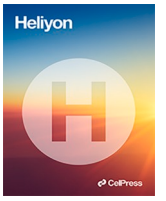
Heliyon 10 (2024) e27214

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Construction and characterization of an infectious cDNA clone of human rhinovirus A89

Hongwei Yang [1], Rui Zhu [1], Zhenhong Zhou, Hao Chen, Yuanyuan Wu, Dongqing Zhang, Che Liu, Ningshao Xia, Longfa Xu [**], Tong Cheng [*]

State Key Laboratory of Molecular Vaccinology and Molecular Diagnostics, National Institute of Diagnostics and Vaccine Development in Infectious Diseases, School of Life Sciences, School of Public Health, Xiamen University, Xiamen, 361102, PR China

ARTICLE INFO

Keywords:
Rhinovirus A89
Reverse genetics
Infectious cDNA clone
Virulence

ABSTRACT

Rhinoviruses (RVs) are major causes of the common cold and are related to severe respiratory tract diseases, leading to a considerable economic burden and impacts on public health. Available and stable viral resources of rhinoviruses for laboratory use are important for promoting studies on rhinoviruses and further vaccine or therapeutic drug development. Reverse genetic technology can be useful to produce rhinoviruses and will help to promote studies on their pathogenesis and virulence. In this study, rhinovirus A89, an RV-A species that has been found to be highly involved in hospitalization triggered by RV infections, was selected to construct an infectious clone based on its sequence as a representative. The viral mRNA produced by a T7 RNA transcript system was transfected into H1-HeLa cells, and the rescued RV-A89 viruses were harvested and confirmed by sequencing. The rescued RV-A89 induced a similar cytopathic effect (CPE) and shared almost identical growth kinetics curves with parental RV-A89. Moreover, 9A7, a pre-screened monoclonal antibody against the parental RV-A89, had a good and specific reaction with the rescued RV-A89, and further characterization showed almost the same morphology and protein composition of both viruses; thus, recombinant RV-A89 with similar biological characterization and virulence to the parental virus was obtained. In summary, the infectious clone of RV-A89 was successfully established, and the development of reverse genetic technology for rhinovirus will provide a framework for further studies on rhinoviruses.

1. Introduction

Human rhinoviruses (RVs) are the most common cause of upper respiratory tract infections (URTIs), generally known as the common cold [1]. However, an increasing number of studies in clinical laboratories have revealed that some low respiratory tract infections (LRTIs) [2], such as severe bronchiolitis [3], pneumonia [4], asthma development [5] and exacerbations of chronic pulmonary diseases [6], are strongly linked to RV infections, particularly in children, elderly patients and immunocompromised hosts [7]. In the COVID-19 pandemic, many anti-transmission measures against respiratory viruses were taken worldwide, and most respiratory viruses were halted except rhinoviruses [8–10], leading to considerable economic burdens and impacts on public health [11].

* Corresponding author.
** Corresponding author.
E-mail addresses: longfaxu@xmu.edu.cn (L. Xu), tcheng@xmu.edu.cn (T. Cheng).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.heliyon.2024.e27214
Received 21 July 2023; Received in revised form 5 February 2024; Accepted 26 February 2024
Available online 28 February 2024

Although RVs are prevalent and impactive [12], we still know little about their pathogenesis and virulence, and there is no vaccine or therapeutic drug available [13–15]; hence, in-depth studies on rhinoviruses are urgently needed.

Since RVs were first discovered in the 1950s, more than 160 serotypes of RVs have been identified and are classified into three species (RV-A, RV-B and RV-C) according to phylogenetic sequence criteria [16]. Recent studies have shown that infections of RV-A and RV-C are commonly detected during childhood, and are more likely related to severe respiratory illnesses [17,18]. In particular, the frequencies of RV-A in RV-positive detections are much higher than those of RV-C in older children [19], suggesting that RV-As are actively circulating and the vaccines against RV-As are desperately needed compared to RV-C [20]. Certain strains of RV-A commonly detected in several studies, such as RV-A89, RV-A12, and RV-A78 [21–23], especially the RV-A89 strain, was found to have an increased specific IgG1 that could be associated with longer respiratory symptoms and hospitalization [24], and the RV-A89 strain is one of the prevalent viruses among all RV detections and RV-associated hospitalization [25]. A study of the epitope of RV-A89 showed that the virus-neutralizing epitopes determined for RV-A89 correspond to other species of rhinovirus, suggesting that RV-A89 has the potential to be a candidate for further vaccine development [26]. However, there are still many difficulties in studies on RVs, and approaches to obtain available and stable viral resources for laboratory use are needed. The traditional approach is to isolate viruses from infections [27], but it is difficult to avoid coinfecting pathogens, and further identification is always needed, which strongly hinders studies on RVs.

Reverse genetics has been thought to be a conventional and useful technology for studying the pathogenesis and virulence of viruses [28,29], and can be a crucial method to help to promote the study of RVs. Recently, complementary DNA (cDNA) clones of many other types of rhinoviruses have been developed and validated, such as RV-C15 [30], and RV-A16 [31,32], while many others are not yet available. Here, we constructed an infectious clone of the species RV-A89 based on its sequence via a T7 RNA transcription system, and later characterization showed that the virulence of the rescued virus was comparable to that of the RV-A89 parental strain from the American Type Culture Collection (ATCC).

2. Materials and methods

2.1. Cells, viruses and antibodies

H1-HeLa (ATCC# CRL-1958) cells were maintained in Dulbecco's modified Eagle's medium (DMEM; BasalMedia) with 10% fetal bovine serum (FBS; Gibco), 100 U/mL penicillin, and 100 μg/mL streptomycin. The parental RV-A89 strain 41,467 Gallo (ATCC# VR-1199) was purchased from ATCC, propagated and passaged on H1-HeLa cells at 33 °C, and the supernatants were harvested by freeze-thaw and stored in aliquots at −80 °C. The viral titer was determined by microtitration using H1-HeLa cells and is expressed as the 50% tissue culture infectious dose ($TCID_{50}$), which was calculated by the Reed-Muench method.

Before this study, 9A7, a mouse monoclonal antibody (mAb) against parental RV-A89, was screened from BALB/c mice immunized intraperitoneally with parental RV-A89 using standard hybridoma technology [33]. The secondary antibody goat-*anti*-mouse IgG conjugated to Alexa Fluor® 488 (GAM-488) was purchased from Invitrogen.

2.2. Construction of the full-length cDNA clone of RV-A89

Two segments (1–3234 bp and 3235–7152 bp) covering the whole genome sequence of RV-A89(GenBank No. M16248) were synthesized by Sangon Biotech (Shanghai, China). The two segments with 15 bp overlapping ends were seamlessly linked together and ligated into the pSVA vector by Gibson Assembly Master Mix (NEB, Ipswich, MA, USA). After transformation into DH5α competent cells (TianGen, Beijing, China), the cells were selected on nutrient agar plates with ampicillin. The resultant pSVA-RV-A89 clone was sequenced by Sangon Biotech (Shanghai, China) and purified by a QIAGEN Plasmid Midi Kit (Qiagen, Beijing, China).

2.3. RNA transfection and viral rescue

The pSVA-RV-A89 plasmid was linearized by *Not* I and *Sal* I (NEB, Ipswich, MA, USA), and the target DNA template was purified by using a TIANGEN DP214 Kit (TianGen, Beijing, China) and then transcribed into mRNA by using a MEGAscript T7 transcription kit (Invitrogen, USA). The mixture containing the RV-A89 mRNA and the TransIT-mRNA Transfection Kit (Mirus, USA) reagents were added into H1-HeLa monolayers and incubated for 48 h at 33 °C. When typical CPE emerged, the viral supernatants were collected after 3 freeze-thaw cycles and passaged on H1-HeLa cells consecutively. The rescued RV-A89 was obtained utill stable 100% CPE was observed in H1-HeLa cells.

2.4. Viral infection

For culture and production of the rescued virus, H1-HeLa cells were seeded in 6-well plates or 10 cm dishes. When the cells reached 70% confluence, the culture medium was replaced by DMEM without FBS, and then, the cells were infected at a multiplicity of infection (MOI) of 1. For analysis of the growth kinetics curves, the rescued virus and parental virus were inoculated in 12-well plates and infected H1-HeLa cells at 0.25 MOI for 4 days. The culture medium supernatant was collected every 12 h after inoculation, and each point had 3 repeats. The virus titer ($TCID_{50}$) at each point was determined by the Reed-Muench method.

2.5. Immunofluorescence assay

H1-HeLa cells were infected with RV-A89 at 0.1 MOI. After 24 h of infection, cells were fixed with 4% paraformaldehyde for 30 min in the dark and then treated with 0.3% Triton X-100 in PBS for 15 min, followed by 2% bovine serum albumin (BSA) blocking at 37 °C for 30 min. For analysis of the specificity of the immunogenicity of the rescued RV-A89, cells were incubated with 9A7 (1 mg/mL) at a dilution of 1:300 in PBS at 37 °C for 1 h. Following three washes with PBST (0.05% Tween 20 in PBS), cells were then incubated with Alexa Fluor® 488 goat anti-mouse IgG at a dilution of 1:1000 in PBS at 37 °C for 30 min. After three washes with PBST, the cells were stained with 4′, 6-diamidino-2-phenylindole (DAPI, Invitrogen, USA) for 5 min. Images were captured using a Leica TCS SP8 X microsystem (Leica, German).

2.6. Virus purification and identification

After infection, 1.6 L of viral supernatant was produced, and the virus was centrifuged at 6000×*g* for 30 min to remove cell debris and precipitated using 8% (w/v) polyethylene glycol (PEG) 8000 and 0.5 M NaCl in PBS at 4 °C overnight. After centrifugation, the virus was resuspended in PBS and then sedimented through a linear 15%–45% (w/v) sucrose density gradient at 120,000×*g* for 2.5 h in a SW41 Ti rotor (Beckman Coulter Inc.,Germany) at 4 °C. The fractions of virions were independently collected and dialyzed in PBS and then concentrated by an Ultra-4 centrifugal concentrator (100 kDa, Millipore). The concentration of virions was measured by the BCA Protein Assay Kit (Thermo Fisher, USA). The protein composition was analyzed by SDS−PAGE. The quantity and quality of RV-A89 particles were assessed by negative-staining transmission electron microscopy.

2.7. Statistical analyses

Significance of differences of the growth kinetics was evaluated using Student's *t*-test, each point of both viral titers showed no significant difference ($P > 0.05$). Statistical analysis was performed using GraphPad Prism 8.0.

3. Results

3.1. Construction and development of an infectious clone of RV-A89

For the infectious clone of RV-A89, segments were designed based on the sequence of RV-A89. The two segments covering the full length of the RV-A89 genome were synthesized with a T7 promoter upstream of the 5′-UTR and a poly(A) tail at the end of the 3′-UTR respectively. Then, both the segments and the pSVA vector were seamlessly assembled and linked together to form the cDNA clone of RV-A89 (Fig. 1A). After *Not* I and *Sal* I cleavage, complementary DNA was generated as a template (Fig. 1B) and then transcribed into mRNA. Following transfection of the viral mRNA into H1-HeLa cells, mild and partial cytopathic effects were observed at 48 h post-transfection, indicating successful rescue of infective virus. Finally, we collected the next generation of the infective rescued virus for genome sequencing, and the result of alignment confirmed that the rescued virus was RV-A89 (data not shown). Thus, we obtained an infective rescued RV-A89 virus.

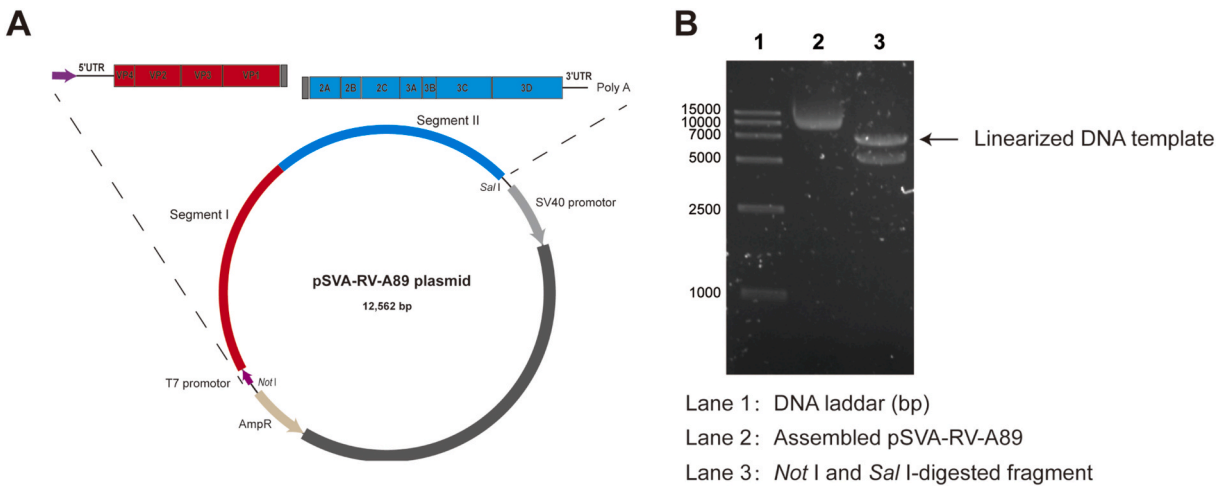


Fig. 1. Construction of cDNA clone of RV-A89. (A) Schematic diagram of the infectious clone containing the full-length cDNA of RV-A89. Segment I consisted of the *Not*I cleavage site and T7 promoter upstream of the 5′-UTR of the viral genome and the sequence coding viral structure proteins; Segment II consisted of the poly(A) tail and the *Sal*I cleavage site downstream of the sequencing coding viral nonstructure proteins and the 3′-UTR of the viral genome. (B) The linearized plasmid provided the DNA template for later transcription, and the target band (arrow) was analyzed and purified by electrophoresis on agarose gel.

3.2. Characterization of the rescued RV-A89

After several passages on H1-HeLa cells, the rescued RV-A89 was propagated and enriched, showing a similar CPE (Fig. 2A) at 36 h post-infection with parental RV-A89 on H1-HeLa cells at the same MOI of 1, and the progeny virus of the rescued RV-A89 (1.78×10^6 $TCID_{50}$/mL) in the supernatant was detected at a similar titer to that of parental RV-A89 (3.80×10^6 $TCID_{50}$/mL). For analysis of the specificity of the composition of viral protein expressed during infection, immunofluorescence was performed using mAb 9A7 to detect the rescued RV-A89. At 24 h post-infection in H1-HeLa cells at an MOI of 0.1, as shown in Fig. 2B, obvious signals around the shrunken nuclei indicated that mAb 9A7 reacted well with the rescued RV-A89 in infected cells. The similar reactivity of 9A7 to both the rescued

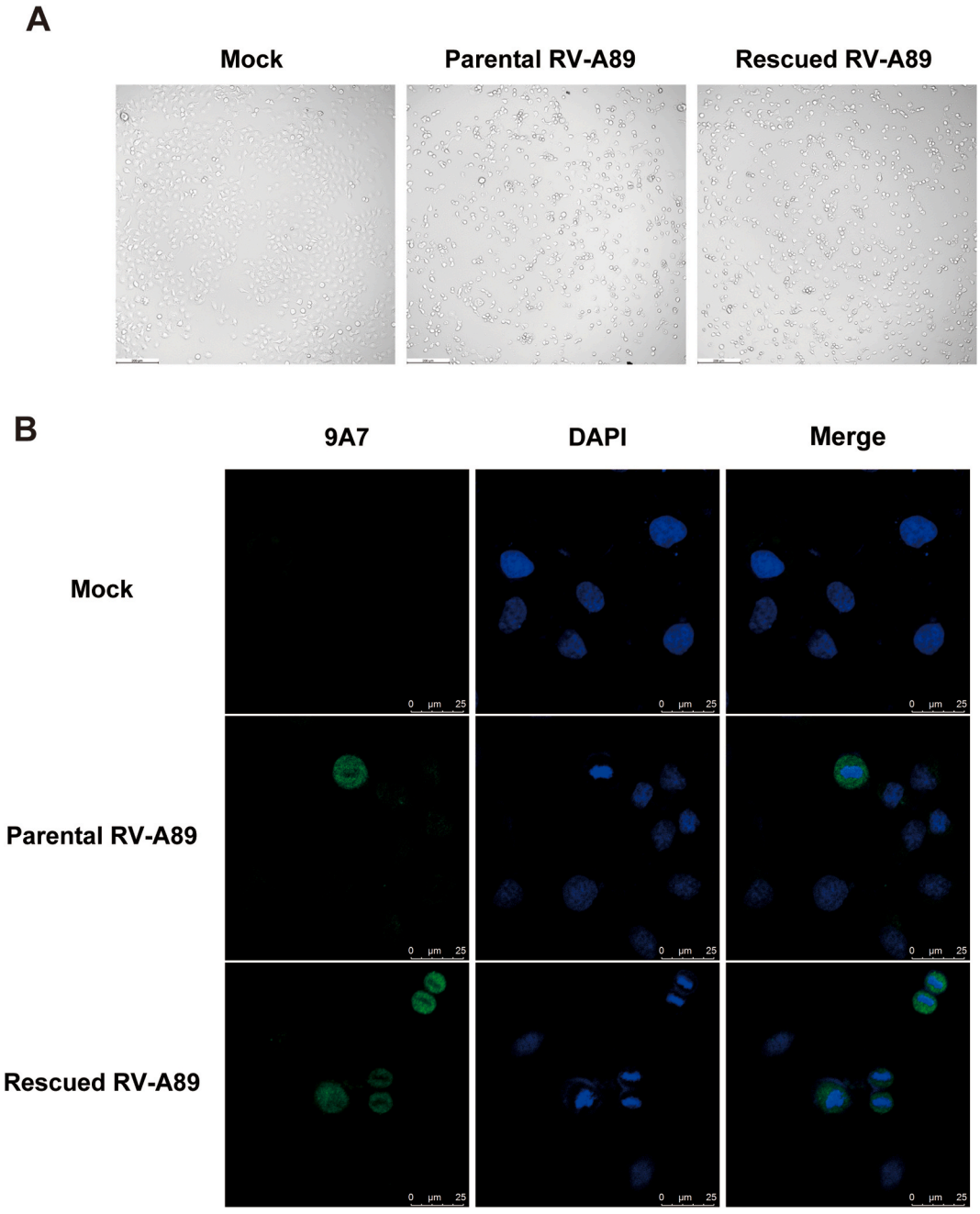


Fig. 2. Characterization of the rescued RV-A89. (A) The cytopathic effect (CPE) of the parental RV-A89 and rescued RV-A89 was detected 36 h post-infection at 1 MOI. Scale bar = 200 μm. (B) Reactivity of 9A7 (monoclonal antibody against the parental RV-A89) to the rescued RV-A89. H1-HeLa cells infected with the rescued RV-A89 were fixed and reacted with 9A7, followed by Alexa Fluor 488 conjugated secondary antibody (green), and the nuclei were stained with DAPI (blue). Scale bar = 25 μm.

RV-A89 and the parental RV-A89 suggested that the same composition of virus was expressed and assembled properly during infection and that the recombinant RV-A89 was successfully rescued.

For further identification of viral replication, the growth kinetics of the rescued and parental viruses were determined. H1-HeLa cells were infected with both viruses at the same MOI of 0.25, and the viral titers of supernatants were monitored every 12 h for 4 days. We observed the same progress of CPE at each point, and the growth kinetics curves of both viruses were almost identical. They shared a similar time-dependent tendency of viral titers, peaking at 72 h post infection and then slowly decreasing until the end of the experiment, indicating that the infection progression of rescued RV-A89 was similar to that of parental RV-A89 (Fig. 3). Herein, the rescued RV-A89 showed similar biological characterization and virulence to the parental strain.

3.3. Purification and verification of the rescued RV-A89

For further characterization of the rescued RV-A89, 1.6 L of viral propagation supernatant of both the rescued RV-A89 and the parental RV-A89 was harvested and purified for TEM analysis. As shown in Fig. 4A, the full particles of both virions were nearly spherical in shape with around 30 nm in diameter, and the rescued RV-A89 showed a similar morphological characterization of viral particles compared to that of parental RV-A89. For analysis of the protein composition of the virus, 1.5 μg of purified virion was used for SDS−PAGE, and both the rescued and parental RV-A89 showed 4 major proteins at the same position on the gel (Fig. 4B). According to the mass of protein stripes, we can distinguish 1 precursor protein (VP0, formed by VP2 and VP4) and 3 viral capsid proteins (VP1, VP2, VP3) from top to bottom, which means that mature virions were produced during the infection of the rescued RV-A89.

4. Discussion

In this study, we provided a standard process of reverse genetics to construct and characterize the infectious clone of RV-A89. Once the complete genome sequence is known, we can produce a certain recombinant virus quickly and easily. These data can help promote more studies on prevalent strains such as RV-A89 detected in the clinic, and provide a framework to compare the virulence and pathogenicity between different serotypes or strains, and notably, the development of reverse genetic tools for rhinoviruses will facilitate further studies on broad-spectrum vaccines or antiviral therapy against rhinoviruses.

The traditional approach to obtain viral resources of rhinoviruses for experiments is to isolate virus from individuals infected with RVs [34]. However, this approach is limited by the detection and identification of RV infections in the clinic. Compared to the traditional approach, there are two main advantages of reverse genetics. First, the window of rhinovirus infection is no more than 7 days, and the onset of symptoms is usually mild and neglected [1], therefore specimens containing high quality and high titer virus are hard to collect. Second, RV coinfection is commonly detected in many epidemiological studies of respiratory viruses [35,36]; thus, specimens such as nasal sections need to be identified to ensure that no other pathogens are contaminated. Reverse genetic tools can simplify the progress to produce a viable virus, ensure that the rescued virus is not contaminated with extraneous pathogens, and help to improve the quality and titer of the virus for experimental use.

Rhinoviruses are a group of nonenveloped viruses with a single-stranded, positive-sense RNA of approximately 7.2 kb, belonging to the genus *Enterovirus* of the family *Picornaviridae* [37]. The RNA genome is not stable and easily accumulates mutations or adaptations during replication, so it is difficult to ensure a high sequence identity of progeny virus for laboratory studies when using the viral resources isolated from nasal sections or from ATCC. The reverse genetics of rhinovirus was originally based on the complementary DNA sequence of the viral genome, and plasmids containing the cDNA sequence and elements for transcription are quite stable and

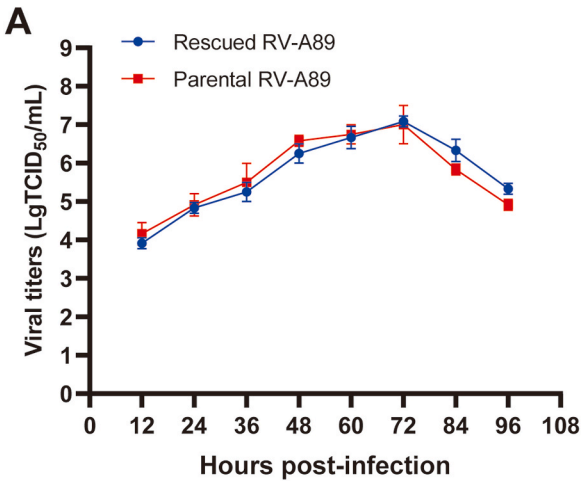


Fig. 3. One-step growth curves of the parental and rescued RV-A89 in H1-HeLa cells. H1-HeLa cells were seeded in 12-well plates and infected with RV-A89 at 0.25 MOI. The viral titer was detected at 12 h intervals for 4 days and each point is shown as $TCID_{50}$. At each time point, titer values are means of the three samples. Error bars represent the standard deviation. *P* value of each point is more than 0.05 ($P > 0.05$).

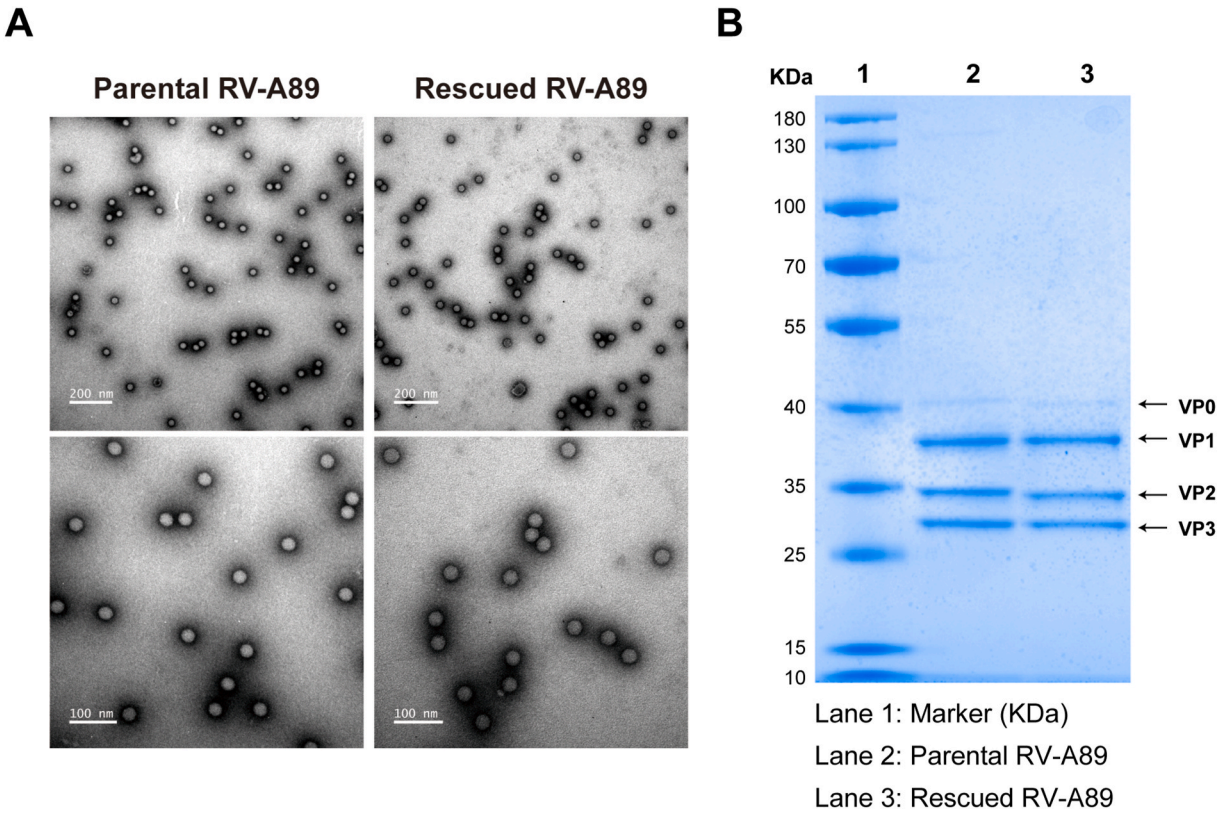


Fig. 4. Purification and verification of the rescued RV-A89. (A) Morphological characterization of the particles of parental and rescued RV-A89. Scale bar (top:200 nm, bottom: 100 nm). (B) The protein composition of particles analyzed by SDS–PAGE. The structural proteins of the parental and rescued RV-A89 were measured according to the masses of bands (VP0: 41 kDa; VP1: 38 kDa; VP2: 34 kDa; VP3: 29 kDa).

reliable for virus production. This issue is an important feature for pathogenesis and virulence studies of viruses, especially for rhinoviruses with such abundant serotypes and epidemic strains. With reverse genetic tools, the differences in pathogenesis and virulence between wild strains and variation strains could be accurately characterized, helping to identify the key factors that influence viral pathogenesis and virulence.

Reverse genetics has been applied to many other viruses belonging to the family *Picornaviridae*, such as EVA71 [38], CVA6 [39], CVA10 [40], and CVB5 [41]. These tools not only provide an available approach to obtain the viral resources of rhinoviruses, but can promote more studies on prevalent and impactive strains. In addition, the newly established infectious clone can be further modified to create reporter viruses, such as luciferase, which can be useful in high-throughput screening of effectors of rhinoviruses or establishing animal models. This clone can also be attenuated or modified as a candidate for vaccine research.

In summary, we successfully established an infectious clone of RV-A89, and the encoded recombinant virus showed similar biological characterization and virulence to the parental strain in vitro. Our results provide a framework for further studies of rhinoviruses on their virulence and pathogenicity, and could be useful for the development of vaccines and therapeutic drugs against rhinoviruses.

Data availability statement

The genome sequence data are available at NCBI (Nucleotide accession is M16248, https://www.ncbi.nlm.nih.gov/nuccore/M16248). The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Additional information

No additional information is available for this paper.

CRediT authorship contribution statement

Hongwei Yang: Writing – review & editing, Writing – original draft, Validation, Methodology, Formal analysis, Data curation, Conceptualization. **Rui Zhu:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Conceptualization.

Zhenhong Zhou: Methodology, Data curation. **Hao Chen:** Data curation. **Yuanyuan Wu:** Data curation. **Dongqing Zhang:** Data curation. **Che Liu:** Data curation. **Ningshao Xia:** Supervision, Resources, Project administration. **Longfa Xu:** Validation, Project administration, Methodology, Conceptualization. **Tong Cheng:** Writing – review & editing, Writing – original draft, Visualization, Validation, Resources, Project administration, Methodology, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

This study was supported by grants from the National Natural Science Foundation of China (82172248 and 82272310). The funders had no role in the study design, data collection and analysis, decision to publish, or manuscript preparation.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e27214.

References

[1] S.E. Jacobs, D.M. Lamson, K. St George, et al., Human rhinoviruses, Clin. Microbiol. Rev. 26 (2013) 135–162, https://doi.org/10.1128/CMR.00077-12.
[2] S.B. Drysdale, A. Mejias, O. Ramilo, Rhinovirus - not just the common cold, J. Infect. 74 (Suppl 1) (2017) S41–S46, https://doi.org/10.1016/S0163-4453(17)30190-1.
[3] K. Hasegawa, T. Jartti, Y.A. Bochkov, et al., Rhinovirus species in children with severe bronchiolitis: multicenter cohort studies in the United States and Finland, Pediatr. Infect. Dis. J. 38 (2019) e59–e62, https://doi.org/10.1097/INF.0000000000002141.
[4] S. Jain, D.J. Williams, S.R. Arnold, et al., Community-acquired pneumonia requiring hospitalization among u.S. Children, N. Engl. J. Med. 372 (2015) 835–845, https://doi.org/10.1056/NEJMoa1405870.
[5] F.J. Rubner, D.J. Jackson, M.D. Evans, et al., Early life rhinovirus wheezing, allergic sensitization, and asthma risk at adolescence, J. Allergy Clin. Immunol. 139 (2017) 501–507, https://doi.org/10.1016/j.jaci.2016.03.049.
[6] J. Cafferkey, J.A. Coultas, P. Mallia, Human rhinovirus infection and copd: role in exacerbations and potential for therapeutic targets, Expet Rev. Respir. Med. 14 (2020) 777–789, https://doi.org/10.1080/17476348.2020.1764354.
[7] W.J. Chen, J.C. Arnold, M.P. Fairchok, et al., Epidemiologic, clinical, and virologic characteristics of human rhinovirus infection among otherwise healthy children and adults rhinovirus among adults and children, J. Clin. Virol. 64 (2015) 74–82, https://doi.org/10.1016/j.jcv.2015.01.007.
[8] R. Jia, L. Lu, S. Li, et al., Human rhinoviruses prevailed among children in the setting of wearing face masks in shanghai, 2020, BMC Infect. Dis. 22 (2022) 253, https://doi.org/10.1186/s12879-022-07225-5.
[9] E. Takashita, C. Kawakami, T. Momoki, et al., Increased risk of rhinovirus infection in children during the coronavirus disease-19 pandemic, Influenza Other. Respir. Viruses 15 (2021) 488–494, https://doi.org/10.1111/irv.12854.
[10] J.M. Mansuy, M. Bourcier, P. Tremeaux, et al., Covid-19 pandemic period, where are the seasonal viruses? J. Med. Virol. 93 (2021) 4097–4098, https://doi.org/10.1002/jmv.26959.
[11] D.A. Rankin, A.J. Spieker, A. Perez, et al., Circulation of rhinoviruses and/or enteroviruses in pediatric patients with acute respiratory illness before and during the covid-19 pandemic in the us, JAMA Netw. Open 6 (2023) e2254909, https://doi.org/10.1001/jamanetworkopen.2022.54909.
[12] H. Ortega, D. Nickle, L. Carter, Rhinovirus and asthma: challenges and opportunities, Rev. Med. Virol. 31 (2021) e2193, https://doi.org/10.1002/rmv.2193.
[13] G.R. Williams, I. Kubajewska, N. Glanville, et al., The potential for a protective vaccine for rhinovirus infections, Expert Rev. Vaccines 15 (2016) 569–571, https://doi.org/10.1586/14760584.2016.1142375.
[14] N. Glanville, S.L. Johnston, Challenges in developing a cross-serotype rhinovirus vaccine, Curr. Opin. Virol. 11 (2015) 83–88, https://doi.org/10.1016/j.coviro.2015.03.004.
[15] A. Grunewaldt, C. Hugel, G.G.U. Rohde, [rhinoviruses], Internist 60 (2019) 1151–1154, https://doi.org/10.1007/s00108-019-00672-4.
[16] A.C. Palmenberg, J.E. Gern, Classification and evolution of human rhinoviruses, Methods Mol. Biol. 1221 (2015) 1–10, https://doi.org/10.1007/978-1-4939-1571-2_1.
[17] K.T. Ng, X.Y. Oong, S.H. Lim, et al., Viral load and sequence analysis reveal the symptom severity, diversity, and transmission clusters of rhinovirus infections, Clin. Infect. Dis. 67 (2018) 261–268, https://doi.org/10.1093/cid/ciy063.
[18] S.Y. Zheng, L.L. Wang, L. Ren, et al., Epidemiological analysis and follow-up of human rhinovirus infection in children with asthma exacerbation, J. Med. Virol. 90 (2018) 219–228, https://doi.org/10.1002/jmv.24850.
[19] T. Choi, M. Devries, L.B. Bacharier, et al., Enhanced neutralizing antibody responses to rhinovirus c and age-dependent patterns of infection, Am. J. Respir. Crit. Care Med. 203 (2021) 822–830, https://doi.org/10.1164/rccm.202010-3753OC.
[20] S.L. Johnston, A, b, and c rhinoviruses: new knowledge from an impressive consortium. A step forward for rhinovirus vaccine efforts or a step back? Am. J. Respir. Crit. Care Med. 203 (2021) 786–788, https://doi.org/10.1164/rccm.202102-0346ED.
[21] D.N. Marcone, A. Culasso, G. Carballal, et al., Genetic diversity and clinical impact of human rhinoviruses in hospitalized and outpatient children with acute respiratory infection, Argentina, J. Clin. Virol. 61 (2014) 558–564, https://doi.org/10.1016/j.jcv.2014.10.006.
[22] L. van der Linden, A.H. Bruning, X.V. Thomas, et al., A molecular epidemiological perspective of rhinovirus types circulating in amsterdam from 2007 to 2012, Clin. Microbiol. Infect. 22 (2016), https://doi.org/10.1016/j.cmi.2016.08.007, 1002 e1009–1002 e1014.
[23] Y.J. Zhao, J. Shen, B.J. Wu, et al., Genotypic diversity and epidemiology of human rhinovirus among children with severe acute respiratory tract infection in shanghai, 2013-2015, Front. Microbiol. 9 (2018), https://doi.org/10.3389/fmicb.2018.01836.
[24] K. Stenberg-Hammar, K. Niespodziana, C. Soderhall, et al., Rhinovirus-specific antibody responses in preschool children with acute wheeze reflect severity of respiratory symptoms, Allergy 71 (2016) 1728–1735, https://doi.org/10.1111/all.12991.
[25] C. Esneau, A.C. Duff, N.W. Bartlett, Understanding rhinovirus circulation and impact on illness, Viruses 14 (2022), https://doi.org/10.3390/v14010141.
[26] K. Niespodziana, C.R. Cabauatan, P. Pazderova, et al., Identification of epitopes on rhinovirus 89 capsid proteins capable of inducing neutralizing antibodies, Int. J. Mol. Sci. 23 (2022), https://doi.org/10.3390/ijms23095113.
[27] D. Proud, Reverse genetics and rhinovirus-a new approach to an old problem? J. Infect. Dis. 220 (2019) 181–183, https://doi.org/10.1093/infdis/jiy630.

[28] W. Wang, X. Peng, Y. Jin, et al., Reverse genetics systems for sars-cov-2, J. Med. Virol. 94 (2022) 3017–3031, https://doi.org/10.1002/jmv.27738.
[29] G. Neumann, Influenza reverse genetics-historical perspective, Cold Spring Harb. Perspect. Med. 11 (2021), https://doi.org/10.1101/cshperspect.a038547.
[30] Y.A. Bochkov, J.E. Gern, Clinical and molecular features of human rhinovirus c, Microb. Infect. 14 (2012) 485–494, https://doi.org/10.1016/j.micinf.2011.12.011.
[31] J.E. Gern, W.M. Lee, C.A. Swenson, et al., Development of a rhinovirus inoculum using a reverse genetics approach, J. Infect. Dis. 220 (2019) 187–194, https://doi.org/10.1093/infdis/jiy629.
[32] W.M. Lee, W. Wang, Y.A. Bochkov, et al., Reverse genetics system for studying human rhinovirus infections, Methods Mol. Biol. 1221 (2015) 149–170, https://doi.org/10.1007/978-1-4939-1571-2_12.
[33] E. Vareckova, T. Betakova, V. Mucha, et al., Preparation of monoclonal antibodies for the diagnosis of influenza a infection using different immunization protocols, J. Immunol. Methods 180 (1995) 107–116, https://doi.org/10.1016/0022-1759(94)00307-i.
[34] C. Savolainen-Kopra, M.L. Simonen-Tikka, P. Klemola, et al., Human rhinoviruses in indis-study material-evidence for recovery of viable rhinovirus from fecal specimens, J. Med. Virol. 85 (2013) 1466–1472, https://doi.org/10.1002/jmv.23593.
[35] Z.J. Li, H.Y. Zhang, L.L. Ren, et al., Etiological and epidemiological features of acute respiratory infections in China, Nat. Commun. 12 (2021) 5026, https://doi.org/10.1038/s41467-021-25120-6.
[36] J. Leotte, H. Trombetta, H.Z. Faggion, et al., Impact and seasonality of human rhinovirus infection in hospitalized patients for two consecutive years, J. Pediatr. 93 (2017) 294–300, https://doi.org/10.1016/j.jped.2016.07.004.
[37] A.C. Palmenberg, J.A. Rathe, S.B. Liggett, Analysis of the complete genome sequences of human rhinovirus, J. Allergy Clin. Immunol. 125 (2010) 1190–1199, https://doi.org/10.1016/j.jaci.2010.04.010, quiz 1200-1191.
[38] Y.W. Ma, S.B. Hao, L.L. Sun, et al., Construction and characterization of infectious cdna clones of enterovirus 71 (ev71), Virol. Sin. 30 (2015) 305–308, https://doi.org/10.1007/s12250-015-3614-3.
[39] L.S. Yang, S.X. Li, Y.J. Liu, et al., Construction and characterization of an infectious clone of coxsackievirus a6 that showed high virulence in neonatal mice, Virus Res. 210 (2015) 165–168, https://doi.org/10.1016/j.virusres.2015.08.002.
[40] M. Wang, J. Yan, L. Zhu, et al., The establishment of infectious clone and single round infectious particles for coxsackievirus a10, Virol. Sin. 35 (2020) 426–435, https://doi.org/10.1007/s12250-020-00198-2.
[41] L. Song, B. Cui, J. Yang, et al., Construction and verification of an infectious cdna clone of coxsackievirus b5, Virol. Sin. 37 (2022) 469–471, https://doi.org/10.1016/j.virs.2022.03.005.