Characterization of the self-assembly of New Jersey polyomavirus VP1 into virus-like particles and the virus seroprevalence in Japan

博士（医学）　周　显凤

論文題目

Characterization of the self-assembly of New Jersey polyomavirus VP1 into virus-like particles and the virus seroprevalence in Japan

(ニュージャージーポリオーマウイルスVP1のウイルス様粒子への自己集合の解析および日本のおける血清抗体陽性率)

論文の内容の要旨

[Introduction]

In general, human polyomaviruses (PyVs) can cause persistent infection, and these infections are asymptomatic. However, a PyV infection can cause serious illnesses, especially in immunocompromised individuals. In the last decade, as a result of improved molecular techniques, nine novel human PyVs have been identified. Among them, New Jersey polyomavirus (NJPyV) was discovered in 2014 in vascular endothelial cells of a pancreatic transplant recipient. Based on the available sequencing data to date and the results of phylogenetic analyses, NJPyV, classified as an Alpha-polyomavirus, is most closely related to chimpanzee PyVs and bat PyVs. To date, little is known about characteristic features of NJPyV particles as well as the viral epidemiology.

[Materials and Methods]

The full-length VP1 DNA of NJPyV was synthesized based on the viral sequence (GenBank accession no. KF954417) and was cloned into a baculovirus transfer vector pVL1393, yielding pVL1393-NJVP1. Sf9 insect cells were co-transfected with linearized wild-type Autographa californica nuclear polyhedrosis virus DNA and pVL1393-NJVP1. The recombinant virus was plaque-purified in Sf9 cells and designated as Ac[NJPyV-VP1]. For the expression of NJPyV VP1, Sf9 and Tn5 insect cells were infected with Ac[NJPyV-VP1] at a multiplicity of infection of 10, respectively. To purify NJPyV-like particles (NJPyV-LPs), the culture supernatants of Ac[NJPyV-VP1]-infected cells were subjected to 3 steps of ultracentrifugation including the cesium chloride density gradient centrifugation. Purified NJPyV-LPs were examined with transmission electron microscope. VLPs of other PyVs; BK virus (BKPyV-LPs), JC virus (JCPyV-LPs), Merkel cell polyomavirus (MCPyV-LPs) and Trichodysplasia spinulosa-associated polyomavirus (TSPyV) were supplied by Department of Virology II, National Institute of Infectious Diseases (NIID), Japan.

Hyperimmune sera against NJPyV-, BKPyV-, JCPyV-, MCPyV- or TSPyV-LPs were obtained by immunizing rats with a dose of 100 μg of each VLP sample per shot including booster shots. VLP-based enzyme-linked immunosorbent assay (ELISA) was developed and used to evaluate the seroprevalence of NJPyV in general Japanese population (529 males and 521 females) aged 1-70 years old. The research proposal was approved by the National Serum Reference Bank of NIID and human sera used were

supplied by the bank.

[Results]

In the baculovirus Ac[NJPyV-VP1]-infected Sf9 cells, a major protein with a molecular mass of 54 kDa (p54), identical to the predicted size of the entire NJPyV VP1, was detectable. In the culture supernatants, a considerable amount of p54 was detectable at 2-10 days post-infection, and spherical ~50-nm-dia. NJPyV-LPs of uniform size with morphology resembling that of the native particles of PyVs were purified from the fraction at 1.33 g/cm^3. In contrast, in the infected Tn5 cells, in addition to a limited amount of p54, a large majority of the proteins observed thereafter appeared to be possible processed forms ranging from 30 to 48 kDa. While 50-nm VLPs were detected in the Tn5 culture supernatants, most of the VLPs observed were 20-30 nm in size.

By using immunized rat sera with 50-nm NJPyV-LPs as well as with BKPyV-, JCPyV-, MCPyV- or TSPyV-LPs, the antigenic property of NJPyV and limited cross-reactivity of anti-NJPyV serum with antisera against other PyVs were shown. As judged by data of the VLP-based ELISA developed, in contrast to the cases with BKPyV, JCPyV, MCPyV, and TSPyV (whose seropositivities were 43-68%), the overall prevalence of anti-NJPyV antibodies in the Japanese population was only 1.8%.

[Discussion]

The results demonstrated that Sf9 cells but not Tn5 cells are suitable for the efficient expression of p54 VP1 and the subsequent formation of NJPyV-LPs that possess the shape and size comparable to native PyV particles with the use of a baculovirus expression system, suggesting that the proteolytic processing of NJPyV VP1 observed mainly in Tn5 cells may lead to the weakening stability of ~50-nm NJPyV-LP or to lessened efficiency of the assembly of the ~50-nm VLPs.

Although the viral seroprevalence may vary depending on several characteristics such as age, immunoprofile and regional features, it is generally accepted that PyVs are ubiquitously distributed; their seroprevalence in adult populations ranges from 40% to 90% throughout the world. Recent studies have shown that seropositivities of NJPyV in general population were approximately 45% and 5% in Italy and Netherland, respectively, indicating its lower seroprevalence compared to most of human PyVs analyzed. The current study demonstrated very low seroprevalence of NJPyV in Japanese population, suggesting low circulation of NJPyV in Japan.

[Conclusion]

The present study demonstrated the expression and processing of NJPyV VP1 and its self-assembly into VLPs in insect cells with a recombinant baculovirus and the development of an NJPyV-LP-based ELISA. The NJPyV seroprevalence was only 1.8% in the Japanese general population examined. This is the first report of a large-scale serological screening for the presence of anti-NJPyV antibodies in Asia. Further studies are required to elucidate the geographical diversity of the virus distribution, as well as the transmission mode of NJPyV and its potential involvement in human diseases.