Supplementary Material

Peptide-based pan-CoV fusion inhibitors maintain high potency against SARS-CoV-2 Omicron variant

Shuai Xia[1]*, Jasper Fuk-Woo Chan[2,3,4]*, Lijue Wang[1]*, Fanke Jiao[1], Kenn Ka-Heng Chik[4], Hin Chu[2,3,4], Qiaoshuai Lan[1], Wei Xu[1], Qian Wang[1], Chao Wang[5], Kwok-Yung Yuen[2,3,4†], Lu Lu[1†], Shibo Jiang[1†]

Affiliations:

[1]Key Laboratory of Medical Molecular Virology (MOE/NHC/CAMS), School of Basic Medical Sciences, Shanghai Frontiers Science Center of Pathogenic Microbes and Infection, Shanghai Institute of Infectious Disease and Biosecurity, Fudan University, Shanghai, China.

[2]State Key Laboratory of Emerging Infectious Diseases, Carol Yu Centre for Infection, Department of Microbiology, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Pokfulam, Hong Kong Special Administrative Region, China.

[3]Department of Clinical Microbiology and Infection Control, The University of Hong Kong-Shenzhen Hospital, Shenzhen, Guangdong, China.

[4]Centre for Virology, Vaccinology and Therapeutics, Hong Kong Science and Technology Park, Hong Kong Special Administrative Region, China.

[5]State Key Laboratory of Toxicology and Medical Countermeasures, Beijing Institute of Pharmacology and Toxicology, Beijing, China.

*These authors contributed equally to this work.

†Corresponding author. Email: shibojiang@fudan.edu.cn (S.J.); lul@fudan.edu.cn (L.L.); kyyuen@hku.hk (K.-Y.Y.)

Methods:

Cells and plasmids

The 293T cells were purchased from ATCC (USA); the Calu-3 cell line and the Caco2 cell line were obtained from the Chinese Academy of Science Cell Bank (China); 293T/ACE2 and VeroE6-TMPRSS2 cells were maintained in our laboratories. All cell lines were cultured in Dulbecco's Modified Eagle's Medium (DMEM) with 10% fetal bovine serum (FBS). Plasmids were synthesized or stored in our laboratories, including pAAV-SARS-CoV-2-S (Omicron/Delta/D614G)-IRES-EGFP, pcDNA-3.1-SARS-CoV-2-S (Omicron/Delta/D614G), pNL4-3.Luc.R-E and pAAV-IRES-EGFP.

S protein-mediated cell-cell fusion and inhibition assays

Effector cells (293T/S/EGFP) co-expressed S protein and EGFP through transfecting plasmid pAAV-IRES-S-EGFP. Calu-3 or Caco2 cells naturally express human ACE2 receptor on their surface or 293T/ACE2 cells were used as target cells, while 293T cells transfected with plasmid pAAV-IRES-EGFP (293T/EGFP) were used as negative control. Effector cells (293T/S/EGFP) were collected and added to target cells for co-incubation for 24 hrs at 37 °C, and then pictures were taken under the fluorescence microscope. The inhibitory activity of peptides tested on S-mediated cell-cell fusion was determined as previously described[1]. Briefly, effector cells (293 T/S/EGFP) and target cells were cocultured in the presence or absence of a test peptide. The percentage of cell-cell fusion was recorded, and the inhibition percentage of cell-cell fusion was calculated using the following formula as described elsewhere[2]: $[1 - (E - N)/(P - N)] \times 100\%$, where "E" represents the percentage of cell-cell fusion in the experimental group, "P" represents the percentage of cell-cell fusion in the positive control group, and "N" represents the percentage of cell-cell fusion in the negative control group. The IC_{50} was calculated using GraphPad Prism 8.0.

Pseudovirus (PsV) infection and inhibition assays

PsVs were produced by co-transfecting plasmids carrying D614G/Delta/Omicron mutant SARS-CoV-2 S protein with pNL4-3.Luc.R−E−. Then pseudovirus supernatant was collected 60 hrs post-transfection. The inhibitory activity of peptides against SARS-CoV-2 PsV was tested on Caco2 cells using a modified standard neutralization assay[3]. Briefly, a serially four-fold diluted peptide (60 μl) was incubated with PsV (50 μl) for ~30 min at 37℃. Then the mixture was transferred into Caco2 cells in a 96-well plate. After 12 hrs, culture medium was refreshed. After an additional 48 hrs, luciferase activity was tested by the Luciferase Assay System (Promega, Madison, WI, USA).

Authentic SARS-CoV-2 Omicron variant inhibition assay

Omicron variant (hCoV-19/Hong Kong/HKU-344/2021; GISAID accession number EPI_ISL_7357684) was isolated from patients in Hong Kong as previously described[4]. To measure the inhibitory activity of peptides tested on authentic SARS-CoV-2 infection, Vero-E6-TMPRSS2 cells were first seeded in a 96 well-plate in cell culture medium (DMEM + 10% FBS + 1% penicillin/streptomycin) overnight at 37 °C under 5% CO_2 to establish a monolayer. The following day, peptide inhibitors were diluted into indicated concentrations, in wells of a 96 well-plate in triplicate in DMEM + 2% FBS and then incubated with 0.01 MOI of SARS-CoV-2 Omicron, at 37 °C for 1 h. Afterwards, the mixture was overlaid onto cells and further incubated at 37 °C under 5% CO_2 for approximately 48 h. Cytopathic effect (CPE) wase then visually assessed in all wells and scored as either negative (100% inhibition) or positive (0% inhibition) for CPE induced by viral replication in a blinded manner as previously described[4].

Conformational analysis of S protein

The structures of SARS-CoV-2 spikes in pre-fusion state (PDB entry: 6XR8) and post-fusion state (PDB entry: 6XRA) were visualized and analyzed using UCSF ChimeraX[5].

The complex structure of SARS-CoV-2 spike HR1 domain and EK1 peptide (PDB entry: 7C53)) were visualized and analyzed using Pymol[6].

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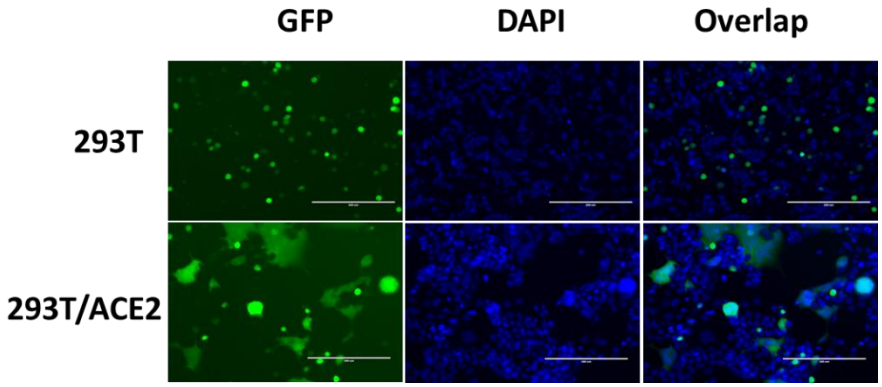
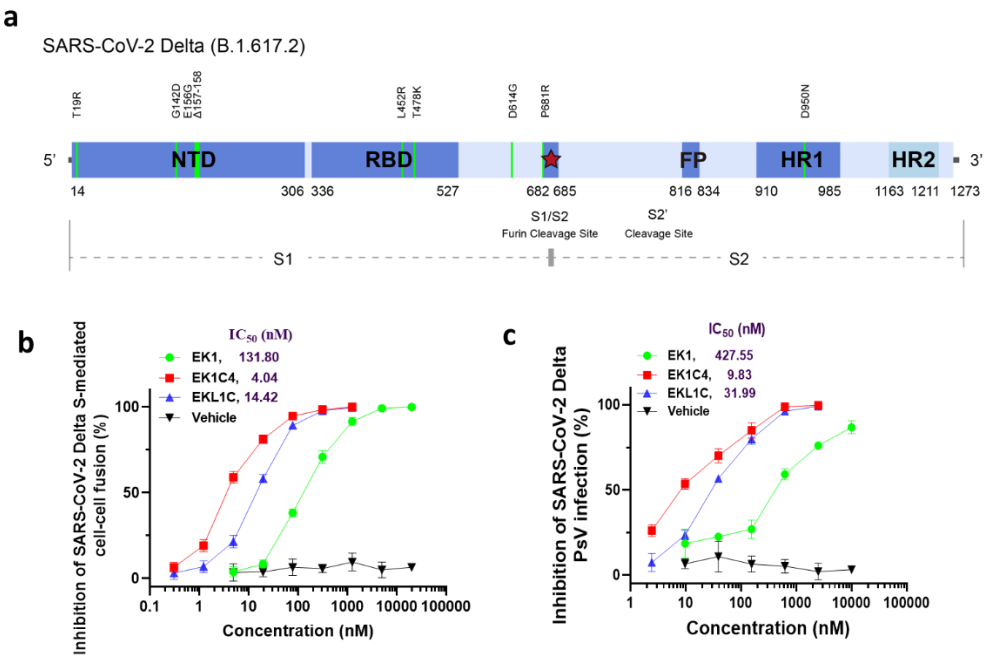
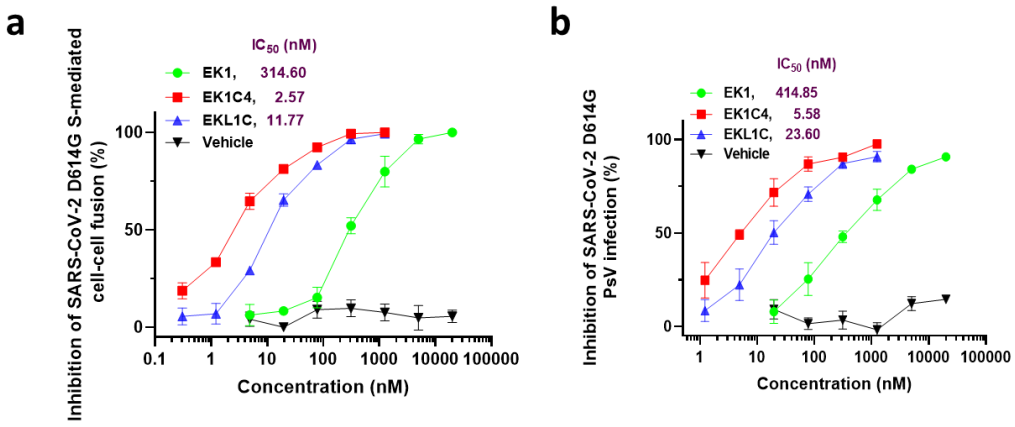


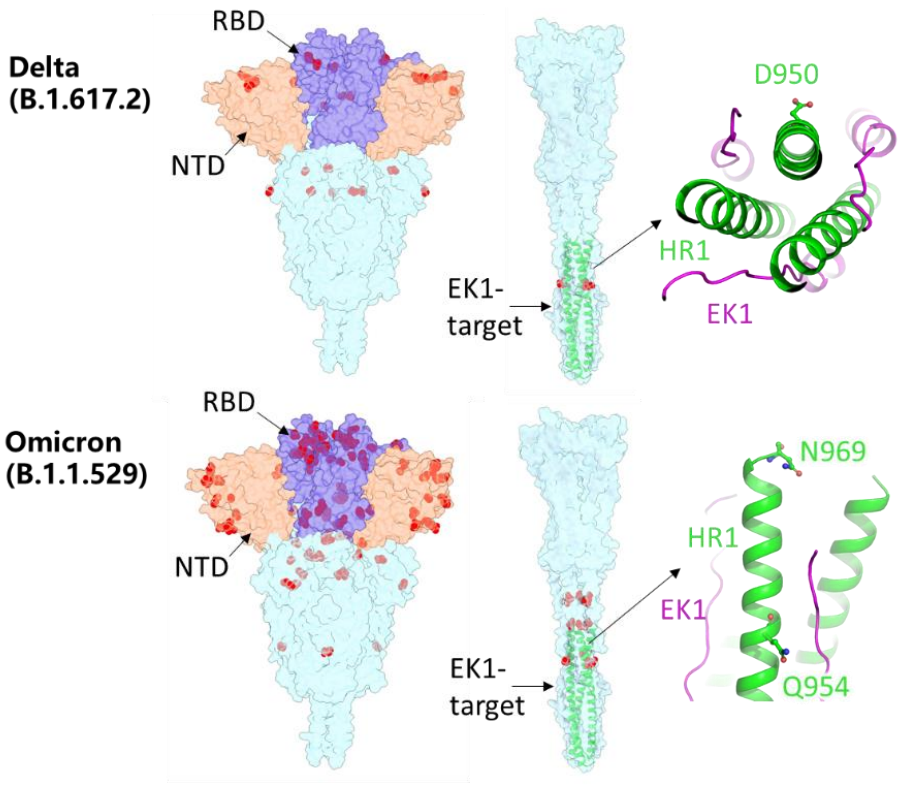
Supplementary Fig. S1. Representative images of cell–cell fusion between 293T/SARS-CoV-2(Omicron)/EGFP, effector cells and target cells (293T or 293T/ACE2) after coculture for 24 hours. Scale bar = 200 μm.



Supplementary Fig. S2. Schematic representation of the Delta SARS-CoV-2 variant S protein (a), and potent inhibitory activity of the pan-CoV fusion inhibitors (EK1, EK1C4 and EKL1C peptides) against Delta S-mediated cell-cell fusion (b) and pseudovirus infection (c). Samples were tested in triplicate, and the experiment was performed twice. Data from a representative experiment are presented in mean±SD.



Supplementary Fig. S3. Potent inhibitory activity of pan-CoV fusion inhibitors (EK1, EK1C4 and EKL1C peptides) against D614G variant S-mediated cell-cell fusion (a) and pseudovirus infection (b). Samples were tested in triplicate, and the experiment was performed twice. Data from a representative experiment are presented in mean±SD.



Supplementary Fig. S4. The structural comparations of spike proteins from different SARS-CoV-2 variants. The trimer structures of spike protein are shown as surface in both prefusion state (PDB entry: 6XR8) and post-fusion state (PDB entry: 6XRA). The colors for NTD, RBD and rest parts of spike protein are light salmon, slate blue and light blue, respectively. The point mutations are shown in red. The complex structure of spike HR1 domain (green) and EK1 peptide (magentas) (PDB entry: 7C53) are shown as cartoon representative. The point mutations are indicated as sticks.