reaction tubes containing HPV28 primer mix A, which recognizes 14 hrHPV types or mix B that identifies five hrHPV types and nine low-risk HPV types, Anyplex Master Mix (Seegene), and genomic DNA purified from specimen. For each run, positive controls of all HPV-type plasmid constructs were included along with a nontemplate control. Results were automatically analyzed in Seegene Viewer version 2.0 (Seegene, Seoul, South Korea) [9,10].

3. Results

We investigated 233 lung carcinoma samples. The demographics of the study patients are presented in Table 1. All members of the study population were of Korean ethnicity. hrHPV DNA ISH analysis was negative in all 233 TMA samples and 20 whole-FFPE SqCC samples (Figure 1).