Virology 476 (2015) 37–42

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Isolation and characterization of a novel arenavirus harbored by Rodents and Shrews in Zhejiang province, China

Kun Li [a,b,1], Xian-Dan Lin [c,1], Wen Wang [a,b], Mang Shi [a,d], Wen-Ping Guo [a,b], Xiao-He Zhang [c], Jian-Guang Xing [d], Jin-Rong He [a,b], Ke Wang [e], Ming-Hui Li [a,b], Jian-Hai Cao [f], Mu-Liu Jiang [g], Edward C. Holmes [a,h], Yong-Zhen Zhang [a,b,*]

[a] State Key Laboratory for Infectious Disease Prevention and Control, Department of Zoonoses, National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Changping, Beijing, China
[b] Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, Hangzhou, China
[c] Wenzhou Center for Disease Control and Prevention, Wenzhou, Zhejiang Province, China
[d] Wencheng Center for Disease Control and Prevention, Wenzhou, Zhejiang Province, China
[e] Lucheng Center for Disease Control and Prevention, Wenzhou, Zhejiang Province, China
[f] Longwan Center for Disease Control and Prevention, Wenzhou, Zhejiang Province, China
[g] Ruian Center for Disease Control and Prevention, Ruian, Zhejiang Province, China
[h] Marie Bashir Institute for Infectious Diseases and Biosecurity, Charles Perkins Centre, School of Biological Sciences and Sydney Medical School, The University of Sydney, Sydney, Australia

ARTICLE INFO

Article history:
Received 10 June 2014
Returned to author for revisions
8 July 2014
Accepted 25 November 2014
Available online 12 December 2014

Keywords:
Arenavirus
Rodents
Shrews
Spill-over
Cross-species transmission
Emergence

ABSTRACT

To determine the biodiversity of arenaviruses in China, we captured and screened rodents and shrews in Wenzhou city, Zhejiang province, a locality where hemorrhagic fever diseases are endemic in humans. Accordingly, arenaviruses were detected in 42 of 351 rodents from eight species, and in 12 of 272 Asian house shrews (*Suncus murinus*), by RT-PCR targeting the L segment. From these, a single arenavirus was successfully isolated in cell culture. The virion particles exhibited a typical arenavirus morphology under transmission electron microscopy. Comparison of the S and L segment sequences revealed high levels of nucleotide (> 32.2% and > 39.6%) and amino acid (> 28.8% and > 43.8%) sequence differences from known arenaviruses, suggesting that it represents a novel arenavirus, which we designated Wenzhou virus (WENV). Phylogenetic analysis revealed that all WENV strains harbored by both rodents and Asian house shrews formed a distinct lineage most closely related to Old World arenaviruses.

Introduction

Arenaviruses (genus *Arenavirus*, family *Arenaviridae*) are enveloped, single-stranded RNA viruses of ambisense polarity (Neuman et al., 2005). The virus genome comprises two segments. The small (S) segment encodes the nucleocapsid protein (NP) and envelope glycoproteins (GP), while the large (L) segment encodes the viral RNA-dependent RNA polymerase (RdRP) and a zinc-binding protein (ZP). Additionally, in both segments, the genes are separated by a non-coding region which has the potential to form hairpin configurations and which plays a role in transcription termination (Gonzalez et al., 2007). Arenaviruses are important human pathogens, and associated with central nervous system disease and hemorrhagic fever (Charrel and de Lamballerie, 2010).

Rodents are the primary natural reservoir hosts of the known arenaviruses. The only known exceptions are a single virus isolated from fruit bats (*Artibeus spp.*) and three recently discovered viruses from snakes (Downs et al., 1963; Stenglein et al., 2012; Bodewes et al., 2013; Hetzel et al., 2013). Each rodent-borne arenavirus species appears to be primarily associated with one (or a few closely related) rodent species, compatible with the long-term co-divergence of these viruses with their hosts (Gonzalez et al., 2007). To date, 25 established arenaviruses have been identified worldwide, largely from the New World, as well as several newly discovered viruses whose taxonomic status has not yet been confirmed by the International Committee on Taxonomy of Viruses (Salvato et al., 2011; Coulibaly-N'Golo et al., 2011; Kronmann et al., 2013; Stenglein et al., 2012; Bodewes et al., 2013; Hetzel et al.,

* Corresponding author at: State Key Laboratory for Infectious Disease Prevention and Control, Department of Zoonoses, National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Changping Liuzi 5, 102206, Beijing, China.
Tel.: +86 10 58900782; fax: +86 10 58900700.
E-mail address: zhangyongzhen@icdc.cn (Y.-Z. Zhang).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.virol.2014.11.026
0042-6822/

2013). These viruses form two phylogenetic groups according to their geographic origins – the New World and the Old World arenaviruses – although Lymphocytic choriomeningitis virus (LCMV) is more globally distributed.

Several viral pathogens are known to cause hemorrhagic fevers in China (Gao et al., 2010; Zhang et al., 2010; Zhang et al., 2012; Chen et al., 2014). Indeed, China is one of the most important endemic areas for Hemorrhagic Fever with Renal Syndrome (HFRS), which is caused by hantaviruses and transmitted by rodents (Zhang et al., 2010). Although more than 103 species of *Muridae* rodents are present in China (Zhang et al., 1997), the only arenavirus that has been described in China to date is LCMV (Morita et al., 1996). The purpose of this study was to determine whether other arenaviruses are present in China and which mammalian hosts act as viral reservoirs.

Results

RT-PCR detection of arenaviruses in rodents and shrews

A total of 623 small mammals comprising rodents and insectivores were captured in Wenzhou city, Zhejiang province, southeast China (Fig. 1). Overall, we sampled 351 rodents from eight species and 272 insectivores of a single species (*Suncus murinus*, the Asian house shrew) as identified by analysis of the mt-*cyt b* gene (Table 1). A nested RT-PCR protocol targeting the RdRP gene within the viral L segment was performed to determine the presence of arenaviruses in stool samples. This revealed an extremely high positive rate for arenavirus RNA. Specifically, in rodents, the arenavirus infection rates in *Rattus norvegicus*, *R. flavipectus*, *R. losea* and *R. rattus* were 17.07%, 15.38%, 11.76% and 75.00%, respectively, with an overall positive rate of 11.97% (42 of 351 animals). In the case of insectivores, 12 of the 272 animals (4.41%) tested positive for arenavirus RNA. The infected animals were found in all four districts and counties of Wenzhou city (Table 1, Fig. 1). In addition to fecal materials, viral RNA was identified in liver, lung, heart, kidney and spleen tissue samples, suggesting that the infected rodents and shrews are experiencing chronic viremic infections.

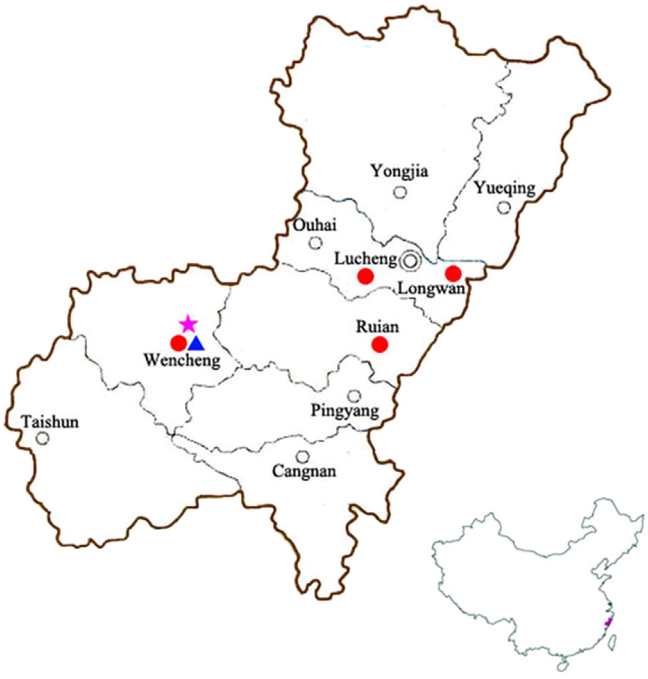

Fig. 1. A map of Wenzhou city, Zhejiang province, China, showing the location of trap sites from which small animals were captured. Red circles: locations of the sampled rodents and shrews. Blue triangle: location where Wenzhou virus was isolated from rodents. Purple star: location where the rodents were sampled to obtain the virus genome.

Isolation of an arenavirus from the liver tissue of the rat Rattus norvegicus

To better characterize the arenaviruses circulating in rodents and shrews, liver tissues from two *R. norvegicus* rats, one *R. flavipectus* rat and one *R. rattus* rat were homogenized and inoculated onto DH82 cell monolayers. After 10 days of viral cultivation, partial L segment sequences were recovered from the harvested culture supernatant inoculated with the liver homogenate of a *R. norvegicus* rat (strain Wencheng-Rn-366), indicating that the virus was successfully isolated. However, amplification from the remaining culture supernatant was unsuccessful. To observe the virus particles, the cells and the supernatant were both subjected to transmission electron microscopy (TEM), which revealed spherical to pleomorphic particles observed in both the cell and supernatant samples (Figs. 2 and S1). The diameter of these virons in each case was approximately 100 nm. In addition, the 'sandy' appearance of the virion particles could be easily observed, further supporting the successful isolation of this arenavirus.

To investigate virus propagation in cultured cells, the isolate was inoculated into fresh DH82 cells, and the extent of virus propagation was approximately estimated by quantitative real-time RT-PCR targeting the RdRP gene. Intracellular viral RNA began to increase steadily at one day post infection (p.i.) (Fig. 3), and levels of viral RNA increased exponentially between day three to five day p.i. However, no increase of viral RNA was observed after seven day p. i.

Genetic analysis of arenavirus sequences recovered from rodents and shrews

The complete viral S and L segments were first successfully recovered from an infected *R. norvegicus* rat (designated strain 'WENV/Wencheng-Rn-242') by high-throughput sequencing and confirmed by RT-PCR. As a typical arenavirus, this virus has two segments of length 7,146 (L) and 3,337 (S) nucleotides (nt), respectively. Highly conserved 3′ and 5′ non-coding regions were identified in both segments (Fig. S2). The S segment has two open reading frames (ORF) encoding a NP of 567 amino acids (aa) and a GP precursor of 493 aa, with a 62 nt non-coding region between the two ORFs. The L segment also has two ORFs encoding a RdRP of 2,228 aa and a ZP of 91 aa, with a 109 nt non-coding sequence between the two ORFs. In addition, complete (or near complete) genome sequences were also recovered from the RNA samples of 1 *R. rattus* rat (Wencheng-Rr-233), 1 *S. murinus* shrew (Wencheng-Sm-247), as well as from the isolate (Wencheng-Rn-366) by RT-PCR. Details of the viral segments and their encoded proteins are presented in (Table 2), while sequence characteristics of the non-coding regions, the 3'-5' exonuclease domain within the C-terminus of NP, and the late domain motifs within the C-terminus of ZP are shown in Fig. S2.

Genetic analysis of the four genome sequences recovered from rodents and shrews in this study indicated that they exhibited less than 7.6% nt difference in the complete S and L segments. This suggests that they are variants of the same virus species, albeit with a relatively high genetic diversity among them. Although the Wenzhou viruses were more closely related to Old World arenaviruses (see below), the differences between them were substantial; $>32.2\%$ and $>39.6\%$ at the nt level, and $>28.8\%$ and $>43.8\%$ at the aa level for the S and L segments, respectively (Table 3). This suggests that the Wenzhou viruses comprise a new