Detection of Merkel cell polyomavirus with a tumor-specific signature in non-small cell lung cancer
（非小細胞肺がんにおける腫瘍特異的特徴を有するメルケル細胞ポリオーマウイルスの検出）

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【Introduction】

Lung cancer is the leading cause of cancer death in the world. Smoking is the most important risk factor for lung cancer, but lung cancers arising in never smokers have increased in recent times. These findings suggest that additional factors other than smoking may have an impact as etiological and risk factors for lung cancer.

In 2008, a new human tumor virus, Merkel cell polyomavirus (MCPyV), was discovered as a causative agent of Merkel cell carcinoma (MCC), a neuroendocrine carcinoma of the skin. While the route of MCPyV transmission has not been established, detection of MCPyV DNA fragments in respiratory tract secretions was reported in 2010. It is likely that long-term exposure of the respiratory tract to this oncogenic virus may cause the cancer in this region. Based on the histological similarities between MCC and small-cell lung cancer (SCLC), the possible association between MCPyV and SCLC has been reported previously. However, the prevalence of MCPyV in non-small cell lung cancer (NSCLC) has not been studied well. This study was performed to determine the possible viral etiology of NSCLCs among Japanese subjects.

【Materials and Methods】

Patients and samples

This study included 112 Japanese patients with primary resectable NSCLC, including 32 squamous cell carcinomas (SCCs), 45 adenocarcinomas (ACs), 32 large-cell carcinomas including 19 large-cell neuroendocrine carcinomas (LCNECs) and 3 pleomorphic carcinomas. Surgically resected frozen tumor samples were obtained from the archives of Kochi University Hospital.

Detection of MCPyV DNA with standard PCR

Standard PCR was performed with 200 ng of extracted DNA from the frozen tissue samplers using three primer sets, commonly used to detect the MCPyV large T (*LT*) and viral protein 1 (*VP1*) genes.

Quantification of MCPyV DNA with real-time PCR

MCPyV-positive samples identified by standard PCR were subjected to a quantitative real-time PCR analysis to determined MCPyV DNA loads. The reactions were performed with 200 ng of extracted DNA using specific labeled probe and primers targeting the MCPyV small T antigen (*ST*) gene.

Reverse transcription (RT)-PCR

RT-PCR was performed on total RNA extracted from the frozen biopsy specimens. Specific primers for analysis of cDNA were used to determine the MCPyV *LT* and *VP1* gene expressions.

Immunohistochemistry

For detecting the MCPyV antigen, immunohistochemistry was performed on formalin-fixed paraffin-embedded tissue sections using a monoclonal antibody targeted to the MCPyV LT antigen.

Analysis of the MCPyV integration site

The integration sites of MCPyV were determined using the DIPS-PCR technique (detection of integrated papilloma sequences).

【Results and Discussion】

MCPyV was detected in 9/32 (28.1%) tissue samples of SCCs, 9/45 (20.0%) of ACs, 1/32 (3.1%) of large-cell carcinomas (including one MCPyV-positive LCNEC), and 1/3 (33.3%) of PL by standard PCR. In total, 20 of 112 NSCLC (17.9%) tissue samples were positive for MCPyV DNA. These results were close to the results reported from North America, but different from those from Chile. The differences in the detection rates may be explained in part by geographic epidemiological diversities in patients with NSCLCs or by the technical approaches used.

The viral DNA loads ranged from 0.0001 to 0.026 copies per cell, shown by quantitative real-time PCR. These DNA loads were lower than those in MCPyV-positive Japanese MCC cases.

Several oncogenic mechanisms for MCPyV development have been proposed in MCPyV-positive-MCC: (1) expression of the MCPyV oncogenic protein, LT antigen; (2) loss of viral replication capacity; and (3) tumor-specific mutations, including the truncating mutation of the *LT* gene and integration of the viral genome into host chromosome.

Expression of the MCPyV LT antigen was examined at the RNA and protein levels by RT-PCR and immunohistochemistry, respectively. The MCPyV *LT* gene transcript was found in 4/10 of MCPyV-positive samples which were suitable for RNA extraction in MCPyV-positive tumors, whereas no *VP1* gene transcript was found in any samples. As the results of immunohistochemistry,

strong diffuse or speckled nuclear signals were observed in tumor cells, indicating that these cancer cells expressed the LT antigen. During viral replication, MCPyV has an orderly gene expression cascade in which the *LT* gene transcript is expressed first (early gene transcription), followed by the expression of the *VP1* gene (late gene transcription). Accordingly, these results suggested that our MCPyV-positive NSCLC tumors had no viral replication activity.

To evaluate integration status of MCPyV, DIPS-PCR was performed. The MCPyV integration was demonstrated in 2 cases, in which the viral genomes were inserted into chromosome 5 and chromosome 11, respectively. Additionally, the virus-host junctions in these cases were both located in the MCPyV *LT* gene, generating the truncated *LT* gene.

【Conclusion】

This study provided the first evidence of prevalence of MCPyV in NSCLCs among Japanese subjects. Furthermore, this study demonstrated the integrated/mutated forms as a tumor-specific signature of MCPyV for the first time in a specific cancer other than MCC. Although the prevalence of MCPyV and viral loads in our NSCLCs were low as compared with those in MCPyV-positive MCCs, our findings suggest that MCPyV is associated with the pathogenesis of NSCLC in a subset of patients.