Identification, molecular characterization, and application of a novel virus isolated from mosquito larvae in Okushiri Island, Japan

Mosquitoes are one of the most important pest insects that have a serious impact on public health; they are a principal vector of serious infectious diseases including malaria, dengue fever, chikungunya, Japanese encephalitis, and yellow fever. Vector control is an essential strategy for reducing the transmission of mosquito-borne diseases. Mosquito-specific viruses with mosquitocidal activity, therefore, may be used as environmentally-friendly biological pesticides for chemical pesticides.

In this study, a small spherical virus, designated Okushiri virus (OKV), was isolated from mosquito larvae and identified as a novel virus belonging to the genus Negevirus, a newly proposed insect-specific virus group. Here, an infectious cDNA clone for OKV and an OKV-based foreign gene expression system were constructed as a basis for reverse genetics approach in basic and applied research of negevirus.

1. Identification and molecular characterization of Okushiri virus

A novel virus was isolated from field-collected Aedes larvae collected on Okushiri Island, Hokkaido, Japan. This virus, designated Okushiri virus (OKV), replicated in the Aedes albopictus cell line C6/36 with severe cytopathic effects and produced a large number of spherical viral particles that were 50-70 nm in diameter and released into the cell culture medium. The OKV had a positive-sense, single-stranded RNA and the genome RNA consisted of 9,704 nucleotides, excluding the poly(A) tail at the 30-terminus, and contained three major open reading frames (ORF1, ORF2, and ORF3). ORF1 encoded a putative protein of approximately 268 kDa that included a methyltransferase domain, FtsJ-like methyltransferase domain, helicase domain, and
RNA-dependent RNA polymerase domain. ORF2 and ORF3 were suggested to encode hypothetical membrane-associated proteins of approximately 45 kDa and 22 kDa, respectively. The genome organization and results of a phylogenetic analysis based on the amino acid sequence predicted from the nucleotide sequence indicated that OKV is a member of a new insect virus group of negeviruses with a possible evolutionary relationship to some plant viruses. This is the first study on a novel negevirus isolated from mosquito larvae in Japan.

2. Generation of an infectious cDNA clone of Okushiri virus and its derivative capable of expressing an exogenous gene

To enable genetic manipulation of the OKV genome, an infectious cDNA clone of OKV was constructed. RNA synthesized in vitro from pFBOKV, a full-length OKV cDNA, in the presence or absence of cap analogue produced infectious progeny viruses effectively in mosquito C6/36 cells. Subsequently, ORF3 in pFBOKV was replaced with GFP coding sequence to generate a construct designated as pO2GFP. C6/36 cells transfected with pO2GFP-derived RNA successfully expressed GFP, but failed to produce progeny viruses. Co-transfection of C6/36 cells with pO2GFP- and pFBOKV-derived RNA revealed that it is possible to produce infectious pO2GFP-derived progeny viruses by supplying OKV genetic elements and/or gene product deleted in pO2GFP even though the infectivity appeared to be low. This is the first report of construction of a negevirus-based foreign gene expression system. The cDNA clones constructed and biological insights obtained in this study will be powerful tools allowing for reverse genetics of OKV and providing a basis to develop efficient negevirus-based expression vector systems.