LETTER TO THE EDITOR

SEQUENCE ANALYSIS OF THE M GENOME SEGMENT OF TWO SEOUL VIRUS ISOLATES FROM SHANDONG PROVINCE, CHINA

Z. TAO, Z. WANG*

Department of Virology, School of Public Health, Shandong University, 44 Wenhuaxilu Rd, Jinan 250012, P.R. China, and Key Laboratory of Experimental Teratology, Ministry of Education, P.R. China

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Hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS) are rodent-borne viral zoonoses caused by hantaviruses (the genus *Hantavirus*, the family *Bunyaviridae*). Hantaviruses have a tripartite, negative-strand RNA genome, of which the small (S), medium (M) and large (L) segments encode nucleocapsid protein (N protein), glycoproteins (Gn and Gc), and RNA-dependent RNA polymerase (L protein), respectively (*1*). More than 20 hantavirus genotypes with specific main rodent hosts have been identified so far (*2*). *Rattus norvegicus* (brown rat) is the primary reservoir of Seoul virus (SEOV) (*3*). In China, HFRS caused by Hantaan virus (HTNV) and SEOV has a broad scope of prevalence. Ninety percent of the total cases worldwide have occurred in China (*4*, *5*). Shandong Province, one of 31 provinces of China, is an area of very severe HFRS epidemics with about one third of total cases in China, and its annual HFRS incidence rate is consistently among the top three in China (*5*). Since 1981, many hantavirus isolates have been characterized genetically and by restriction and sequence analyses of 300-nts regions of M segments (*3*, *6*). Only the sequences of M segments of about 7 Chinese SEOV isolates, which did not originate from Shandong Province and were isolated mostly before 1990, have been sequenced over 2000 nts. In order to obtain an up-to-date information on SEOVs from Shandong Province, we cloned, sequenced and phylogenetically analyzed 2353-nts regions of M segments of two SEOV isolates (ZB8 and GM04-38) from Shandong Province.

The isolates ZB8 and GM04-38 were identified as SEOV by an indirect immunofluorescence assay in the lung tissue of two brown rats captured in different areas of Shandong Province in 2004. To avoid a potential genomic alteration during growth in cell cultures (*7*), total RNA was extracted from lung tissue of the rats with Total RNA Isolation System (Promega) according to the manufacturer's instructions. Cloning and sequencing was done using an RT-PCR. The RT step was performed with random primers and AMV reverse transcriptase (TaKaRa) in standard manner. The cDNAs were PCR-amplified by LA Taq polymerase (TaKaRa) using SEO virus-specific primers 5'-TAGTAGTA GACTCCGCAA-3' (forward, nt 1–18) and 5'-TGGGCAATC TGGGGGGTTGCATG-3' (reverse, nt 2353–2331) in 30 cycles consisting of 94°C/1 min, 51°C/1 min, and 72°C/150 secs. The PCR products of 2353 bp were gel-purified and cloned into the pUCm-T vector (Sanger). Sequencing of the clones was performed by Sangon Biocompany. The sequences of the ZB8 and GM04-38 isolates were deposited at GenBank under Acc. Nos. DQ469396 and DQ469397, respectively. The nucleotide and deduced amino acid sequences of these isolates were aligned and compared with

*Corresponding author. E-mail: Zhiyu.wang@sdu.edu.cn; Zywang01@yahoo.com; fax: +86531-88382553.

Abbreviations: SEOV = Seoul virus; HTNV = Hantaan virus; HFRS = hemorrhagic fever with renal syndrome; HPS = hantavirus pulmonary syndrome

those of other SEOVs deposited at GenBank: L99 (AF288298), B-1 (X53861), KI-88-15 (D17594), Gou3 (AF145977), HB55 (AF035832), IR461 (AF458104), K24-v2 (AF288654), SR-11 (M34882), 80-39 (NC_005237), Z37 (NC_006436), R22 (S68035 with corrections) (*8*), ZT10 (DQ159911), and 76-118 (M14627). Phylogenetic analysis of 2353-nts regions of M segments of the isolates was performed by the neighbour-joining method using the Mega3 version 3.

Homologous comparison showed that ZB8 and GM04-38 had a 98.9% identity with each other and an 84.4–97.5% identity with other SEOV isolates at nucleotide level, and a 99.7% identity with each other and a 96.9–99.7% identity with other SEOV isolates at amino acid level. Except Gou3, the nucleotide and amino acid sequences of all compared SEOV isolates were highly conserved though they were isolated in various countries and in different years, indicating a genetic stability of these viruses. Among all nucleotide substitutions in the isolates, transitions represented 76.9% and transversions 23.1%. Whereas C→T transitions were most common (45.1%), C→G transversions were rare (1.7%). The L→F substitution at the position 6 was common for both isolates. Two amino acids substitutions at the positions 258 (A→T) and 543 (C→R) were unique for ZB8 and differed from other SEOVs.

Three potential N-glycosylation sites (N132, N345, and N397) (*9*) were conserved among all SEOV isolates, while N233 of IR461 glycoprotein Gn was substituted by I, implying the glycosylation at this site was not crucial to the folding and function of Gn. In phylogenetic analysis (the figure), the two Shandong isolates formed together with two isolates from Zhejiang Province P.R. China (Z37 and ZT10) in the subtype 3 cluster an independent lineage distinct from other SEOV isolates from other Chinese provinces or other countries (*10*, *11*).

Viruses of some species of hantaviruses, such as HTNV or Puumala, are characteristic of virus-host co-evolution and geographical clustering (*10*, *12*). Although Zhejiang and Shandong are geographically remote Chinese provinces, both are situated on the east coast of the country. The fact that the respective isolates clustered together was probably related to their common host, *Rattus norvegicus,* which can also migrate via trains, ships or long-distance buses. Some isolates dated to different years grouped into the same clusters, such as the abovementioned subtype 3 isolates, indicating a slow mutation rate of SEOV. Three frame shifts in the sequence of M segment of R22 isolate (*7*), caused by erroneous sequencing, were later corrected (*8*). In this study, we found that the corrected sequence of R22 M segment still had two frame shifts due to a missing G at the position 897 and a T inserted at the position 905. Considering the high identities of R22 with other SEOV isolates, e.g. a 99.2% identity with L99 at the nucleotide level, we assume that also the two frame shifts are sequencing errors and require a re-sequencing.

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