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Analysis of single nucleotide polymorphism among Varicella-Zoster Virus and identification of vaccine-specific sites

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ABSTRACT

Varicella-zoster virus (VZV) is a causative agent for chickenpox and zoster. Live attenuated vaccines have been developed based on Oka and MAV/06 strains. In order to understand the molecular mechanisms of attenuation, complete genome sequences of vaccine and wild-type strains were compared and single nucleotide polymorphism (SNP) was analyzed. ORF22 and ORF62 contained the highest number of SNPs. The detailed analysis of the SNPs suggested 24 potential vaccine-specific sites. All the mutational events found in vaccine-specific sites were transitional, and most of them were substitution of AT to GC pair. Interestingly, 18 of the vaccine-specific sites of the vaccine strains appeared to be genetically heterogeneous. The probability of a single genome of vaccine strain to contain all 24 vaccine-type sequences was calculated to be less than 4%. The average codon adaptation index (CAI) value of the vaccine strains was significantly lower than the CAI value of the clinical strains.

1. Introduction

Varicella–zoster virus is a herpesvirus that causes chickenpox (varicella), and shingles (zoster) in human (Gilden et al., 2000). Primary VZV infection usually occurs in childhood and causes varicella, characterized by fever and vesicular rash. During the resolution of varicella, VZV establishes latency in the trigeminal and dorsal root ganglia where, in most cases, it remains latent throughout the lifetime of its host. Approximately 25% of varicella-infected individuals develop zoster later in life due to VZV re-activation. The virus multiplies and spreads within the ganglion, causing neuronal necrosis and intense inflammation, often resulting in severe neuralgia. The virus is then released from sensory nerve endings in the skin where it spreads to produce the characteristic cluster of zoster vesicles (Quinlivan and Breuer, 2006). Zoster is characterized by pain and a vesicular eruption on an erythematous based in one or several dermatomes.

VZV infection could be prevented by immunization with live attenuated vaccines. Takahashi and colleagues developed a vaccine strain vOka, attenuated by *in vitro* cell culture passaging in human embryo lungs, guinea pig embryonic lungs and WI38 cells from Japanese pOka strain (Takahashi et al., 1974). vOka strain supplied by the Biken Institute was licensed for use in healthy subjects in Japan since 1986. Several manufacturers have since developed vaccines derived from vOka strain, for example, Varilrix from GlaxoSmithKline (GSK) Biologics and Varivax from Merck were originated from vOka. The vaccines were licensed for routine use against varicella in 1995 and against herpes zoster in 2006 in the United States (CDC, 1996; Mitka, 2006). Zostavax from Merck is a zoster vaccine and contains 14-fold higher viral titers than that of the varicella vaccine. Besides vOka strain, another attenuated VZV vaccine strain MAV/06 was developed by Green Cross in Korea (Sohn et al., 1994) and has been used to manufacture Suduvax against varicella. Suduvax has been marketed in Korea since 1994 and internationally since 1998.

Multiple genome sequences of vaccine strains, including vOka, Varilrix, Varivax and Suduvax, have been reported by several groups (Gomi et al., 2002, Tillieux et al., 2008, Kim et al., 2011). A comparative genome analysis of vOka vaccine sequences with those of clinical pOka strain suggested 42 substitution sites, which may be potently involved in the attenuation of varicella vaccine (Gomi et al., 2002; Yamanishi, 2008; Quinlivan et al., 2011). Further analysis of vOka-derived vaccine strains Varilrix and Varivax reduced the numbers of potential vaccine-specific sites to 23 (Tillieux et al., 2008). However, the information seems to be

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limited since the vaccine strains analyzed were of one kind, vOka or vOka-derived Varilrix and Varivax. Thus, it is important to include the comparative genome analysis of another vaccine strain, MAV/06-based Suduvax, and this study would facilitate the identification of vaccine-specific sites and attenuation mechanisms.

The goal of our study is to compare all the complete genome sequences of clinical strains and vaccine strains available in order to find single nucleotide polymorphism (SNP) among currently known VZV strains. Using the SNP information, we were able to define and characterize genomic variations among VZV stains and provide the insights into the sties specific for vaccine strains.

2. Methods

2.1. Acquisition of complete genomic DNA sequences

Complete genomic DNA sequences of VZV strains were retrieved from the NCBI GenBank database. VZV strains analyzed in this study include the reference strain Dumas (NC001348), Japanese pOka (AB097933) and pOka-derived vaccine strains vOKa (AB097932), Varilrix (DQ008354), Varivax (DQ008355), 1002/2008 (JN704697) and Korean vaccine strain Suduvax (JF306641). GenBank accession number for other clinical strains are DQ452050, DQ457052, DQ479953～63, DQ674250, EU154348, AY548170～1, AJ871403, JN704690～96, JN704698～JN704710, JQ972913～4. The key information about multiple VZV strains was analyzed and summarized in Supplemented Table 1.

2.2. Isolation and propagation of virus in vitro cell culture

Virus samples were obtained from patients, who were admitted to Yeungnam University Hospital in Daegu, South Korea from June to August 2012. VZV strain YC01 was isolated from a 40-year old man with herpes zoster. VZV YC02 was isolated from a 3-year old boy with chickenpox. VZV YC03 was isolated from an 8-year old girl who was vaccinated, but contracted later with chickenpox and came to the hospital with zoster. A vesicular fluid of the patients was collected and inoculated into MRC-5 cell monolayer grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% antibiotic-antimycotic (Gibco, Grand Island, NY, USA) at 37 °C in a CO_2 incubator. When VZV-infected cell culture showed 50–60% cytopathic effect (CPE), cells were trypsinized and 1/2–1/16 of the infected cells were used to infected a new monolayer of MRC-5 cells. VZV strain Ellen (ATCC VR-1367) was also propagated by *in vitro* cell culture.

2.3. DNA sequencing

Samples for DNA sequencing of clinical strains (YC01 passage13, YC02 passage14, YC03 passage6, Ellen) were obtained by repeated freezing and thawing of the infected cell culture for 3 times, followed by 12 cycles of sonication (Sonics, model VCX130, Newton, CT, USA) at 20% Amp for 10 s and 30 s of cooling down. The resultant was centrifuged at 8000 rpm for 5 min at 4 °C. In order to enrich virus particles and removing as much as cellular DNA, ammonium sulfate-mediated precipitation was applied. An equal volume of 0.8 g/ml of ammonium sulfate was added to supernatant, and the mixture was stirred for 4 h. After centrifugation at 10,000 × *g*, 4 °C for 30 min, the pellet was resuspended with 200 μl of serum-free DMEM and stored in 1.5 ml tube.

DNA samples from vaccine strains were extracted from commercial vials of Varilrix (Lot No. A70CB482A), Varivax (Lot No. R1291), and Suduvax (Lot No. 08-027) with QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacture's protocol at a concentration of 5.5 μg/100 μL. DNA was extracted with GenAll DNA kit (Daejeon, Korea) according to the protocol provided by the manufacturer.

Next Generation Sequencing (NGS) was conducted in order to get complete genome sequence and relative proportions of the 4 bases (A, C, G, T) at each position of the complete genome of high-passaged YC01, YC02, and YC03, as well as resequencing of the strains Ellen, Varilrix, Varivax and Suduvax. NGS systems using Illumina Mi-Seq of Solexa (Illumina, San Diego, CA, USA) or Genome Sequencer FLX Titanium System of Roche Diagnostics (Roche, CT, USA) were employed. The construction of DNA libraries and NGS were performed by Chun Lab (Seoul, South Korea). After quality check and data trimming, CLC Genomics Workbench (version 5.0, http://www.clcbio.com/products/clc-genomics-workbench/) program was used to assemble the sequences. Finally a single contig without a gap for each strain was obtained. The completed sequences were annotated and deposited into NCBI GenBank database: KJ767491 (YC01), KJ767492 (YC02) and KJ808816 (YC03).

2.4. Genetic distance

Nucleotide and amino acid sequences were multi-aligned with ClustalW (ver. 2.1, http://www.clustal.org/clustal2/). Aligned sequences were compared with SeqAid program developed by our group to estimate genetic distances among sequences belonging to different groups. Then, mean and standard deviation values for each pair of the comparison were calculated. Statistical analysis was performed with SPSS (ver. 10) in order to get statistical significance of the data when needed.

2.5. Codon adaptation index (CAI)

There are 61 codons for 20 amino acids, indicating an average of 3 codons for each amino acid and these codons are called synonymous codons. However, not all synonymous codons are used equally in the majority of genes of any organism. There is a bias in the use of synonymous codons. One indicator of the codon usage bias is codon adaptation index (CAI). CAI is a simple, effective measure of synonymous codon usage bias and uses a reference set of highly expressed genes from species, such as *Homo sapiens*, to assess the relative merits of each codon (Sharp and Li, 1987). CAIcal program (ver.1.4, http://genomes.urv.cat/CAIcal) was used to get CAI values. Input file for CAIcal program was fasta file of the coding DNA sequence of target gene(s), concatenated entire ORFs or ORF62 in this study. Input file was codon usage of *Homo sapiens* (http://genomes.urv.es/CAIcal/CU_human_nature).

3. Results

3.1. Sequence diversity among VZV strains

VZV strains are relatively homogeneous in their genomic DNA sequences, compared with other viruses. The sequence diversity at the genome level among 50 currently known VZV strains was calculated to be $1.40 \pm 0.58 \times 10^{-3}$ and $3.39 \pm 4.29 \times 10^{-3}$ at nucleotide and amino acid level, respectively (Table 1). These 50 VZV strains could be divided into two groups, clinical and vaccine types. The sequence diversity among 5 vaccine strains (vOka, Varilrix, 1002/2008, Varivax and Suduvax) was $0.27 \pm 0.21 \times 10^{-3}$ at the nucleotide level, which was significantly lower, compared with other 45 clinical strains ($1.31 \pm 0.54 \times 10^{-3}$, Table 1). Similar result was obtained at the amino acid level. These results indicate that the vaccine strains are more conserved in their nucleotide and amino acid sequences than that of clinical strains. It is plausible