

水痘帯状疱疹ウイルスを用いた汎用性のある生ワクチンベクターの開発とその応用

森 康子 (医薬基盤研究所)

Development of live attenuated vaccine vector based on varicella vaccine

Yasuko MORI, National Institute of Biomedical Innovation

1. Matsuura M, Takemoto M, Yamanishi K, Mori Y. (2011)

Human herpesvirus 6 major immediate early promoter has strong activity in T cells and is useful for heterologous gene expression.

Virology, 8:9.

BACKGROUND: Human herpesvirus-6 (HHV-6) is a beta-herpesvirus. HHV-6 infects and replicates in T cells. The HHV-6-encoded major immediate early gene (MIE) is expressed at the immediate-early infection phase. Human cytomegalovirus major immediate early promoter (CMV MIEp) is commercially available for the expression of various heterologous genes. Here we identified the HHV-6 MIE promoter (MIEp) and compared its activity with that of CMV MIEp in various cell lines.

METHODS: The HHV-6 MIEp and some HHV-6 MIEp variants were amplified by PCR from HHV-6B strain HST. These fragments and CMV MIEp were subcloned into the pGL-3 luciferase reporter plasmid and subjected to luciferase reporter assay. In addition, to investigate whether the HHV-6 MIEp could be used as the promoter for expression of foreign genes in a recombinant varicella-zoster virus, we inserted HHV-6 MIEp-DsRed expression cassette into the varicella-zoster virus genome.

RESULTS: HHV-6 MIEp showed strong activity in T cells compared with CMV MIEp, and the presence of intron 1 of the MIE gene increased its activity. The NF- κ B-binding site, which lies within the R3 repeat, was critical for this activity. Moreover, the HHV-6 MIEp drove heterologous gene expression in recombinant varicella-zoster virus-infected cells.

CONCLUSIONS: These data suggest that HHV-6 MIEp functions more strongly than CMV MIEp in various T-cell lines.

2. Somboonthum P, Koshizuka T, Okamoto S, Matsuura M, Gomi Y, Takahashi M, Yamanishi K, Mori Y. (2010)

Rapid and efficient introduction of a foreign gene into bacterial artificial chromosome-cloned varicella vaccine by Tn7-mediated site-specific transposition.

Virology, 402: 215-221.

Using a rapid and reliable system based on Tn7-mediated site-specific transposition, we have successfully constructed a recombinant Oka varicella vaccine (vOka) expressing the mumps virus (MuV) fusion protein (F). The backbone of the vector was our previously reported vOka-BAC (bacterial artificial chromosome) genome. We inserted the transposon Tn7 attachment sequence, LacZ α -mini-attTn7, into the region between ORF12 and ORF13 to generate a vOka-BAC-Tn genome. The MuV-F expressing cassette was transposed into the vOka-BAC genome at the mini-attTn7 transposition site. MuV-F protein was expressed in recombinant virus, rvOka-F infected cells. In addition, the MuV-F protein was cleaved in the rvOka-F infected cells as in MuV-infected cells. The growth of rvOka-F was similar to that of the original recombinant vOka without the F gene. Thus, we show that Tn7-mediated transposition is an efficient method for introducing a foreign gene expression cassette into the vOka-BAC genome as a live virus vector.

3. Somboonthum P, Yoshii H, Okamoto S, Koike M, Gomi Y, Uchiyama Y, Takahashi M, Yamanishi K, Mori Y. (2007)

Generation of a recombinant Oka varicella vaccine expressing mumps virus hemagglutinin-neuraminidase protein as a polyvalent live vaccine.

Vaccine, 25: 8741-8755.

We constructed a recombinant varicella-zoster virus (VZV) Oka vaccine strain (vOka) that contained the mumps virus (MuV) hemagglutinin-neuraminidase (HN) gene, inserted into the site of the ORF 13

gene by using the bacterial artificial chromosome (BAC) system in *Escherichia coli*. Insertion of the HN gene into the VZV genome was confirmed by PCR and Southern blot. The infectious virus reconstituted from the vOka-HN genome (rvOka-HN) had a growth curve similar to the original recombinant vOka without the HN gene. The mumps virus HN protein expressed in rvOka-HN infected cells was expressed diffusely in the cytoplasm, and modification of the protein was similar to that seen in MuV-infected cells. Electron microscopic examination of infected cells revealed that HN was expressed on the plasma membrane of the cells but not in the viral envelope, suggesting that the tropism of rvOka-HN would be unchanged from that of the original vOka strain. Immunization of guinea pigs with rvOka-HN induced VZV- and HN-specific antibodies. Interestingly, the induced antibodies had a strong neutralizing activity against virus-cell infections of both MuV and VZV. Therefore, the novel varicella vaccine expressing MuV HN protein is suitable as a polyvalent live attenuated vaccine against VZV and MuV infections.

4. Yoshii H, Somboonthum P, Takahashi M, Yamanishi K, Mori Y. (2007)

Cloning of full length genome of varicella-zoster virus vaccine strain into a bacterial artificial chromosome and reconstitution of infectious virus.

Vaccine, 25: 5006-5012.

The complete genome of the varicella-zoster virus (VZV) Oka vaccine strain (vOka) has been cloned into a bacterial artificial chromosome (BAC), and several BAC clones with the vOka genome have been obtained. Infectious viruses were reconstituted in MRC-5 cells transfected with the vOka-BAC DNA clones. The clones were distributed into two groups based on differences in amino acids found in ORF 62/71 region among the vOka-BAC clones. The recombinant vOka (rvOka) grew slower than recombinant Oka parental virus (rpOka), pOka and vOka. This is the first report that the vOka genome was cloned into BAC vector. The rvOka-BAC system would be useful as a vector for construction of recombinant live vaccines.

5. Nagaike K, Mori Y, Gomi Y, Yoshii H, Takahashi M, Wagner M, Koszinowski U, Yamanishi K. (2004)

Cloning of the varicella-zoster virus genome as an infectious bacterial artificial chromosome in *Escherichia coli*.

Vaccine, 22: 4069-4074.

The complete genome of the varicella-zoster virus (VZV) Oka strain has been cloned as a bacterial artificial chromosome (BAC). Following electroporation into *Escherichia coli* (*E. coli*) strain DH10B, the VZV BAC was stably propagated over multiple generations of its host. Human embryonic lung (HEL) cells transfected with VZV BAC DNA recovered from DH10B showed cytopathic effect (CPE), and virus spread to neighbouring cells was observed. BAC vector sequences are flanked by loxP sites and, coinfection of the reconstituted virus, with a recombinant adenovirus expressing Cre recombinase removed the bacterial sequences. The resulting recombinant rv02 grew as well as the parental virus in HEL cells. The recombinant VZV will promote VZV research and increase use of the viral genome as an investigative tool.