Characterization of replication and variations of genomic segments of a bat reovirus, BatMRV/B19-02 by RNA-seq in the infected Vero-E6 cells

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Abstract

Mammalian Orthoreoviruses have been isolated from a wide variety of mammalian species that can cause enteric, respiratory and encephalitis infections. We report a novel MRV type 1 detected in *Miniopterus schreibersii* may have resulted from reassortment events. Using next-generation sequencing RNA-seq, we found that the ratios of relative abundances in RNA levels of the ten reovirus segments are constant during the late stages of infection in infected cells. We also discovered that the related RNA abundance level of each gene differed. Remarkably, the relative abundances of total RNA of M2 (coding μ1 protein) and S4 (coding σ3 protein) genes were higher than others throughout infection time. In addition, massive junctions were defined. These are evidence to support the hypothesis of generating defective genome segments and cross-family recombination mechanisms. These findings may support further the study of gene function, viral replication and virus evolution in the host.

1. Introduction

Mammalian Orthoreoviruses (reoviruses – respiratory and enteric orphan viruses - MRVs) are associated with gastrointestinal enteritis, respiratory illness and encephalitis and have been isolated from a wide variety of mammalian species including bats, minks, pigs and humans [12, 19, 35]. Reoviruses were not associated with any known disease when they were first discovered in the 1950s [33]. Reovirus is also one of many oncolytic viruses as an ideal candidate for development for cancer therapeutics [25]. In recent years, a number of zoonotic diseases have been identified. Bats have been recognized to have several viruses that are closely related with zoonotic viruses that can infect humans and other mammals and cause numerous emerging infectious diseases such as Filovirus disease (Ebola virus disease and Marburg virus disease) and highly pathogenic emerging Coronaviruses relevant to humans (MERS and SARS) [1]. Novel bat MRVs have recently been identified in Europe and China [9], and have high similarity to the MRVs that were detected from a child hospitalized with acute gastroenteritis and a diarrheic pig [23, 30].

Reoviruses are members of the Reoviridae family, non-enveloped, icosahedral viruses that contain a segmented genome consisting of ten double-stranded RNAs. The reovirus genome comprises 23.5 kb in ten segments containing three large (L1 – L3), three medium (M1 – M3), and four small (S1 – S4) genomic segments. These ten segments encode for eight structural proteins (λ1–3, μ1–3, and σ1–3 protein) and four nonstructural proteins (μNS, μNSC, σ1s, and σNS). Two major outer capsids (μ1 and σ3 protein) surround the core and are composed of 600 heterodimers each proteins [33]. The S1 gene segment encodes the σ1 protein that is located on the outer capsid of the virion and is responsible for viral attachment to cellular receptor, and defines the MRV serotype [7]. To date, four reovirus serotypes have been determined, serotype 1 (MRV1), serotype 2 (MRV2), serotype 3 (MRV3), and novel serotype 4 (MRV4) with only one strain (Ndelle virus) [5]. In the most recent study, reovirus strains were isolated from humans, mouse and swine in South Korea, belonged to serotype 2 and 3 [13, 16]. However, the MRVs serotype 1 has not been identified in bat, human or other mammals in Korea.

Recombination is a pervasive process generating diversity in most viruses involving the exchange of genetic information [14]. Reassortment is a form of genetic recombination that is exclusive to segmented RNA viruses in which co-infection of a host cell with different viral strains may result in segments shuffling to generate progeny viruses with novel genome combinations [22]. Recently, reassortment MRV strains have been reported from different animal species including bat, human, pig, mink and deer, which could generate new pathogenic virus strains associated with human and pig diseases [9, 23, 24, 30, 35].

Human reovirus have been studied extensively as a potential oncolytic virus. However, the model and the mechanism involved in ten reovirus mRNA synthesis and/or transcription needed using modern approaches for precise RNA quantitation still remain. With next-generation sequencing, RNA-seq of virus-infected cells can be used to study, in great detail, the patterns of virus transcription and replication and microbe-host interaction.

In this study, we report the first isolation of a novel MRV strain from bat in South Korea. The genome sequence of BatMRV/B19-02 was determined and analyzed using phylogenetic analysis. We investigated the abundance level of viral RNA which reflected the transcription and replication of reovirus in infected cells using the next-generation sequencing RNA-seq. Furthermore, the variations/SNP was defined within all the gene segments to support the elucidative of recombination mechanisms on viruses.

2. Materials And Methods

Viral isolation

BatMRV/B19-02 was isolated from bat feces (Miniopterus schreibersii) in Jeju Island, South Korea. 10% (w/v) fecal samples in transport medium (Universal Transport Medium, Noble Biosciences™, Korea) were centrifuged at 3000 × g for 15 min at 4°C. The clarified supernatant was diluted 10-fold with fresh DMEM and filtered with a 0.22 μm pore-size filter before inoculated in confluent monolayers of Vero-E6 and MARC-145 cells. Cells were incubated at 37°C with 5% CO2 and observed daily for 10 days. The infected cells and supernatant were broadly tested for virus families including Coronaviridae, Paramyxoviridae, Flaviviridae, Filoviridae, and Influenza A virus as previously described [4, 10, 28, 32, 34]. When a cytopathic effect (CPE) was observed, the supernatant was harvested by three freeze-thaw cycles, centrifuged at 6000 × g for 5 min at 4°C, and stored at -80°C.

Plaque assay

Vero E6 cells prepared in 6-well plates were inoculated with 1ml of 10-fold serially diluted virus samples and incubated at 37°C for 1h. Cells were then cultured with 2.5 ml per well of DMEM containing 2% (v/v) FBS and 0.8% (w/v) agar for 5 ~ 7 days. Cells were fixed with 2ml of 10% formaldehyde per well for 1h. After removing the culture medium, cells were stained with crystal violet. Titration was done in duplicate and infectivity was displayed by plaque-forming units (PFU).

Virus growth and titration

The virus was inoculated in a monolayer of Vero-E6 cells at an MOI of 10 and maintained in DMEM with 2% FBS for 7 days. At different time points, the virus was collected from infected cells by three freeze-thaw cycles, was centrifuged at 3000 × g for 10 min, and stored at -80°C. The infectious units were quantified from the prepared samples using the tissue culture infectious dose 50 (TCID50) method. Briefly, the prepared samples were 10-fold serially diluted and inoculated to monolayers of Vero-E6 cells in 8 wells. The highest dilution showing 50% of viral infection was obtained by observing CPEs with a light microscope. The TCID50 was calculated using the Spearman–Kärber method.

Sequencing and phylogenetic analysis

A total of 22 primer pairs were designed to amplify the complete genome of BatMRV/B19-02. All information regarding the primers is available upon request. Two-step RT-PCR for the amplification of each viral genome segment was carried out following by synthesized cDNA with the M-MLV reverse transcriptase kit (Promega, USA) and PCR performance with 2x AccuPower→ PCR Master Mix (Bioneer, USA). The PCR products were revealed by electrophoresis on 1.5% agarose gels and were gel purified using QIAquick Gel Extraction kit (Qiagen, Venlo, Netherlands) and then were submitted to the Cosmogenetech company in Seoul, Korea for sequencing.

Nucleotide sequences were assembled using ClustalW software implemented in BioEdit (version 7.1.9). The complete nucleotide sequence of B19-02 has been submitted to GenBank under accession number MW582622 – MW582631. Searching using the BLAST algorithm were performed on the NCBI server (https://blast.ncbi.nlm.nih.gov). The sequence alignments of the 10 gene segments were compared with those of closest reference strains and other reoviruses downloaded from GenBank using the program ClustalW. The phylogenetic analyses using the Maximum Likelihood method based on the Kimura 2-parameter model were conducted in MEGA 7. The support for the tree nodes was calculated with 1000 bootstrap replicates.

RNA isolation for RNA-seq

Vero-E6 cells were infected with strain BatMRV/B19-02 at an MOI of 10 (PFU/cell). Infected-cells were harvested at different times point and used to isolate total RNA using RNeasy Mini Kit (Qiagen, Cat # 74104). The total RNA was submitted to the Macrogen Co., Ltd. (Seoul, South Korea) for cDNA library construction using a TruSeq Stranded total RNA LT Sample Prep Kit (Gold) following the manufacturer's protocol. Sequencing was performed using a NovaSeq 6000 platform.

Bioinformatic analysis

The sequencing data were demultiplexed, and the reads were trimmed to remove adapter sequences and filtered to remove low-quality reads using Geneious Prime ver.2020.1 (https://www.geneious.com/) with the cutoff threshold for average base quality score set at 30 over. The paired reads created from the R1 and R2 files were mapped independently to the plus-stand of the reference sequence, which is genome sequence of BatMRV/B19-02 were obtained from the Sanger sequencing method (Sequencing and

phylogenetic analysis part). Relative RNA abundances were calculated from fragments per kilobase per million mapped reads (FPKM) and transcripts per million (TPM) on each gene. FRKM and TPM values normalize were visualized using a small multiples chart made with ggplot2 in R (ver. 1.3.959). The variations/SNP were found by mapping trimmed reads to the ten nucleotide sequences of the genome virus with the minimum variant frequency 0.1 and 0.25 and quality 30 (99.9% correct). The junction analysis was used with default settings. For this operation, two passes were made during mapping. On the first pass each read mapped generated candidate junctions based on where fragments of the read aligned to different regions of the reference sequence. The second pass involved mapping reads using the discovered junctions. By default, at least two reads must support the discovery of a junction in order for it to be used during the next pass.

3. Results

Isolation of a mammalian orthreovirus

During surveillance of bat viruses in South Korea, we isolated the strain BatMRV/B19-02 from a fecal sample, B19-02 (from Miniopterus schreibersii), also positive with alpha-coronavirus and paramyxovirus, collected from Jeju Island in January 2019. Bat species were identified as previously described [21]. The isolate caused clear cytopathic effects (CPE) at 3-4 days post-inoculation at the first passage in both MARC-145 and Vero-E6 (Figure 1). Virus identification was first confirmed by real-time RT-PCR specific for the MRV S1 gene [12]. Both the supernatant and the infected cells were negative by RT-PCR specific for Coronaviridae, Paramyxoviridae, Flaviviridae, Filoviridae, and Influenza A.

Genetic Characterization of BatMRV/B19-02

The whole genome of BatMRV/B19-02 was amplified and sequenced from the cell culture supernatants passage 2. The nucleotide sequences of all 10 genome segments were deposited in GenBank under Accession Number MW582622 – MW582631.

Phylogenetic tree reconstructions by Bayesian inference and maximum likelihood analyses were based on the nucleotide sequence of the complete 10 segments (Figure 2). The phylogenetic reconstruction of the S1 segment on the complete ORF (nt) encoding for the s1 protein revealed that BatMRV/B19–02 was a novel MRV serotype 1. When compared to the S1 sequences available in GenBank, the BatMRV/B19-02 shared the highest identity with isolate MRV 1 TS (MG451067.1) from rodent (Tupaia sp. – Tree shew) and strain JS2017 (MN788310.1) from swine (86.6 and 86.4% respectively). The S1 segments of the BatMRV/B19-02 had lower nucleotide identity (lower 82.2%) to that of MRV strains isolated from the bats. Phylogenetic analysis of the L1, L2, L3, M1, M2, M3, S2, S3 and S4 segments were very highly similar whole-genome strains available in GenBank as shown in Table 1. L1 and M3 segments showed the highest nucleotide identity (95.8 and 96.0%, respectively) and amino acid identity (98.6 and 98.0%, respectively) to an MRV2Tou05 strain detected in children with acute necrotizing encephalopathy in France [24]. Similarly, L2, M1 and S2 segments, closely related to that of Neth/85 and Lang strains, were isolated from humans with 96.3 – 98.4% nucleotide identities. However, L3, M2, S3 and S4 segments

shared a relatively high identity (92.2 – 98.9%) with that of the strains detected from animals (including rodent, swine and lion).

Viral RNA expression analysis from the infected cell line

The Vero-E6 cells were infected with BatMRV/B19-02 at a multiplicity of infection (MOI) of 10 PFU per cell. Total RNA was extracted from infected cells harvested at the early stage (2h and 6h post-infection) and the late stage (18h, 24h and 48h postinfection). The cDNA library was constructed based on the mammalian orthoreovirus genome sequences prior to high throughput sequencing on the NovaSeq 6000 platform. The result of virus transcriptome sequencing is shown in table S1. A total of 290.57 million clean reads were generated with an average of 63.33 million reads per infectious time group with Q20 and Q30 greater than 98.74% and 95.66%, respectively. However, only 1.08 million reads were mapped to the BatMRV/B19-02 which was assembled to a linear by ten segments. Unfortunately, 128 and 46 reads were assembled from 2h and 6h groups. Hence, the RNA-seq data of 18h, 24h and 48h groups were used with 94,544, 101,315 and 886,241 mapped reads, respectively, to compare expression level (reads) of the viral RNA made during infection.

The relative abundances of viral RNAs of individual segment RNA using values for fragments per kilobase million mapped (FPKM), which normalize for sequencing depth and gene length in order to allow the total RNA from individual genes to be compared. Transcripts per kilobase million (TPM) allow comparing the proportion of reads that mapped to a gene in each sample. In this study, we sequenced total of viral RNAs, including mRNA and genomic dsRNA. Although the term “expression level” was usually specific for mRNA, the relative abundances of viral RNA levels were analyzed based on the expression level FPKM and TPM.

During the late stage (18h to 48h), the ratio of the expression level FPKM and TPM of ten gene segments was invariable. The FPKM value of M2 and S4 genes, which are coding for the major proteins of the virion, were higher than the value of 8 other genes, greater than 93000 and 100000 transcripts, respectively (Figure 3). The coverage depth of those genes was also greater than that of other genes (Figure S2). In contrast, the lowest expression level was observed in the M1 gene (under 20,000 transcripts at three time points). The TPM value of each genomic segment showed that there was no difference at expression level through the time points when sufficient numbers of new virions are produced and emerge from the host cells from 18 to 48 hpi (The viral growth kinetics, Figure S1).

The variations/SNPs analysis during the viral infection in the cell line

To detect reovirus variations/SNP, we analyzed the total reads of the 48h group and mapped and called the variations/SNP to individual 10 nucleotide sequences of genome virus. Only transition SNP (T -> C) was found at 817 position in the S4 gene with variant frequency 28.2. Rearrangement junction is a deletion longer than 1,000 bp or structural variant, and deletion junction type is the deletions from 3 bp to 1,000 bp (by the Geneious manual). By definition, the rearrangement junction with the junction source and destination sites located within a segment is an inner segment junction, whereas an outer segment

junction is a junction with junction source and destination sites in two different viral genomic segments. Remarkably, we detected massive junctions including a deletion junction and rearrangement junction within individual viral gene segments (Table 2). The junctions were more frequency in M2, M3, S3 and S4 gene segments (4.9 – 6.4%) and at low frequencies in L1 and M1 (1.3 – 1.7 %). Based on mapped read, the junctions in the M3 and S3 gene were more frequent with 0.15 % and 0.20 %, respectively. The junctions per mapped read were around 0.05% to 0.10% in the other segments. The rearrangement junction had a high-density across all the gene segments (Figure 4). The rearrangement junction with the destination within the segment shown higher counts compared to the outer segment destination junction (Table 2). Deletion junctions also were defined in all segments, detected within coding domain sequences (Figure 4). The number of deletions were higher in L3 and M2 gene segments with 33 and 36 deletions, respectively, whereas only 6 deletions were found in the M1 gene segment. Small deletions were less than 200 bp with the shortest size of 3 bp, were abundantly prevalent in all segments. Long deletions, greater than 500 bp in length, were detected in the L1, L3, M2, S1 and S4 gene segments (Figure 5). The largest deletion junction was of 946 bp in the M2 gene segment (677 – 1624 nucleotide position, MW582626).

4. Discussion And Conclusion

Over the last two decades, since the outbreak of Severe Acute Respiratory Syndrome coronaviruses (SARS-CoV) in 2003, several surveillance studies of viruses prevalent in wildlife and especially in bats have been conducted, revealing a number of novel viruses associated with newly emerging diseases. A few of these viruses originating from bats are thought to be associated with severe human diseases, including Ebola virus, Middle East respiratory syndrome coronavirus (MERS-CoV), Nipah and Hendra viruses, and the current pandemic viruses - Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) [3, 6, 36, 37]. Reoviruses were not known to be associated with severe human diseases and were studied as oncolytic viruses. MRVs not only efficiently replicate in many mammalian species but also can change drastically via gene reassortment events. Almost all novel reovirus isolates are hypothesized to have resulted from a reassortment event [9, 19, 20, 23, 24, 30, 35]. An isolate can contain genome segments from different serotypes and/or different hosts. It could increase the zoonotic potential of these viruses in the future.

Based on sequence comparison and phylogenetic analysis, we hypothesize that the novel type 1 BatMRV/B19-02 isolate may have resulted from a reassortment of the bat, human, rodent and/or swine MRV strains. Five of ten segments (L1, L2, M1, M3 and S2) were highly similar to those segments of MRVs isolated from human, including two classical MRV type 1 strain and a novel MRV type 2 strain caused acute necrotizing encephalopathy. Only segment S4 was closer to that of the bat MRV WIV5 isolate from Hipposideros sp. in China [35]. However, due to limited number of references, we could not exclude the possibility of genetic drift from the MRV that had not found before in bats or other species.

In the previous reports, all three MRV serotypes are circulating in bat species in Europe and China. However, MRV serotypes 2 and 3 are more prevalent in bat populations [12, 17, 20, 35]. In South Korea, MRVs have been detected in swine, human and mouse belonging to serotype 2 and 3 [13, 16]. This study

is the first description of the bat MRV serotype 1 strain found in South Korea, and almost all of the genome segments were more related to those of human MRVs than those of bat MRVs identified. Since Melaka virus was identified in 2006, several novel reovirus strains have been discovered which cause severe respiratory and enteric and encephalitis diseases in both animals and humans [27]. Thus, the pathogenesis of the novel reovirus infections is needed to be further studied.

It is important to distinguish between genomic RNA and synthesized viral mRNA in infected cells. Unfortunately, reovirus mRNAs lack poly(A) tails [31]. However, the viral genome comprises ten dsRNA segments, with a single copy of each viral gene segment incorporated per virion, encoding for eight structural proteins and four nonstructural proteins [33]. In addition, the minus strands were synthesized using mRNA as templates, resulting in formation of nascent genomic dsRNA, concomitantly [15]. The relative proportions of the 10 nascent genomic dsRNA segments are equimolar [2]. Thus, the relative abundances of total RNA could reflect the expression level.

The level of viral mRNA synthesis could be divided into two stages: the early viral mRNA is synthesized before the newly viral genome is replicated, and the late mRNA. The previous data indicated that the S4 mRNA, which encodes the σ3 protein, is the most efficiently translated in both in vitro translation reactions and reovirus-infected cells [18]. In this study, both the M2 gene and S4 gene, encoding for the two most abundant proteins in the reovirus virion (μ1 and σ3 protein, respectively), were related to the highest expression levels of mRNA during the late stage. In contrast, the genes encoding for enzymes related to the lower gene expression levels. The dsRNA activates innate immune response in host cells, which may be one disadvantage for MRVs replicating in the infected cells. As the M2 and S4 were related to apoptosis and inhibition of host RNA and protein synthesis [33], the high expression-associated level of M2 and S4 RNA might be associated with evading host cell innate immune responses. However, there should be additional studies to demonstrate this hypothesis and on whether those differences of RNA levels were due to either induced RNA synthesis or delayed RNA decay. In addition, further studies based on dsRNA-seq and/or strand-specific sequencing for preparing libraries could be useful for not only the role of junctions in RNA virus recombination and rearrangements but also the level gene expression in the virus life cycle.

Both recombination and reassortment are an evolutionary mechanism of segmented RNA viruses. The previous study demonstrated that recombination junctions affected non-canonical RNA synthesis. They used RNA-seq to analyze viral RNA packaged in reovirus particles, which reovirus strains rsT1L and rsT3D were engineered using plasmid-based reverse genetics [29]. We also utilized RNA-seq to analyze viral RNA genomic reovirus type 1 isolated from bats using the Geneious software platform to analyze and visualize the RNA-seq data, an easy-to-use and flexible desktop software application framework [11]. We identified massive junctions within individual viral genome segments in the infected cell. The percentage of deletion and rearrangement junctions based on size and mapped read was high in both M3 and S3 genes. In contrast, the junctions per size were the highest, while the junctions per mapped read were low in the S4 segment. The rates of junctions per size and mapped read were low (2.4% and 0.06%, respectively) in the S1 gene that is the most variant. The relative abundance of deletions and

rearrangement varies seems not only increase along with the read coverage. Most studies on RNA viruses suggested that a large diverse population of defective viral genomes are generated by high MOI. In the case of Zika virus, the large deletions were more abundant under high MOI, while the small deletions were common in both low and high MOI passaging conditions [26]. At a high MOI condition, our results also suggested that the relative abundance of the deletion or rearrangement junctions varies lead to the distribution of defective viral genomes (DVGs) diversity during infections. The defective gene segments featuring internal deletions undergoes sequence-directed recombination at distinct site [29]. The deletions could lead to the mechanism of recombination. The role of deletion and rearrangement junctions in the recombination pathways may be explained based on the studies on the interaction between viral RNA and host-cell proteins or viral structure/non-structure proteins in the replication cycles of viruses. Moreover, deletions in different lengths could be donors for the recombination mechanisms within species or even cross family. A novel gene likely originated from the segment S1 gene of a bat orthoreovirus in a novel coronavirus isolated from Rousettus leschennaulti bat in China, which suggested the heterologous inter-family recombination between a single positive-strand RNA virus and a double-stranded segmented RNA virus [8]. In the long-term, surveillance of prevalent viruses from bats, virus co-infections can be observed in a few cases. For instance, coronavirus frequently co-infect with paramyxovirus or reovirus (Data not shown). Thus, the cross-family recombination events could have probably occurred during co-infection in the reservoir host – bat.

In conclusion, the BatMRV/B19-02 strain, which belonged to the MRV type 1 isolated from Miniopterus schreibersii, may have resulted from a reassortment of the bat, human, rodent and swine MRV strains. The ratios of relative abundances in RNA levels of the ten reovirus segments is constant during the late stage, with higher-levels of abundance of M2 and S4 gene segments. Moreover, the massive junctions, including deletion and rearrangement junctions, were identified within all viral genome segments in the infected cell that supported the explanation of the recombination and reassortment mechanism of segmented RNA viruses. Considering that mammalian orthoreovirus isolated from bat was highly similar to human MRV strains, it could increase the zoonotic potential. The pathogenesis of the novel reovirus infections is needed for further study.

Declarations

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author contributions

All authors contributed to the study conception and design. Hye Kwon Kim, Sun-Woo Yoon and Dae Gwin Jeong conceived and supervised the study. Ji Yeong Noh and Seong Sik Jang investigated and analysised the data. Van Thi Lo performed data, designed and wrote manuscript. Daesub Song and Woonsung Na performed data interpretation and assisted in writing. All authors read and approved the final manuscript.

Data Availability

The genome sequences generated in this study have been deposited into Genbank under accession numbers MW582622 – MW582631. The RNA-seq data have been deposited into Sequence Read Archive (SRA) under accession number PRJNA718116. ***Supplementary Materials:*** Figure S1. Viral growth kinetics, Figure S2. Visualization of RNA-seq coverage across the MRV/B19-02 genome (10 segments). Table S1. RNA-seq trimmed data.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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Tables

Table 1. Highest nucleotide and amino acid identities for each gene segment of the strain MRV/B19-02.

Genes	Sequences/Isolate (Highest identity)	Serotype	Host species	Similar identities (%)	
				nt	aa
L1	MRV 2 Tou05 (GU196306.1)	MRV 2	Human	95.8	98.6
L2	MRV 1 Neth/85/Human (AF378004.1)	MRV 1	Human	96.3	98.9
L3	MRV 2 TRALAU2004 (JX204740.1)	MRV 2	Rodent	92.2	98.5
M1	MRV 1 Lang (AF461682.1)	MRV 1	Human	95.4	97.9
M2	MRV 1 TS (MG451065.1)	MRV 1	Rodent	94.7	99.4
M3	MRV 2 Tou05 (GU196314.1)	MRV 2	Human	96.0	98.0
S1	MRV 1 TS (MG451067.1)	MRV 1	Rodent	86.6	91.0
S2	MRV 1 Lang (L19774.1)	MRV 1	Human	98.4	99.2
S3	MRV 3 strain SC-A (DQ411553.1)	MRV 3	Swine	94.6	98.0
S4	MRV 2 Panthera leo (LC121912.1)	MRV 2	Panthera leo	98.9	99.1

Table 2. Number of junctions.

Gene	Junction type			Total junctions	Segment size (bp) (junction per size)	Mapped reads (junctions per reads)
	Deletion	Rearrangement (Junction destination)				
		Inner segment	Outer segment			
L1	18	22	9	49	2852 (1.7%)	77,444 (0.06%)
L2	9	54	14	77	3902 (2.0%)	95,891 (0.08%)
L3	33	82	17	132	3892 (3.4%)	134,947 (0.10%)
M1	6	16	8	30	2293 (1.3%)	38,181 (0.08%)
M2	36	52	20	108	2198 (4.9%)	177,790 (0.06%)
M3	20	66	25	111	2229 (5.0%)	72,612 (0.15%)
S1	16	14	5	35	1460 (2.4%)	61,493 (0.06%)
S2	13	10	7	33	1323 (2.5%)	70,949 (0.05%)
S3	10	44	13	67	1184 (5.7%)	33,927 (0.20%)
S4	16	46	14	76	1180 (6.4%)	126,118 (0.06%)

Supplementary Table

Table S1. RNA-seq trimmed data.

Index	Sample ID	Total read bases	Total reads	Total viral reads	GC(%)	Q20(%)	Q30(%)
1	2h	5,848,163,915	58,270,688	128	53.25	98.81	95.91
2	6h	5,394,290,996	53,750,568	46	50.90	98.81	95.89
3	18h	6,828,770,304	68,033,136	94544	48.45	98.74	95.66
4	24h	6,917,493,296	68,917,758	101215	53.57	98.76	95.76
5	48h	6,796,736,287	67,662,374	886241	49.84	98.84	95.95

Figures

Figure 1

Cytopathic effects of the BatMRV/B19-02 in infected cells. (a, c), mock Vero E6 and MARC cell. (b,d), Infected Vero E6 and MARC cell 4 dpi at the first passage. Bar is indicating 50 μm.

Figure 2

Phylogenetic trees of 10 segments of isolate BatMRV/B19-02 (•) and most related whole-genome strains from GenBank (14 reference isolates). Maximuum Likelihood was used for the construction of phylogenetic tree with boostrap values of 1000 replicates using MEGA 7. Scale bar shows the evolutionary distance of nt substitutions per position. GenBank accession numbers are shown for each isolate.

Figure 3

FPKM (a) and TMP (b) values for 10 gene segments of BatMRV/B19-02 in infected cells at different time points.

Figure 4

Maps of the total of rearrangement and deletion junctions in 10 gene segments. Black lines represent for each segment in length (bp). Each green symbol represents a junction. The “” symbol represent for a junction and green lines represent for the deletion size. The symbol “¦” and “¦” represents for the rearrangement junction with plus and minus junction destination, respectively. The plus junction destination is from the source junction to the destination junction in the 5'-to-3' direction, whereas the minus junction destination is in the 3’-to-5’ direction. Annotations are colored from blue to green based on increasing values of reads supporting discovery.

Figure 5

The deletion size (nt) of all junctions in the 10 gene segments. Each dot represents a unique junction.

Supplementary Files

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- FigureS1.tif

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