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Original Article

No evidence for a role of Merkel cell polyomavirus in small cell lung cancer among Iranian subjects

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ABSTRACT

Merkel cell polyomavirus (MCPyV), as a new member of polyomaviruses, has recently been discovered as a possible etiologic factor for human cancer. It was first detected in Merkel cell carcinoma (MCC). Small cell lung cancer (SCLC) is a malignant lung tumor which shares histopathological and genetic features with MCC, as both are of neuroendocrine origin. In this study, we investigated the presence of MCPyV DNA in SCLC specimens by real-time PCR. Our null hypothesis was that MCPyV is an etiologic factor in SCLC, as previously seen in MCC. Formalin-fixed and paraffin-embedded (FFPE) specimens were obtained from 50 patients, who underwent bronchoscopic biopsy and were diagnosed with SCLC between March 2010 and March 2012. Similarly, we obtained bronchoscopic biopsy specimens from 29 patients, who were diagnosed with non-small cell lung cancer (NSCLC). All samples were obtained at a single center (Masih Daneshvari Hospital, Tehran, Iran). Real-time PCR was done to detect the presence of MCPyV DNA. After excluding one specimen from the SCLC group due to loss of tumor tissue, we did not detect MCPyV DNA in samples from patients with either SCLC (the mean age 58.9 years, male/female ratio: 7.3/1) or NSCLC. Our results suggest that MCPyV does not play a role in the pathogenesis of SCLC, which is in accord with the results from other prior investigations.

Introduction

Cancer is a major public health problem in many parts of the world. Currently, one in four deaths in the United States is due to cancer [1]. With more than 1.1 million deaths annually worldwide, lung cancer is the most common, and one of the deadliest types of cancer [2–4]. Patients with SCLC are different from those with NSCLC in that their presenting symptoms are frequently caused by distant metastases [2]. Unfortunately, because of the poor survival in patients with SCLC, most studies continue to focus on other types of lung cancer.

Polyomaviruses are small double-stranded DNA viruses that are suspected to be the etiologic factors in many different human malignancies. Four of these viruses, BKV (first isolated in 1971 from the urine of a renal transplant patient, initials B.K), JCV (John Cunningham virus), KIV (Karolinska Institute virus), and WUV (Washington University virus), belong to the SV40 (Simian vacuolating virus 40) subgroup [5,6]. However, a direct oncogenic role in humans has never been proven [7]. Recently, Feng et al. reported the identification of a fifth human polyomavirus, which was designated Merkel cell polyomavirus (MCPyV) based on its detection in Merkel cell carcinoma (MCC), an aggressive, metastatic form of

Abbreviations: MCPyV, Merkel cell polyomavirus; MCC, Merkel cell carcinoma; SCLC, Small cell lung cancer; PCR, Polymerase chain reaction; FFPE, Formalin-fixed and paraffin-embedded; NSCLC, Non small cell lung cancer; IHC, Immunohistochemistry; H&E, Hematoxylin and eosin; COPD, Chronic Obstructive Pulmonary Disease.

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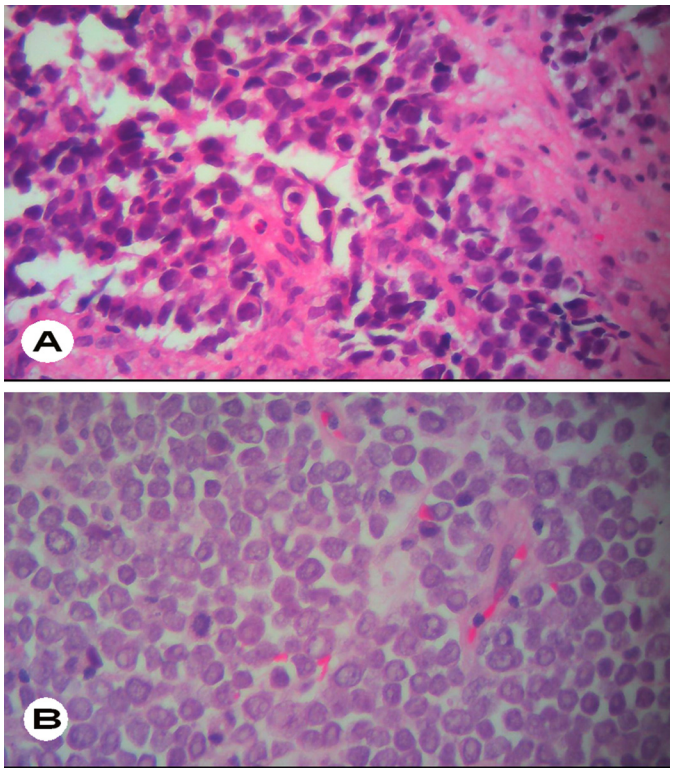

Fig. 1. Histologic slides of small cell lung cancer (A) and Merkel cell carcinoma (B), both characterized by small round to oval cells with fine granular nuclei and scanty cytoplasm (H&E, 400×). These diagnoses were confirmed by IHC studies.

skin cancer [8]. Merkel cell polyomaviurs is detected in 39%–80% of patients with MCC [8–10]. However, its association with malignancies that are histologically similar to MCC, specifically SCLC, and other high-grade neuroendocrine tumors, has yet to be investigated [11].

Merkel cell carcinoma and SCLC are neuroendocrine tumors with remarkable histopathological similarities (Fig. 1). They are made of small round cells that often contain dense core granules, and express several identical immunohistochemical markers [12]. Once a diagnosis of MCC has been made, it is recommended that patients should undergo screening for metastatic SCLC [13].

Knowing the unfavorable outcome of SCLC, and a 5-year survival rate less than 5% [4,14], few etiologic factors for this cancer have been identified. However, a viral etiology for a subset of patients developing lung cancer has been suggested [15].

Although there has been a reduction in the prevalence of smoking, and a decrease in the incidence of SCLC, there has been loss of a decline noted among women and nonwhites [16]. Interestingly, females with lung cancer have a better survival rate than males with lung cancer, despite the higher incidence of SCLC in women, which has a worse prognosis than the other types of lung cancer [3]. On the other hand, MCC with very low or undetectable levels of MCPyV DNA has a tendency for poorer survival [17].

Herein we investigated the prevalence of MCPyV as a possible etiologic factor in patients with SCLC.

Materials and methods

Design

This was a single center observational case–control study, conducted between March 2010 and March 2012 at Masih Daneshvari Hospital, Tehran, Iran. The prevalence of MCPyV in FFPE samples of bronchoscopic biopsies diagnosed as SCLC was determined by PCR. Additionally, we included FFPE samples from NSCLC with relatively similar characteristics to those of the SCLC group as our control group. Subsequently, patients' clinical information were correlated with radiologic studies and pathologic reports.

All previously performed immunohistochemistry (IHC) studies for thyroid transcription factor 1 (TTF1), and cytokeratin (CK), along with the hematoxylin and eosin (H&E) stained slides, were reviewed by two expert pathologists to confirm the diagnosis.

Studied variables

Demographic and histological data collected included sex, age, smoking status (at the time of diagnosis), histopathological diagnosis after re-examination of H&E stained slides, and presence or absence of MCPyV DNA, as detected by PCR. Treatment status of the patients and their survival were obtained from medical records.

Subjects and data collection

We used 50 FFPE samples from patients diagnosed with SCLC as the case group, and 29 FFPE samples from patients with NSCLC as our control group. These samples were obtained from the archives of the Pathology Department of the Masih Daneshvari Hospital, a teaching affiliate of Shahid Beheshti University of Medical Sciences, Tehran, Iran. All samples were collected for diagnostic purposes between 2010 and 2012. In all cases the diagnosis of either SCLC or NSCLC was confirmed after re-examination of H&E stained slides by two expert pathologists. Of the 50 SCLC patients, samples from 49 patients underwent PCR (one sample was excluded due to loss of tumor tissue after re-examination of H&E slides). Similarly, all 29 samples from patients with NSCLC underwent PCR. After re-examining H&E slides, samples that included evidence of a different malignancy, and those with necrosis of more than 50% of the tissue were excluded.

DNA extraction

Paraffin was removed from the FFPE samples after undergoing xylene treatment, followed by two washes with pure ethanol. Total DNA was extracted from the air-dried pellet tissues according to the QIAamp DNA Mini Kit procedure. The extracted DNA was stored at −20 °C until it underwent PCR.

PCR

To assess the quality of the extracted DNA, as well as inhibition of PCR test, all extracted and stored DNA underwent beta-globin PCR, using the PCO3/PCO4 primer set, as described previously [18]. To detect MCPyV, real-time PCR was performed using VP2 primer sets amplifying 82 bp gene region of the virus genome (forward primer: 5′-GCCTAGAGGTAGGAGATAAAGAATTAAAAA-3′, reverse primer: 5′-CTAGATCCTCTGCAGTGGGAAAA-3′, with an internal probe 5′FAMTTGCCCCACAGAATGCAGCAAGC-TAMRA3′) [19]. Amplification of MCPyV VP2 genes was performed in reaction volumes of 20 μL with under the following conditions: first the samples underwent denaturation at 95 °C for 10 min, followed by denaturation at 95 °C for 10 s, followed by annealing and extension at 60 °C for 1 min, 50 cycles. Real-time PCR System CFX-96 (BIO-Rad) with *Maxima Probe* qPCR *Master Mix* TaqMan reagent (Thermo Scientific) was used in Real-time PCR assay. The limit detection of five genome equivalent of MCPyV per reaction, was determined by the real-time assay, using the serial dilutions of cloned VP2 PCR fragment plasmid. In addition, we used the genome extracted from the two different FFPE MCC tissues (diagnosed between 2009 and 2011), as well as sputum samples from four patients with chronic