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# Identification of the amino acid residue important for fusion of severe fever with thrombocytopenia syndrome virus glycoprotein

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## ABSTRACT

Severe fever with thrombocytopenia syndrome (SFTS) is an infectious disease with a high fatality rate, caused by SFTS virus (SFTSV). Because little is known about the nature of SFTSV, basic studies are required for the developments of vaccines and effective therapies. In the present study, we identified the amino acid residue important for membrane fusion induced by the SFTSV glycoprotein (GP). Syncytium formations were observed in cells expressing the GPs of SFTSV Japanese strain (YG-1 and SPL030). In contrast, no or only weak syncytium formations were induced in cells expressing GP of SFTSV Chinese strain (HB29). The replacement of arginine at amino acid residue 962 with serine in HB29 GP (R962S) induced membrane fusion, while the replacement of serine at residue 962 with arginine in YG1 GP (S962R) did not. These data indicate that serine at residue 962 in the SFTSV-GP is critical for inducing membrane fusion and viral infection.

## 1. Introduction

Severe fever with thrombocytopenia syndrome (SFTS) virus, SFTSV, is an emerging tick-borne pathogen that is prevalent in the environment in China (Xu et al., 2011; Yu et al., 2011), Japan (Takahashi et al., 2014) and South Korea (Kim et al., 2013). Major clinical symptoms of SFTSV infection include fever, gastrointestinal symptoms, hemorrhage, and deterioration in consciousness. Total blood cell tests reveal the presence of thrombocytopenia and leukopenia (Liu et al., 2014). The antiviral drug, favipiravir, was reported to have efficacy in treating animals infected with SFTSV (Tani et al., 2016a, 2018). A clinical study to evaluate the efficacy of favipiravir in the treatment of human patients with SFTS has been initiated in Japan. SFTSV has three genomic segments of negative-sense or ambisense RNA, designated L, M, and S. The L-RNA segment encodes the viral RNA-dependent RNA polymerase, the M-RNA segment encodes the glycoprotein precursor (GPC), and the S-RNA segment encodes the nucleoprotein NP and the nonstructural protein NS (Elliott and Brennan, 2014). The GPC is cleaved into Gn and Gc, which form a spike complex known as the envelope glycoprotein, GP. GP are incorporated into viral particles and are involved in binding to cellular receptors and fusion with the cell membrane during endocytosis. Neither Gn nor Gc alone is sufficient for host-cell entry; Gn facilitates Golgi transport and virion incorporation of Gc (Plegge et al., 2016). These glycoproteins are also the targets for neutralization antibodies. Recently, we and other groups demonstrated that a pseudotyped virus possessing SFTSV-GP, based on vesicular stomatitis virus (SFTSVpv), exhibited GP- and pH-dependent entry of target cells (Hofmann et al., 2013; Plegge et al., 2016; Tani et al., 2016b). Furthermore, C-type lectins, such as DC-SIGN, DC-SIGNR, and LSECtin, were demonstrated to be crucial for the entry of SFTSVpv and SFTSV and the growth of SFTSV in susceptible cells (Hofmann et al., 2013; Tani et al., 2016b). Syncytium formation in SFTSV-infected cells was observed under low pH conditions by microscopic examination. It has also been reported that syncytium formation was observed with a highly-passaged Japanese strain of SFTSV, SPL030, in mild low-pH conditions, compared with that of the original strain (Taniguchi et al., 2017). Another group demonstrated that a sub-clone established from the Japanese isolate, YG1, exhibited strong cell-fusion activity under acidic conditions (Nishio et al., 2017). This sub-clone has several mutations compared with the sequence of the original YG1 strain. Strong

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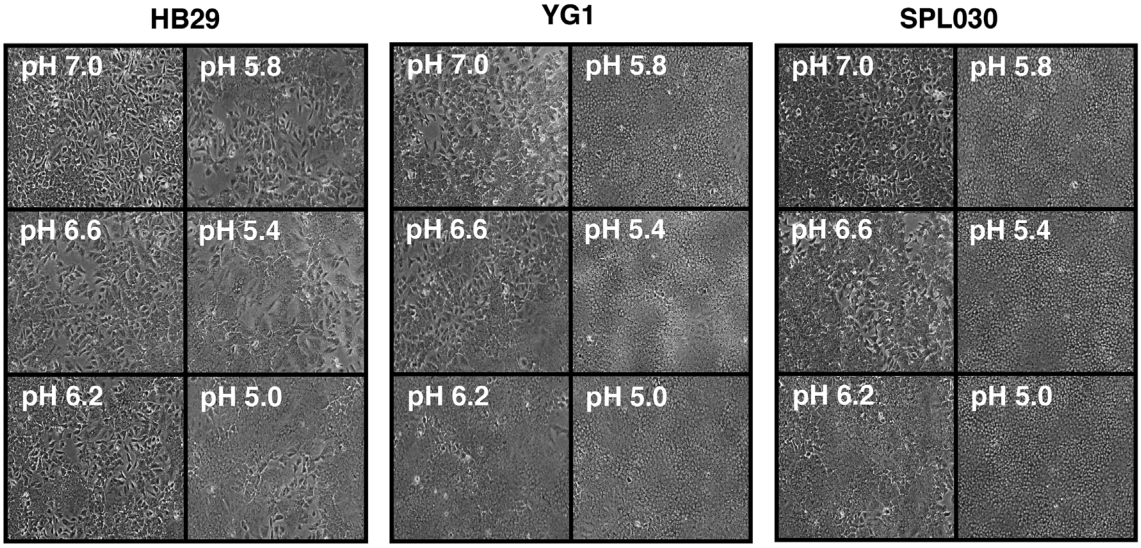

**Fig. 1. Syncytium formation in Huh7 cells infected with SFTSV.** To examine cell-fusion activity among SFTSV strains, SFTSV (HB29, YG1, or SPL030 strain) were inoculated into Huh7 cells. After incubation for 48 h, the cells were treated with the indicated pH citrate-phosphate buffer for 2 min, which was then replaced with culture medium. The syncytium formations of monolayer cells were observed under a phase-contrast microscope 4 h following the treatment.

cell-fusion activity in this sub-clone was associated with an amino acid substitution at position 624 in the Gc (Tsuda et al., 2017). Furthermore, amino acids, such as tryptophan, serine, glycine, or aspartic acid, at position 624 were shown to be important for inducing low-pH-dependent cell-fusion. In an analysis of a crystal structure model of SFTSV-Gc, the amino acid at residue 624, together with the basic and acidic amino acids located around residue 624, were suggested to be stabilized cell fusion in post-fusion step (Tsuda et al., 2017).

In this study, we determined that another amino acid residue, which is also important for membrane fusion, is induced by SFTSV-GP. The amino acid was identified by comparing visible syncytium formation and cell-fusion assays under low-pH conditions among various strains. Although cell fusion was not clearly observed in the HB29 strain, it was clearly observed in other SFTSV strains. We found that arginine at residue 962 in the GP was crucial for virus-induced cell-fusion.

## 2. Materials and Methods

### 2.1. Plasmids, cells, and viruses

Glycoproteins (pKS-HB29-GP, pKS-YG1-GP, and pKS-SPL030-GP) were prepared as previously described (Taniguchi et al., 2017). The mutant GP-cloned plasmids were constructed using a QuikChange II Site-Directed Mutagenesis Kit (Agilent Technologies, Santa Clara, CA), using pKS-HB29-GP or pKS-YG1-GP as a template. The resulting plasmids were designated pKS-HB29-GP-E114G, pKS-HB29-GP-H394Q, pKS-HB29-GP-G525R, pKS-HB29-GP-R962S, pKS-HB29-GP-R962N, pKS-YG1-GP-Q394H, and pKS-YG1-GP-S962R. A schematic representation of the mutant-GP plasmids was described in Fig. 4A. Each mutant GP sequence of the plasmids was confirmed to be identical to the nucleotide sequence, based on the GenBank accession numbers KP202164 (HB29 strain) and AB817987 (YG1 strain), using an ABI PRISM® 3500-Avant Genetic Analyzer (Applied Biosystems).

Huh7 and 293T cells were obtained from the American Type Culture Collection (Summit Pharmaceuticals International, Japan). These cells were grown in Dulbecco's Modified Eagle's Medium (DMEM; Sigma-Aldrich, St. Louis, MO), containing 10% heat-inactivated fetal bovine serum (FBS).

SFTSV (Chinese strain HB29, and Japanese strains YG1 and SPL030) was amplified, and infectious titers were determined using a focus-forming assay, as previously described (Tani et al., 2016b).

### 2.2. Generation of pseudotyped VSVs

Pseudotyped VSVs bearing each GP of SFTSV were generated as previously described (Tani et al., 2016b). Briefly, 293T cells were grown to 70% confluence on collagen-coated tissue-culture plates and then transfected with each of the expression plasmids. Following incubation for 48 h at 37 °C, the transfected cells were infected with G-complemented VSVΔG/Luc (*G-VSVΔG/Luc) (Tani et al., 2010), at a multiplicity of infection (MOI) of 0.5 per cell. The virus was adsorbed and then washed four times with 10% FBS-DMEM. After incubation for 24 h, culture supernatants containing pseudotyped VSVs were centrifuged to remove cell debris and stored at −80 °C until use. Resultant pseudotyped VSVs were referred to as HB29pv, YG1pv, HB29(E114G)pv, HB29(H394Q)pv, HB29(G525R)pv, HB29(R962S)pv, YG1(Q394H)pv, and YG1(S962R)pv derived from the respective plasmid, respectively.

The infectivity of each pseudotyped VSV was assessed by luciferase activity. The relative light unit (RLU) values of luciferase were determined using a Bright-Glo Luciferase Assay System (Promega Corporation, Madison, WI), according to the manufacturer's protocol.

### 2.3. Syncytium formation and low-pH treatment in SFTSV-infected cells

To examine whether syncytium formation of SFTSV-infected cells is induced by exposure to low pH, Huh7 cells were infected with each SFTSV strain (HB29, YG1, and SPL030) at an MOI of 0.1. Following incubation for 48 h, cells were rinsed once with phosphate-buffered saline (PBS) and then incubated with citrate-phosphate buffer (0.1 M citric acid, 0.2 M sodium dihydrogen orthophosphate) adjusted to the indicated pH value (pH7.0, 6.6, 6.2, 5.8, 5.4, and 5.0) for 2 min. The citrate-phosphate buffer was replaced with 10% FBS-DMEM, and incubated for 4 h. Cell fusion within monolayers was examined under a phase-contrast microscope.

### 2.4. Comparison of amino acid sequences of SFTSV strains

The amino acid sequences of HB29, YG1, SPL032A, SPL030A, SPL010A, SPL005A, SPL004A, and SPL003A GPs, and the GenBank accession numbers HM745931, AB817987, AB817993, AB817992, AB817991, AB817990, AB817989, and AB817988 respectively, were compared using the genetic information processing software, Genetyx

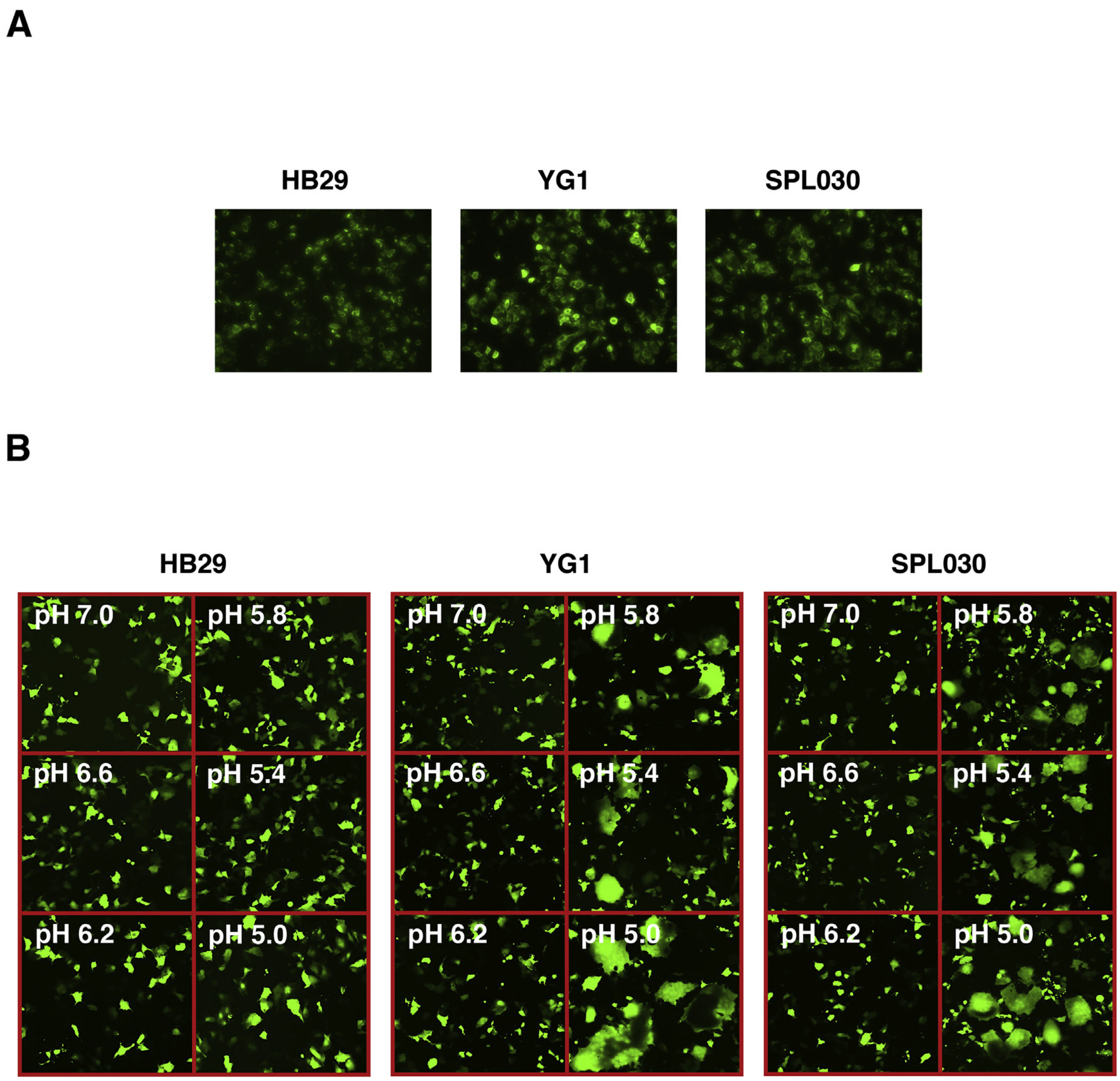

**Fig. 2. Syncytium formation in Huh7 cells expressing SFTSV-GP.** (A) To examine expression of each SFTSV-GP (HB29, YG1, or SPL030 strain), GP-expressing plasmids were transfected to Huh7 cells. Expression of SFTSV-GP was detected by immunofluorescence assay at 48 h post-transfection. (B) To examine cell-fusion activity in cells transiently expressing SFTSV-GP proteins, GP-expressing and eGFP-expressing plasmids were co-transfected to Huh7 cells. At 48 h post-transfection, cells were treated with the indicated pH citrate-phosphate buffer for 2 min, which was then replaced with culture medium. The syncytium formations of monolayer cells were observed under a fluorescence microscope 24 h following the treatment.

(Genetyx Co., Tokyo, Japan). Amino acid sequences were aligned at the beginning of the coding codons in nucleotide sequences.

### *2.5. Immunofluorescence assay*

For immunofluorescence staining of SFTSV-GP-expressing cells with antibodies, Huh7 cells transfected with the indicated expression plasmids were fixed with acetone–methanol (1:1) at room temperature for 10 min. Fixed cells were stained with mouse monoclonal anti-SFTSV-GP (Immune Technology Corp., New York, NY) as a primary antibody. After incubation for 1 h, the cells were rinsed with PBS and incubated with goat anti-mouse Alexa Fluor 488 antibodies (Life Technologies, Carlsbad, CA). After washing with PBS, the stained cells were observed under a fluorescence microscope (BZ-X710, Keyence, Osaka, Japan).

### *2.6. Syncytium formation and quantitative reporter assay for cell fusion in GP-expressing cells*

To compare the fusion activity of each GP in low-pH conditions, Huh7 cells were transfected with the indicated GP-expression plasmids together with pCAGGS-eGFP. At 48 h post-transfection, the cells were rinsed once with PBS and incubated with citrate-phosphate buffer adjusted to the indicated pH value for 2 min. The citrate-phosphate buffer was replaced with 10% FBS-DMEM and incubated for 24 h. The cell monolayers were then observed for the induction of cell fusion under a fluorescence microscope. To quantify cell-fusion induced by each GP, a reporter assay was carried out as follows. Huh7 cells were grown on 24-well tissue-culture plates and transfected with indicated GP-expression plasmids together with pCAGT7pol (Tani et al., 2014), an expression plasmid encoding the T7 RNA polymerase gene under the control of the CAG promoter (Tani et al., 2014). The Huh7 target cells were grown on 12-well tissue-culture plates and transfected with pT7EMCVLuc (Tani et al., 2014), a reporter plasmid encoding a firefly luciferase gene under the control of the T7 promoter. At 24 h post transfection, the target cells were collected by trypsinization and regrown in 96-well tissue-culture plates. Huh7 cells that expressed GP were treated with 0.1% trypsin and suspended in 10% FBS-DMEM. The cells were overlaid onto target Huh7 cells and incubated for 4 h. The co-cultured cells were bathed in citrate-phosphate buffer adjusted to the indicated pH value for 2 min and then incubated with 10% FBS-DMEM for 12 h. Cell-fusion activity

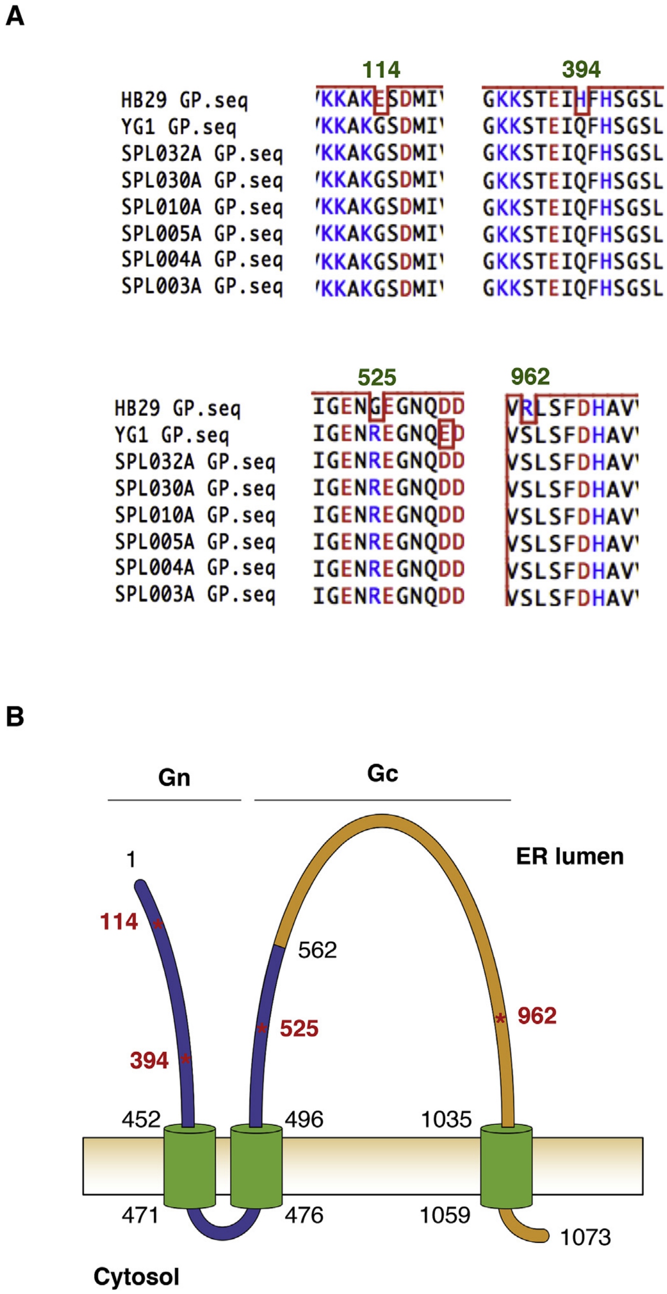

**Fig. 3. Comparison of amino acid sequences among SFTSV strains.** (A) The alignment of amino acid sequences on the Gn/Gc proteins among SFTSV strains HB29, YG1, SPL032A, SPL030A, SPL010A, SPL005A, SPL004A, SPL003A is indicated. The numbers above show the amino acid positions of the Gn/Gc protein. (B) A schematic diagram showing the hypothetical structure of the Gn/Gc protein. The upper side shows the ER lumen and the lower side shows the cytoplasmic region. The Gn/Gc protein has three transmembrane regions. Gn (blue) is from amino acid positions 1 to 562 and Gc (orange) is from amino acid positions 563 to 1073. Asterisks represent mutations.

was quantitatively determined by measuring luciferase gene expression in the lysates of the co-cultured cells. The RLU values of luciferase were determined using a Bright-Glo Luciferase Assay System and normalized to the values of cells treated with pH 7 buffer.

### 2.7. Infectivity and neutralization of pseudotyped VSVs bearing SFTSV mutant-GPs

To examine infectivity and neutralization of pseudotyped VSVs bearing wild-type and mutant GPs of SFTSV using a novel anti-SFTSV-GP monoclonal antibody with neutralizing activity (Tani et al., unpublished data), each SFTSVpv was preincubated with the anti-SFTSV-GP monoclonal antibody for 1 h at 37 °C. This monoclonal antibody was made from the immunization of cell lysates from the HB29 strain SFTSV-infected cells and broadly neutralized with several strains of SFTSV (Tani et al., unpublished data). Huh7 cells were then inoculated with each of the SFTSVpv. After 2 h at 37 °C to allow adsorption, cells were washed with 10% FBS-DMEM, and infectivity was determined by luciferase activity following incubation for 24 h at 37 °C. The rate at which each SFTSVpv was inhibited by anti-SFTSV-GP monoclonal antibodies was standardized by the infectivity of each SFTSVpv without treatment with the antibodies.

### 2.8. Statistical analysis

The results were expressed as means ± standard deviations. The significance of any differences in the means was determined using the Student's *t*-test.

### 2.9. Three-dimensional structures of SFTSV Gc

The pre-fusion structure of SFTSV Gc was generated by homology modeling based on the Gc of Rift Valley Fever Virus (PDB code: 4HJ1). Once 100 models of pre-fusion SFTSV Gc had been generated using MODELLER 9v6 (Sali and Blundell, 1993), a model with the best score for probability density function was chosen. The coordinate of the post-fusion structure of SFTSV Gc was taken from the crystal structure (PDB code: 5G47).

## 3. Results

### 3.1. Syncytium formation in SFTSV-GP-expressing cells exposed to low pH conditions

To examine the formation of syncytia in SFTSV-GP-expressing cells, Huh7 cells were infected with HB29, YG1, or SPL030 strains of SFTSV (Fig. 1), or transfected with SFTSV-GP expression plasmids (Fig. 2) and cultured for 48 h. The cells were then treated with a buffer at the indicated pH for 2 min. The expression of SFTSV-GP was confirmed by immunofluorescence staining using anti-SFTSV-GP (Fig. 2A). Syncytia were observed in Huh7 cells infected with YG1 and SPL030 strains of SFTSV, following treatment with pH 6.2 or lower; in contrast, few syncytia were observed in cells infected with the SFTSV HB29 strain at the same pH (Fig. 1). In SFTSV-GP-expressing Huh7 cells, syncytia were also observed in cells transfected with YG1 and SPL030 strains of SFTSV-GP expression plasmids at a treatment with pH 5.8 or lower, but were not observed in cells transfected with the HB29 strain SFTSV-GP expression plasmid, even at pH 5.0 (Fig. 2). These results indicate that the HB29 strain GP has different properties compared with those of the YG1 or SPL030 strains.

### 3.2. Comparison of amino acid sequences among SFTSV strains

The amino acid sequences on the Gn/Gc proteins among SFTSV Chinese strain HB29, or Japanese strains YG1, SPL032A, SPL030A, SPL010A, SPL005A, SPL004A, SPL003A was compared. The nature of amino acids of the position number 114, 394, 525, or 962 of the Gn/Gc protein of HB29 was different from all Japanese strains we examined (Fig. 3A). All of these amino acids are considered to be located in the ER lumen (Fig. 3B).

### 3.3. Expression, localization, and syncytium formation of SFTSV mutant-GPs

To examine the expression and localization of SFTSV mutant-GPs,

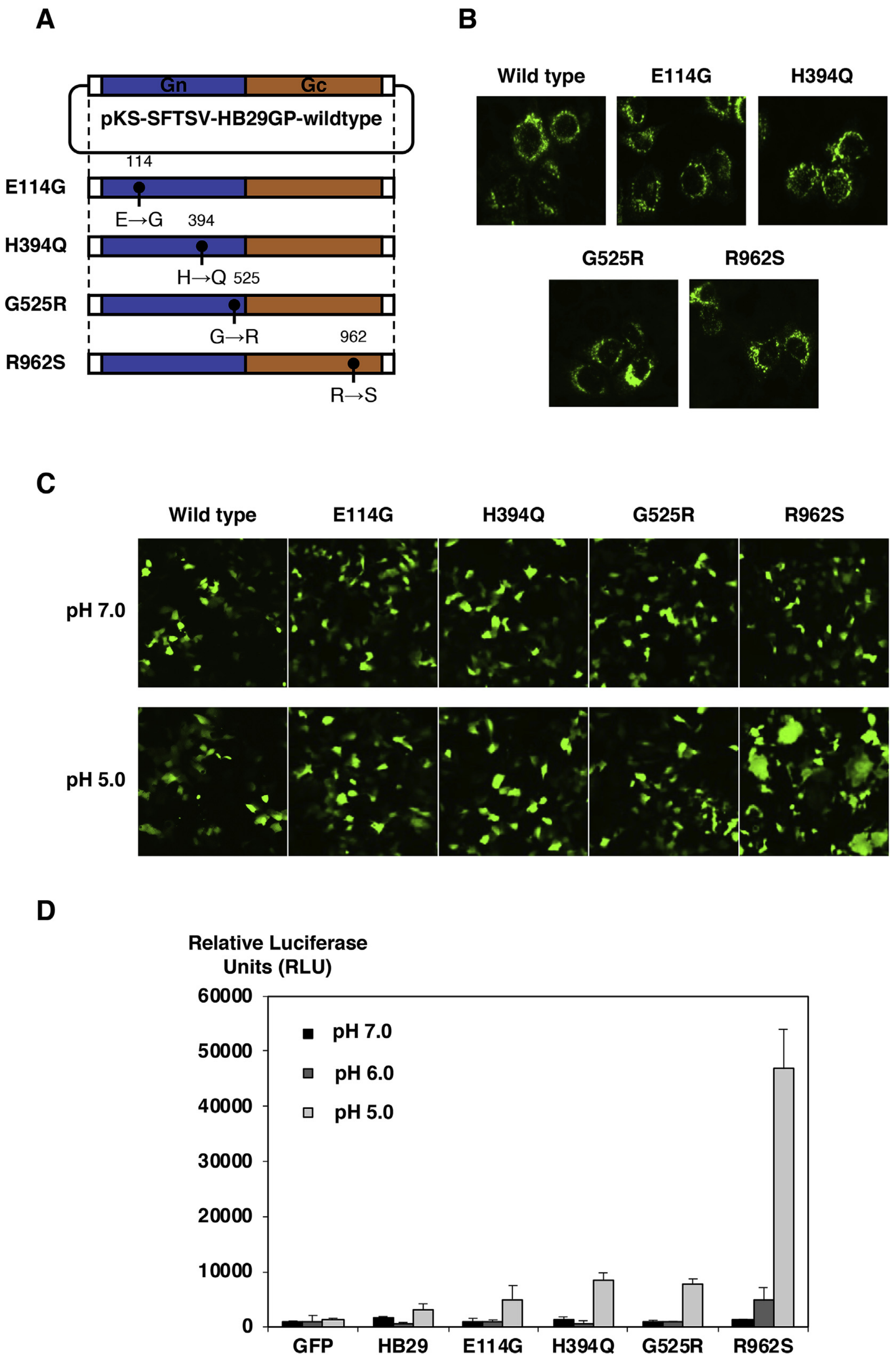

**Fig. 4. Analysis of SFTSV HB29 mutant-GPs expressed in Huh7 cells.** (A) A schematic representation of HB29 mutant-GP plasmids. (B) Expression and localization of mutant-GPs in cells transfected with mutant plasmids was examined using anti-SFTSV-GP monoclonal antibodies. (C) Syncytium formation of mutant-GPs was observed under low-pH conditions, as described in the Materials and Methods section. (D) Syncytium formation of mutant-GPs was quantitatively examined using a cell-fusion reporter assay under low-pH conditions, as described in the Materials and Methods section. The results shown are from three independent assays, with error bars representing standard deviations.

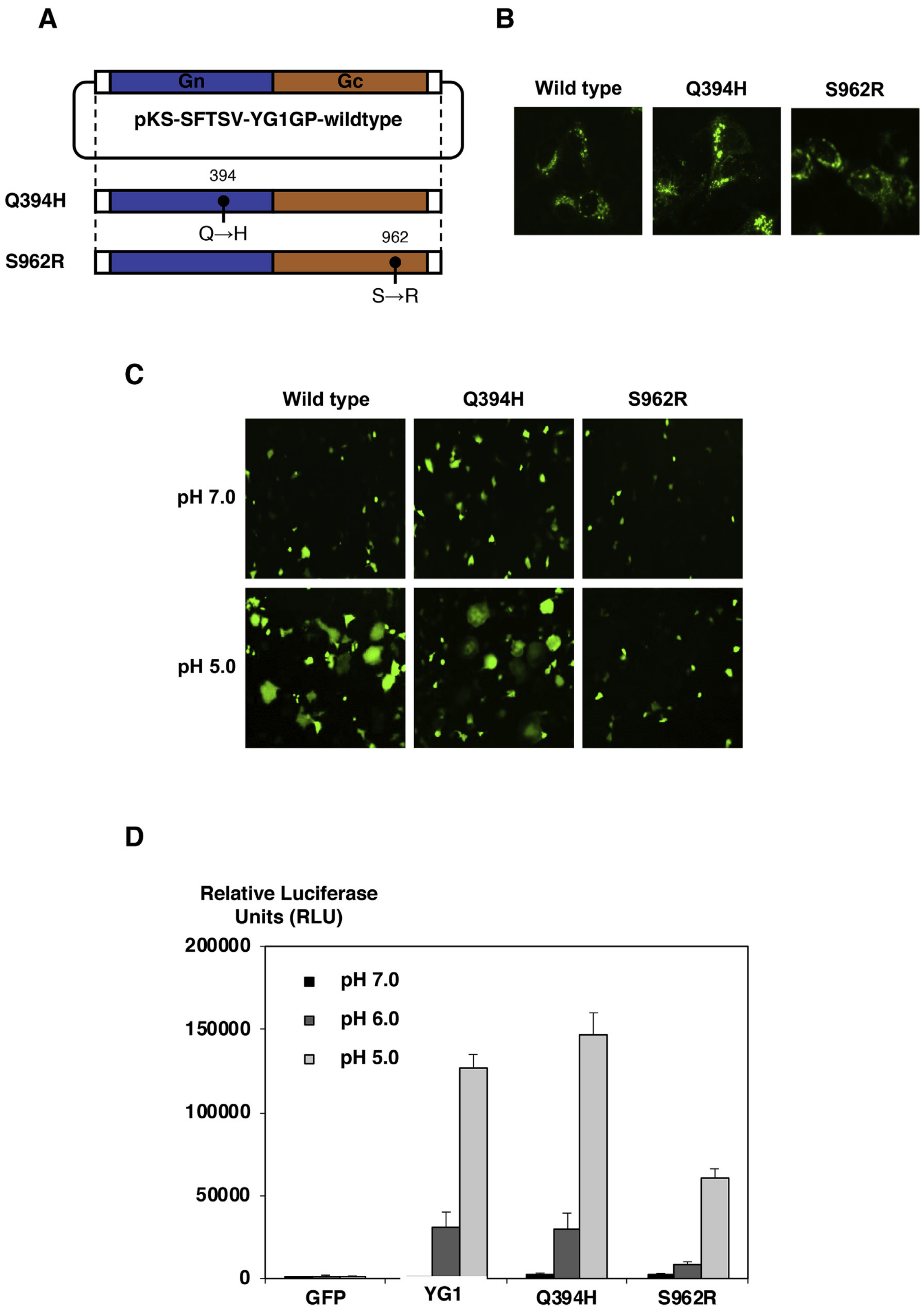

**Fig. 5. Analysis of SFTSV YG1 mutant-GPs expressed in Huh7 cells.** (A) A schematic representation of YG1 mutant-GP plasmids. (B) Expression and localization of the mutant-GPs in cells transfected with mutant plasmids was examined using anti-SFTSV-GP monoclonal antibodies. (C) Syncytium formation of mutant-GPs was observed under low-pH conditions, as described in the Materials and Methods section. (D) Syncytium formation of mutant-GPs was quantitatively examined using a cell-fusion reporter assay under low-pH conditions, as described in the Materials and Methods section. The results shown are from three independent assays, with error bars representing standard deviations.

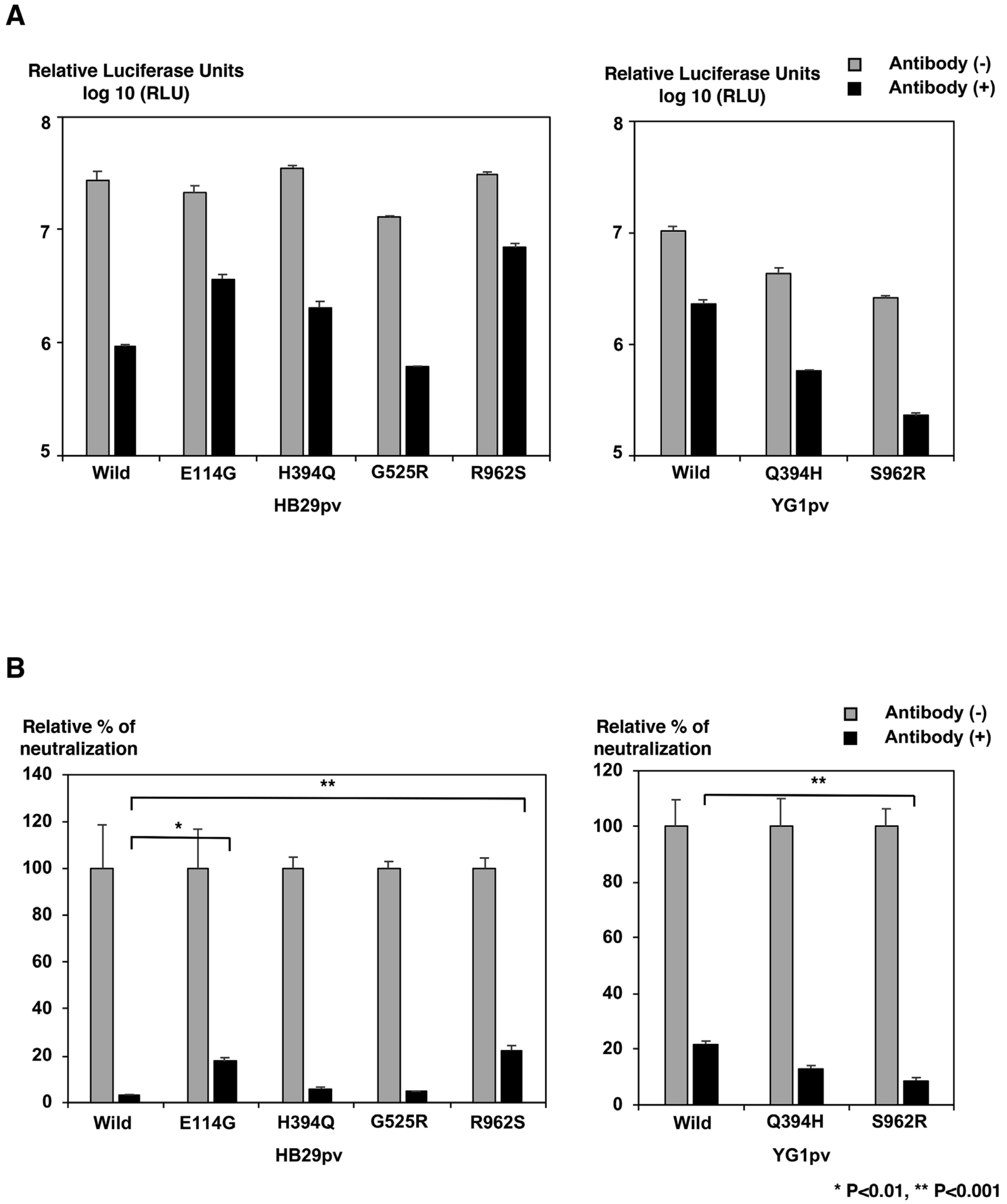

**Fig. 6. Neutralization of pseudotyped viruses with SFTSV mutant-GP by monoclonal antibodies.** (A) Infectivity and neutralization of SFTSVpv in Huh7 cells was determined by luciferase activity. The left panel shows infectivity and neutralization of SFTSVpv-bearing HB29 mutant-GPs with or without anti-SFTSV-GP monoclonal antibodies, while the right panel shows those of SFTSVpv-bearing YG1 mutant-GPs with or without anti-SFTSV-GP monoclonal antibodies. (B) The effects of anti-SFTSV-GP monoclonal antibodies on the infectivity of SFTSVpv in Huh7 cells. The relative percentage of neutralization of SFTSVpv was analyzed on the basis of the data in Fig. 6A. The results shown are from three independent assays, with error bars representing standard deviations. *, $P < 0.01$, **, $P < 0.001$.

Huh7 cells were transfected with SFTSV-GP mutant plasmids. Expression of all the mutant GPs was observed in the transfected cells and their localization in cells seemed to be identical to that of wild type SFTSV-GP HB29 strain (Fig. 4B) and YG1 strain (Fig. 5B). To examine syncytium formation in the mutant-GP expressing cells, Huh7 cells transfected with expression plasmids encoding SFTSV mutant-GPs were cultured for 48 h, then treated with the indicated pH buffer for 2 min. Among HB29-based mutant-GP expressing cells, syncytia were only observed after treatment with pH 5.0 buffer in the HB29-GP (R962S) expressing cells (Fig. 4C). On the other hand, among YG-1-based mutant GP expressing cells, syncytia were observed in YG1-GP (Q394H) expressing cells as in the YG1-GP (wild type) expressing cells, but not in the YG1-GP (S962R) expressing cells with pH 5.0 buffer treatment (Fig. 5C). To quantitatively examine syncytium formation further, a cell-fusion reporter assay was employed. Luciferase activity in the HB29-GP (R962S) expressing cells was highest compared with other cells expressing the HB29 mutant-GPs under pH 5.0 conditions (Fig. 4D). In contrast, lower luciferase activity was observed in the YG1-GP (S962R) expressing cells compared with the YG1-GP (wild type) and the YG1-GP (Q394H) expressing cells (Fig. 5D). These data indicate that

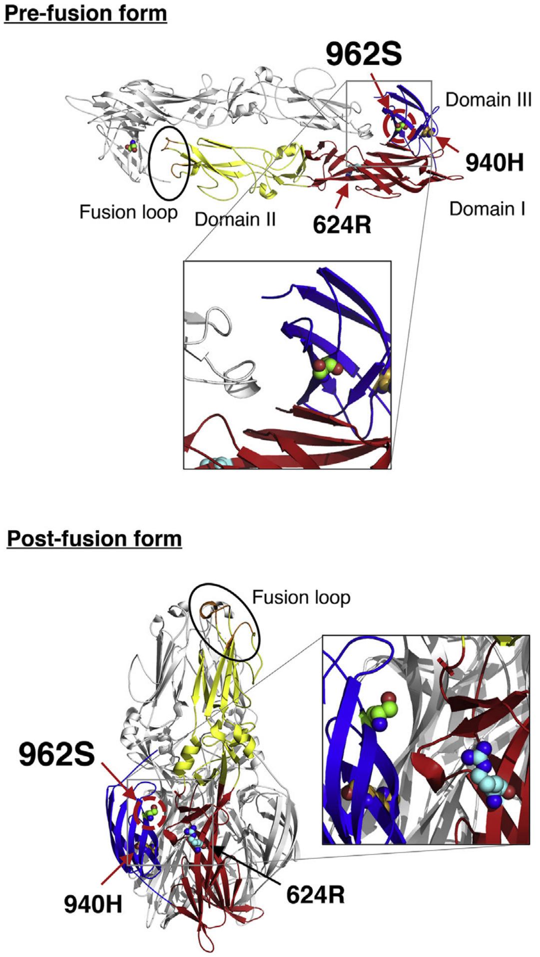

**Fig. 7. Structural models of SFTSV Gc.** Structural rearrangement of SFTSV Gc from pre-to post-fusion structures. The amino acids at positions 624, 940, and 962 are described. Domain I is in red, Domain II is in yellow, Domain III is in blue, and the fusion loop in Domain II is in orange. Magnified images of the region around position 962 are shown in the box.

serine at residue 962 in the SFTSV-GP is critical for membrane fusion under low pH conditions.

### 3.4. *Infectivity and neutralization of pseudotyped VSVs bearing SFTSV mutant-GPs*

To examine the infectivity of pseudotyped VSVs bearing wild and mutant GPs of SFTSV, Huh7 cells were infected with HB29pv, HB29(E114G)pv, HB29(H394Q)pv, HB29(G525R)pv, HB29(R962S)pv, YG1pv, YG1(Q394H)pv, or YG1(S962R)pv. All pseudotyped VSVs bearing SFTSV mutant-GPs exhibited almost same the infectivity in Huh7 cells (Fig. 6A). Next, we examined whether the infectivity of the pseudotyped VSVs bearing SFTSV mutant-GPs was neutralized by a monoclonal antibody to SFTSV GP (Fig. 6). Neutralization of HB29(R962S)pv and HB29(E114G)pv in Huh7 cells was weaker compared with wild type HB29pv or other pseudotyped VSVs (Fig. 6B). In contrast, neutralization of YG1(S962R)pv was higher compared with the wild type YG1pv or YG1(Q394H)pv. These results suggest that serine at position 962 in the SFTSV-GP is important for viral infection and partially involved in the recognition of neutralizing antibodies. The crystal structure of the pre- and post-fusion forms of SFTSV Gc was analyzed (Halldorsson et al., 2016). Dimeric pre- and post-fusion structural models of SFTSV Gc were represented in order to ascertain where the amino acid at position 962 is structurally located (Fig. 7). The amino acids at positions 624 and 940, which are important for the membrane fusion (Halldorsson et al., 2016; Tsuda et al., 2017), were also represented. Position 962 was located in Domain III, which was present on the outside of the structure. Both position 624 in Domain III and position 940 in Domain II were contiguous with position 962 in the post-fusion structural models.

## 4. Discussion

SFTS is an emerging infectious disease that represents a serious global health concern because of its high fatality rate and lack of effective vaccine and therapeutic treatment. An understanding of the life cycle of this virus will help to inform the development of antiviral strategies. Knowledge is accumulating about the activity and structure of the SFTSV glycoprotein. The Gc of SFTSV is a class II viral-fusion protein; membrane fusion occurs under acidic conditions. Viral entry can be inhibited by lysosomotropic agents, suggesting that low-pH conditions in endosomes are essential for viral infection. Recently, it was reported that SFTSV required a corrective glucosylceramide formation in host cells for efficient cell-entry (Drake et al., 2017).

In our previous study, syncytium formation in cells infected with SFTSV strain HB29 was observed following treatment with low-pH buffer. In the present study, syncytium formation was not observed in HB29-GP transfected cells, even at pH 5.0, whereas syncytium formation in cells infected with SFTSV strain HB29 was observed at pH values of less than 6.2. It is difficult to evaluate the ability of infected cells to form syncytia, because viruses, including mutations of the GP region, emerge while the viruses replicate in the cells. This discrepancy may be due to the presence of mutations that are already capable of forming syncytia in infected cells. Cell fusion is generally often induced when viral GP is present at the plasma membrane. Previous and present studies revealed that SFTSV-GP is considered to be mainly present in the cells. Nevertheless, cell fusion is observed in the SFTSV-GP-expressing cells because it is considered to be caused by leakage of GP due to disruption of the part of the cell membrane both in the SFTSV infected cells and in GP-overexpressing cells. Unfortunately, the amount of expression of GP on the cell surface seems to be very small and would not be confirmed by immunofluorescence assay or fluorescence activated cell sorting analysis.

The degree of cell-fusion activity associated with the replacement of arginine by other amino acids in HB29 strain GP has not been confirmed, but there may be amino acid mutations that increase the likelihood of cell fusion occurring. Although there is no means of quantifying the amount of GP incorporated into pseudotyped viruses bearing each mutant-GP, the amount of GP incorporated into virions does not seem to change, because the infectivity of each SFTSVpv appeared to be similar (Fig. 6A). Generally, entry steps, including membrane-fusion by a virus, may be associated with an envelope glycoprotein affected by the modification of a sugar chain. Serine or threonine has the potential to undergo O-linked glycosylation. Therefore, it is only speculated that the serine at position 962 in the SFTSV GPs undergoes O-linked glycosylation. Regarding the degree of neutralizing activity against neutralizing antibodies, the amino acid at position 962 is likely to affect the recognition site of the antibody. To investigate the influence on antibody neutralization, it will be necessary to construct mutants that have amino acids other than serine.

In a comparison of pre- and post-fusion structural models of Gc, the amino acid at position 962 was located in Domain III (Fig. 7). Previous reports suggested that an arginine at position 624 and located in Domain I, and a histidine at position 940 in Domain III, play a critical role in low-pH-dependent cell fusion and viral production. Although the amino acids at positions 962 and 940 are located close to each other, and the arginine at position 624 seems distant from the serine at position 962 in the pre-fusion form, the arginine at position 624 is close to the amino acids at positions 962 and 940 in the post-fusion form. These results imply that the region around these amino acids is important for membrane fusion, in addition to the region of the putative fusion-loop.

In the pre-fusion form, it takes a positional relationship very close to the amino acids located in the fusion loop when forming a dimer. Arginine at position 624 located in Domain I and histidine at position 940 in Domain III might interact via the fusion loop region to influence viral infectivity (Halldorsson et al., 2016; Tsuda et al., 2017). In particular, histidine at residue 940 critically affects viral infectivity, and it is possible that serine at residue 962 is closely related to or interacts with the region, including residue 940.

In conclusion, we clarified that the amino acid at residue 962 of SFTSV-GP is crucial for virus–cell fusion. Several amino acids at other residues were shown to be important for cell fusion of SFTSV in addition to serine at residue 962, but the findings of our study afford us a better understanding of the fusion activity of the SFTSV-GP and contribute to the future analysis of cell entry mechanisms of SFTSV.

## Conflicts of interest

The authors declare no conflicts of interest in association with the present study.

## Acknowledgments

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