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Single amino acid mutation of nectin-1 provides remarkable resistance against lethal pseudorabies virus infection in mice

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ABSTRACT. An approach to genetically engineered resistance to pseudorabies virus (PRV) infection was examined by using a mouse model with defined point mutation in primary receptor for alphaherpesviruses, nectin-1, by the CRISPR/Cas9 system. It has become clear that phenylalanine at position 129 of nectin-1 is important for binding to viral glycoprotein D (gD), and mutation of phenylalanine 129 to alanine (F129A) prevents nectin-1 binding to gD and virus entry *in vitro*. Here, to assess the antiviral potential of the single amino acid mutation of nectin-1, F129A, *in vivo*, we generated genome-edited mutant mouse lines; F129A and 135 knockout (KO). The latter, 135 KO used as a nectin-1 knockout line for comparison, expresses a carboxy-terminal deleted polypeptide consisting of 135 amino acids without phenylalanine 129. In the challenge with 10 LD_{50} PRV via intranasal route, perfect protection of disease onset was induced by expression of the mutation of nectin-1, F129A (survival rate: 100% in F129A and 135 KO versus 0% in wild type mice). Neither viral DNA/antigens nor pathological changes were detected in F129A, suggesting that viral entry was prevented at the primary site in natural infection. In the challenge with 50 LD_{50} PRV, lower but still strong protective effect against disease onset was observed (survival rate: 57% in F129A and 75% in 135 KO versus 0% in wild type mice). The present results indicate that single amino acid mutation of nectin-1 F129A provides significant resistance against lethal pseudorabies.

KEYWORDS: genome-edited mouse, herpesvirus, nectin-1, pseudorabies, resistance

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Pseudorabies virus (PRV) is a representative member of genus *Varicellovirus* of the subfamily *Alphaherpesvirinae* [19]. It is the swine pathogen causing lethal encephalitis in neonates and latent infection in surviving pigs. PRV infection causes severe economic losses to the swine industry. PRV is also known to cause acute and often fatal infections in other domestic and wild animals. The alphaherpesvirus subfamily including PRV, herpes simplex virus types 1 and 2 (HSV-1 and HSV-2), and bovine herpesvirus 1, is characterized by primary infection in epithelium, followed by neurotropic viral spread and establishment of latent infections in the ganglion neurons. Binding of alphaherpesviruses to cells occurs primarily by the interaction of virion glycoproteins (gC and/or gB) with heparan sulfate on the cell surface [11, 20, 29, 38], while fusion between the virion envelope and the plasma membrane requires glycoprotein gB, gD, gH, and gL [2, 5, 18, 28]. So far, five alphaherpesvirus receptors have been identified: herpesvirus entry mediator (Hve) A (HveA; HVEM), HveB (nectin-2), HveC (nectin-1), HveD (CD155), and 3-*O*-sulfated heparan sulfate [3, 4, 7, 30, 35]. In particular, nectin-1 has the broadest specificity for mediating alphaherpesvirus entry and is present in a wide range of tissues and cells [4, 6, 32].

Protecting livestock from infectious diseases avoids large-scale culling of animals for disease control, prevents significant economic losses, reduces the risk of zoonotic diseases, and of course enhances animal welfare. However, vaccines for infection control are not always available, and other biosecurity strategies do not necessarily work. Furthermore, vaccination alone generally cannot eliminate viral infections from animal populations, even though it can suppress disease manifestations. Therefore, other strategies need to be developed to protect animals from infectious disease outbreaks; the CRISPR/Cas9 system has already contributed to the generation of disease-resistant animals for infectious disease prevention. The simplest approach to produce resistant livestock is to restrict susceptibility to pathogens [37]. For example, mutating or deleting a receptor essential for viral entry could protect animals from

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infection. The first resistant livestock to be successfully produced by genetic engineering were porcine reproductive and respiratory syndrome resistant pigs generated by knocking out the porcine CD163 receptor using the CRISPR/Cas9 system [1, 10, 36, 37, 39]. However, CD163 has important biological functions such as anti-inflammatory regulations [9, 16]. In general, the potential for adverse effects of knocking out target genes in the host must be carefully evaluated. On the other hand, we have previously reported that some types of soluble nectin-1 consisting of an extracellular domain of nectin-1 and the Fc portion of IgG1 provide a significant resistance against PRV infection in cultured cells and mice [21–25]. The goal of these studies was to propose a novel approach to combat pseudorabies by generating livestock expressing the soluble nectin-1 as an alternative to vaccine strategies. It would be better if disease resistance could be conferred by expressing molecules that are as close to the wild type as possible rather than such chimeric molecules and knocking out of the target genes. Therefore, we focused on the role of specific amino acids within receptor molecules. The generation of point mutations is a major tool for evaluating the roles of specific amino acids within the regulatory or functional molecule. The CRISPR/Cas9 system greatly facilitates the generation of genetically modified animals with point mutations within such target molecules.

Recent studies have shown that phenylalanine 129, located at the tip of extracellular loop structure of the nectin-1, protrudes to bind viral gD and is key to alphaherpesvirus-cell binding [8, 17, 27]. Furthermore, the phenylalanine 129 to alanine (F129A) mutation was reported to almost completely inhibit gD/nectin-1 binding and to affect cell fusion. Thus, the functional essentiality of nectin-1 F129 in the binding of alphaherpesvirus gD to cells has been demonstrated in previous *in vitro* studies. In the present study, we indicated that single amino acid mutation of nectin-1 F129A confers disease resistance against lethal pseudorabies in genome-edited mice and proposed the *in viv*o model system for pseudorabies-resistant livestock.

MATERIALS AND METHODS

Animals

C57BL/6N and CD1 mice were purchased from Japan SLC, Inc. (Hamamatsu, Japan) and Charles River Laboratories Japan, Inc. (Yokohama, Japan), respectively. Mice were kept in plastic cages (4–5 per cage) under specific pathogen-free conditions in a room maintained at 23 ± 3°C and 50 ± 15% humidity under a 12:12-hr light: dark cycle. Mice were allowed free access to commercial food and water throughout the study. Animal experiments were conducted humanely in accordance with the animal experiment regulations of Kyushu University and Tottori University, and the Basic Guidelines for the Proper Implementation of Animal Experiments in Academic Research Institutions under the jurisdiction of the Ministry of Education, Culture, Sports, Science and Technology, and with the approval of the institutional animal experiment committee (approval number: A19-129 and A21-006 in Kyushu University, 22-T-10 in Tottori University).

Microinjection

The following mutation in the nucleotide number 385–386 TT to GC (Phe 129 Ala) of the nectin-1 gene was introduced into the mouse genome using the CRISPR/Cas9 method. The sgRNA was selected using the CRISPRdirect software. Artificially synthesized two sgRNAs; M-nectin-1 F1: GAATTTGCCACCTTCCCTACguuuuagagcuagaaauag caaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcacggacucggugcuuuu and M-nectin-1 F2: GACGGTTGCCCGTAGGGAAGG guuuuagagcuagaaauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcacggacucggugcuuuu were purchased from FASMAC Co., Ltd. (Atsugi, Japan). The single-stranded oligo DNA (ssODN), CTCTCCGGTCTGGAGCTGGAGGACGAGGGCAT GTACATCTGTGAATTTGCCACCGCCCCTACGGGCAACCGTGAAAGCCAGCTCAATCTCACTGTGATGG, was designed to insert the desired mutations and synthesized by Integrated DNA Technologies (IDT; Coralville, IA, USA). Female C57BL/6N mice were injected with pregnant mare serum gonadotropin (PMSG) and human chorionic gonadotropin (hCG) with a 48-hr interval and mated with male C57BL/6N mice. The fertilized one-cell embryos were collected from the oviducts. Then, 25 ng/μL of the sgRNAs, 2.5 ng/μL ssDNA and 75 ng/μL Guide-it™ Recombinant Cas9 protein (Takara Bio Inc., Kusatsu, Japan) were injected into the pronuclei of these one-cell-stage embryos. The next day two-cell embryos were then transferred into pseudopregnant CD1 mice. All experiments on genome editing were conducted in accordance with Cartagena protocol on biosafety and the regulations of Kyushu University and Tottori University, and with the approval of the institutional gene modification experiments safety committee (approval number: 29-22 in Kyushu University, 34-038 in Tottori University).

DNA extraction and sequencing

Genomic DNA of tails collected from mice was extracted using NucleoSpin Tissue (Macherey-Nagel, Düren, Germany). The targeted fragments around the sgRNA targeting site from the extracted genomic DNA as a part of the nectin-1 gene was amplified with TAKARA Ex Taq (Takara Bio) and the following primers: M-nectin-1_1st_F (5′-GTGGTGCAGGTGAACGACT-3′) and M-nectin-1_1st_R (5′- CCATCACAGTGAGATTGAGC-3′) as 1st primers pair; M-nectin-1_2nd_F (5′- CTCCATGTATGGCTTCATCG-3′) and M-nectin-1_2nd_R (5′- CAGTGAGATTGAGCTGGCTTT-3′) as 2nd primers. The PCR product was purified with a Fast Gene Gel/PCR Extraction Kit (Nippon Genetics, Tokyo, Japan) and were additionally purified by agarose gel electrophoresis and Monarch Gel Extraction Kit (New England BioLabs, Inc., Beverly, MA, USA). The purified PCR products were subsequently sequenced with M-nectin-1_2nd_F primer, using a BigDye Terminator v3.1 Sequencing Kit (Applied Biosystems, Inc., Foster City, CA, USA).

Histology

For histological analysis, mice were euthanized using an excess of isoflurane (FUJIFILM Wako Pure Chemical Corp., Osaka, Japan)

and the tissue samples including brain and lung were excised. The tissues were immediately fixed in 10% neutral buffered formalin (FUJIFILM Wako Pure Chemical) and processed via routine methods then embedded in paraffin wax. Paraffin sections (4 µm thick) were prepared and stained with haematoxylin and eosin for histopathological examination.

Analysis of nectin-1 expression

To confirm the nectin-1 expression, Western blotting was also performed by the method of Towbin *et al* [34]. For the collection of protein samples, mice were euthanized using an excess of isoflurane (FUJIFILM Wako Pure Chemical). Total proteins of brains were extracted by using CelLytic MT Cell Lysis Reagent (Sigma, St. Louis, MO, USA) with Protease Inhibitor Cocktail (Sigma). Two µg of each sample was fractionated by 4–15% Mini-PROTEAN® TGX Gel (Bio-Rad Laboratories, Hercules, CA, USA). Then the separated proteins were electrophoretically transferred to an Immobilon transfer membrane (Millipore, Cork, Ireland). Blotted membrane was treated sequentially with blocking solution (5% skim milk in phosphate buffered saline with 0.05% Tween 20), primary antibody, and finally with HRP-labeled secondary antibody. Anti-nectin-1 rabbit polyclonal antibody (H-62; Santa Cruz Biotechnology, Santa Cruz, CA, USA) corresponding to aa 451–512 at C-terminus of human nectin-1 were used as primary antibodies. Anti-Rabbit IgG (H+L) HRP Conjugate (BioRad) were used as secondary antibodies. Detection was performed using ECL Plus Western Blotting Detection System (GE Healthcare, Piscataway, NJ, USA) as a substrate. For histological analysis of nectin-1 expression, mice were euthanized using an excess of isoflurane (FUJIFILM Wako Pure Chemical) and the tissue samples were excised. The tissues were immediately embedded in Tissue-Tek OCT compound (Sakura Finetek Japan Co., Ltd., Tokyo, Japan) after harvest and slowly frozen with liquid nitrogen. Sections (7 µm thick) were cut on a cryostat (CM1850; Leica, Wetzlar, Germany) and were mounted on microslides (MAS coat; Matsunami Glass). The sections were fixed by immersion with 4% Paraformaldehyde Phosphate Buffer Solution (Fujifilm Wako Pure Chemical) for 30 min. Subsequently, indirect immunofluorescence was performed by using anti-nectin-1 rabbit polyclonal antibody (H-62; Santa Cruz Biotechnology) corresponding to aa 451–512 at C-terminus of human nectin-1 as a primary antibody and goat anti-rabbit IgG (H+L) cross-adsorbed ReadyProbes™ secondary antibody, Alexa Fluor™ 488 (Invitrogen, Carlsbad, CA, USA). Finally, sections were coverslipped with Vectashield with DAPI (Vector Laboratories, Burlingame, CA, USA) and images were taken by using a fluorescence microscope equipped with CCD camera (DP73; Olympus, Tokyo, Japan).

Virus infection

Experimental infection in mice was carried out in the BSL-2 facility at Tottori University. The 50% lethal doses (LD_{50}) of PRV strain YS-81 in C57BL/6N mice were determined as described previously [26]. F129A, 135 KO and C57BL/6N mice were used for experimental infection. Mice were anesthetized by intraperitoneal injection of three types of mixed anesthetic agents [14] and then intranasally inoculated with 10 µL of PBS containing 10 LD_{50} (F129A, n=5; 135 KO, n=4; C57BL/6N, n=10) or 50 LD_{50} (F129A, n=7; 135 KO, n=4; C57BL/6N, n=10) of PRV. Survival and signs of the infected mice were recorded for 14 days. Apart from the experiment for survival observation, mice were intranasally inoculated with 10 LD_{50} of PRV in the same manner as above, and the tissues were sampled for viral DNA detection and pathological analysis at three or seven days after inoculation. Mice were similarly inoculated with the same volume of PBS as negative control.

Detection of viral DNA and antigen from infected mice tissue

For detection of viral DNA, mice were euthanized using an excess of isoflurane (FUJIFILM Wako Pure Chemical Corp.) and the tissue samples including trigeminal nerve and brain were excised. Porcine kidney cell line (CPK cells) inoculated with PRV (approximately 5 plaque-forming unit/cell) or PBS were prepared as positive or negative controls. Genomic DNA was isolated from the tissues or cells by a routine procedure, and PRV immediate-early gene (IE180) DNA was detected by PCR analysis using specific primers, as described previously [31]. For detection of viral antigen, formalin-fixed, paraffin-embedded specimens were prepared in the same manner as above. Subsequently, immunohistochemical analyses using anti-IE180 antibody were performed using a Simple Stain MAX-PO (R) kit (Nichirei Bioscience Inc., Tokyo, Japan) and 3,3′-Diaminobenzidine tetrahydrochloride (DAB) detection. Anti-IE180 antibody produced in rabbit was used at the concentration of 1 µg/mL, as described previously [33].

Statistics

Statistical analysis was performed by Kaplan-Meier method and generalized Wilcoxon test using Mac Statistical Analysis ver. 3.0 (Esumi Corp., Tokyo, Japan).

RESULTS

Genomic sequences of founder mice

Schematic representation of each nectin-1 protein is shown in Fig. 1A. Sequencing analyses with 31 founder mice showed that optional substitution (TT385-386GC) was induced in only one mouse (Fig. 1B) and designated as F129A mouse. Twelve of the remaining 30 founders carrying various deletions and a nucleotide insertion was detected in three of them. Among them one founder was selected for further analyses. The 16 nucleotides deletion (381–396) mutant mouse expresses a carboxy-terminal deleted polypeptide consisting of the first 128 amino acids of the intact nectin-1 and 7 amino acids (VKASQSH). The mouse line was designated as 135 KO mouse as a control of nectin-1 knockout mouse line. Each homozygous mouse line was established and used for analyses.

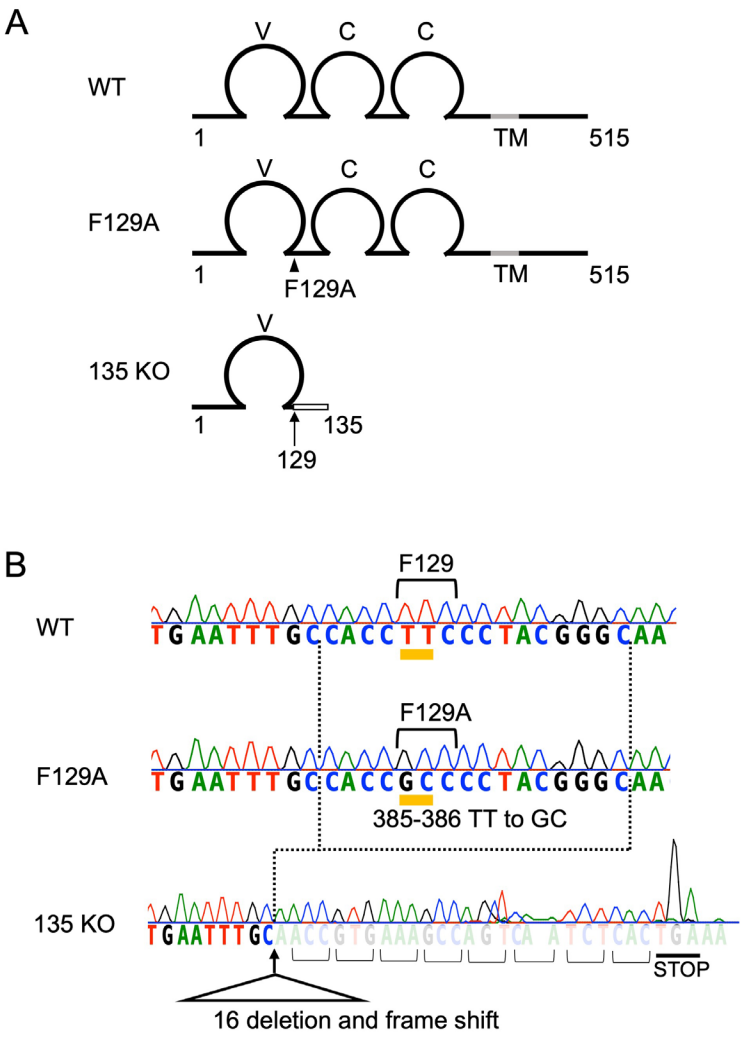

Fig. 1. Generation of genome-edited nectin-1 mutant. (**A**) Schematic representation of each nectin-1 protein in the genome-edited nectin-1 mutant mouse; F129A, 135 knockout (KO), and control wild type C57BL/6N. The location of the single amino acid mutation in F129A is indicated by black arrowheads. The frameshift mutation site (129 to 135 amino acids) due to 16 nucleotides deletion (381-396) in 135 KO is indicated by a white square. (**B**) Genomic sequences around the mutation site of nectin-1 in each mouse line.

Histology and expression of nectin-1 in each mouse strain

Histological images of the brain and lungs of F129A, 135 KO, and control wild-type C57BL/6N mice are shown in Fig. 2 (A–C, brain; D–F, lung). Homozygous mice of F129A and 135 KO showed grossly smaller size and microphthalmia compared to C57BL/6N mice, but no significant differences in any internal organs including brain and lung, histologically. Western blot analysis using anti-nectin-1 antibody against C-terminus of nectin-1 revealed that the F129A mice expressed nectin-1 with the same molecular weight as the wild-type C57BL/6N (Fig. 2G). And it confirmed that the 135 KO mice did not express intact nectin-1 recognized by the same antibody (Fig. 2G).

To confirm expression pattern of nectin-1 in the nervous and respiratory systems, immunohistochemistry using anti-nectin-1 antibodies against C-terminus was performed. The expression pattern of nectin-1 in brain of F129A mice were same as that of wild-type C57BL/6N (Fig. 3A vs. 3C). The bronchoalveolar epithelial cells in the lungs of F129A mice, especially the cell surface, were specifically stained with the antibodies, in the same manner as the wild-type mice (Fig. 3D vs. 3F). On the other hand, no staining was observed in those tissues of the 135 KO mice (Fig. 3B and 3E).

Resistance to lethal infections with pseudorabies virus in each mouse lines

To find out whether the single amino acid mutation of nectin-1, F129A, protects the mice from PRV infection, three strains mice were infected intranasally with 10 LD_{50} or 50 LD_{50} of PRV strain YS-81. The survival data were summarized in Fig. 4A and 4B. In the intranasal challenge with 10 LD_{50} (Fig. 4A), significant protections were observed in both mutant strains, F129A and 135 KO mice: 100% (5/5 and 4/4) of the animals from F129A and 135 KO mice survived, respectively. In contrast, 100% (10/10) of control wild-type C57BL/6N died after three days post inoculation (dpi). F129A and 135 KO mice showed a significant resistance as compared with wild-type C57BL/6N (P<0.001). These resistances were also substantially confirmed in higher-titer challenges; intranasal inoculation with 50 LD_{50} was lethal for 100% of all control C57BL/6N mice (10/10) at three-dpi, while 57% (4/7) and 75% (3/4) of the animals from F129A and 135 KO mice survived during the 14-day observation period, respectively (Fig. 4B). Both F129A and 135 KO mice showed a significantly higher survival rates as compared with wild-type C57BL/6N (P<0.001). The mice that developed neurological symptoms such as depression and neuropathic itch died within a day of onset, but the surviving mice did not show any symptoms at all. No significant differences were found between F129A and 135 KO (P=0.789). In the challenge with 10 LD_{50} PRV, no viral DNA was detected in F129A and 135 KO mice at three- and seven-dpi, whereas viral DNA was detected in trigeminal nerve and brain of C57BL/6N mice at three-dpi (Fig. 4C). Figure 4D shows the representative histopathology of brain in F129A and C57BL/6N mouse at three-dpi. Neuronal degeneration and necrosis, which sometimes accompanied by viral antigen-positive signals in neurons and microglia, were observed in the brain of C57BL/6N, while no specific lesions and no viral antigen were detected in F129A. No histopathological lesions and no viral antigens were detected in both C57BL/6N and F129A lungs.

DISCUSSION

In the present study, we indicated that genome-edited mouse line expressing single-amino-acid mutated nectin-1 (Phe 129 Ala) showed significant resistance to pseudorabies. Indeed, mutation of F129A completely protected animals against 10 LD_{50} PRV infection,

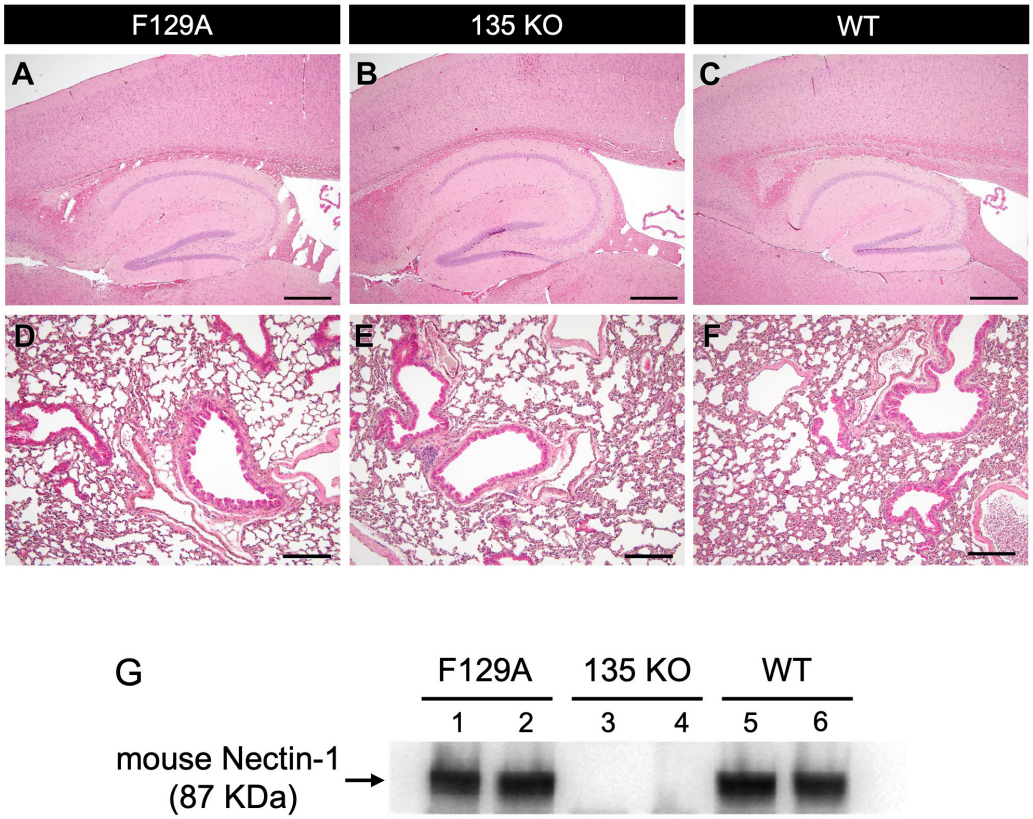

Fig. 2. Histological images and expression of nectin-1 in the tissue of genome-edited nectin-1 mutant mouse. H.E. staining. F129A (**A** and **D**), 135 KO (**B** and **E**), and control wild type C57BL/6N (C and F). Brain (**A**–**C**) and lung (**D**–**F**). Bars, 50 µm. (**G**) Detection of nectin-1 in F129A (lane 1, female; lane 2, male) and wild type C57BL/6N (lane 5, female; lane 6, male), by Western blot analysis using anti-nectin-1 antibody against C-terminus of nectin-1. No detected in 135 KO (lane 3, female; lane 4, male).

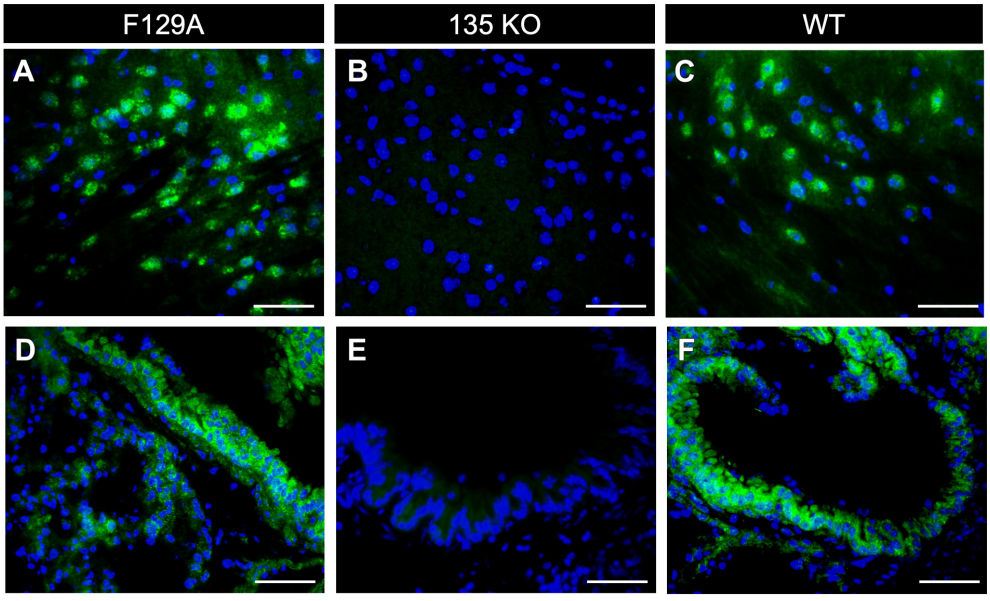

Fig. 3. Localization of nectin-1 in the lung and brain of genome-edited nectin-1 mutant mouse; F129A (**A** and **D**), 135 KO (**B** and **E**), and control wild type C57BL/6N (**C** and **F**). Brain (**A**–**C**) and lung (**D**–**F**), immunofluorescence staining with anti-nectin-1 antibody against C-terminus of nectin-1. Nectin-1 (green) and DAPI (blue). Bars, 50 µm.

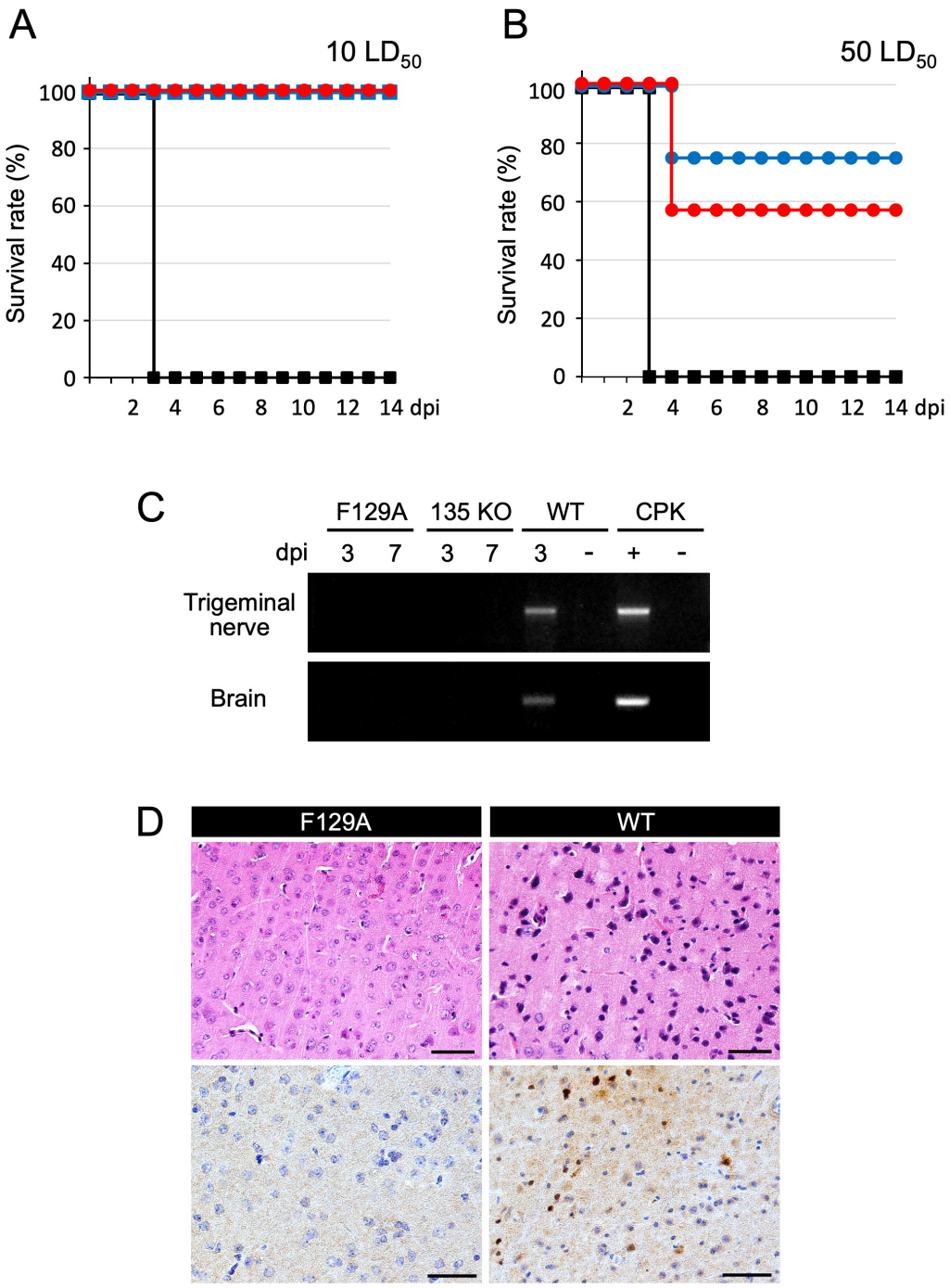

Fig. 4. Experimental infection. Survival rates of genome-edited nectin-1 mutant mouse after the intranasal challenge with pseudorabies virus (PRV) strain YS-81, 10 lethal doses 50 (LD_{50}) (**A**) and 50 LD_{50} (**B**). The F129A (red line), 135 KO (blue line), and C57BL/6N (black line) mice. Deaths were recorded daily until the termination of the experiments at day 14. PCR detection of viral DNA in the mice inoculated with PRV YS-81 (**C**). Dpi 3, 7: three or seven days after inoculation; -: PBS inoculated; +: PRV inoculated; CPK: porcine kidney cell line, CPK cellular DNA control. Histopathological images (**D**, upper) and immunohistochemical detection of viral IE180 in the brain tissue (**D**, lower). Brain from the mice at three-dpi, with PRV. Bars, 50 µm.

as same as 135 KO mutation, which include the first 128 amino acids but not the phenylalanine 129. There is no significant difference between F129A and 135 KO in the resistances against intranasal infection with higher dose of PRV (50 LD_{50}) (P=0.789). Phenylalanine 129 of nectin-1 V-domain is most important as interfacing to the gD of alphaherpesvirus including PRV *in vitro* [8, 17, 27]. In particular, it has been indicated that mutation of F129A inhibits nectin-1 binding to gD and viral entry [8, 17, 27]. Our present results seen with F129A nectin-1 mouse strongly support the results provided by these *in vitro* experiments.

All the mice of F129A and 135 KO which died of 50 LD_{50} PRV infection died one day later than wild type mice. The surviving mice

of F129A and 135 KO with 10 or 50 LD_{50} PRV infection did not show any symptoms, including respiratory and neurological ones, and were healthy and active throughout the observation period. Previously, it has been shown that nectin-1 KO mice have attenuated pathogenesis of HSV-1 infection via the corneal scarification [13] and HSV-2 infection via the vaginal or intracranial route [15]. These findings support the present study that 135 KO mice showed a remarkable resistance against pathogenesis of PRV infection. The resistance may be due to the absence of the intact nectin-1 on the cell surface at the early stage of virus infection. Immunofluorescent staining revealed the expression of mutated nectin-1 with F129A on the respiratory epithelial cells and neurons in the CNS, but that of mutated nectin-1 with 135 KO was not observed. This expression pattern of mutated nectin-1 with F129A was identical to that of normal nectin-1 in control wild-type mice. These sites are primary virus entry site or target for the viral to amplify and spread. Taken together, we infer that virus binding to and entry into mutant nectin-1-expressing cells in the respiratory epithelium were almost completely inhibited at the early stage of virus infection in the surviving F129A.

Recently, Yang *et al*. generated mice with the F129A mutation in nectin-1 as in our study, infected them with PRV via the subcutaneous route, and showed that the mortality rate and viral loading was decreased in the mutant mice [40]. The results of our study essentially support their findings. However, in their study, a few percent of F129A mouse died and virus was detected in the brain tissues and serum, despite infection conditions that were not 100% lethal to wild-type control. On the other hand, in the present study, all F129A mice survived the infection experiment with a viral titer of 100% lethal to wild-type mice, and no viral DNA or antigen was detected, confirming stronger protection than their results. The fact that such stronger protection was observed when mice were infected by intranasal route like natural infection would lead to the above discussion, which viral binding and entry were blocked at the primary infection sites in F129A mice. Whereas 43% and 25% of the animals from F129A and 135 KO mice died respectively in the 50 LD_{50} infection experiment, there was no statistically significant difference between these lines. Considering that both lines are homozygous, we supposed that both were infected using more minor receptors other than nectin-1 such as HVEM.

Homozygous mice of F129A and 135 KO exhibit microphthalmia, produce fewer litters, and must be maintained in heterozygous breeding pairs. Knockout studies of nectin-1 and nectin-3 have demonstrated that the heterologous trans-interaction between nectin-1 and nectin-3 is essential for ciliary body morphogenesis [12]. Taken together, these findings support the hypothesis that the Phe129 of nectin-1 is key to the inhibition of eye development and spermatogenesis in the dominant-negative mutants, although the detailed mechanism remains to be determined.

Although F129A mice showed significant resistance to pathogenesis of PRV infection and no abnormal traits other than microphthalmia morphologically, and life span was not different from that of the wild type, they were not suitable for breeding maintenance in homozygous pairs, because of difficulty in reproduction. We have previously generated several transgenic mouse lines with the ultimate purpose of developing virus-resistant livestock, and we have shown that transgenic mouse lines expressing various types of soluble nectin-1 are highly resistant to PRV infection [21, 22, 24–26]. Compared to these lines, F129A mice showed equal or stronger resistance, indicating that this approach is enough to confer a remarkable prevention of the pathogenesis to mice. It is necessary to solve the problem of the side effects such as microphthalmia and difficulty in reproduction for application of this approach to the development of virus-resistant livestock.

CONFLICT OF INTEREST. The authors declare that there are no conflicts of interest.

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