Establishment of an integrated assay platform for supporting the discovery of antivirals and vaccines against human metapneumovirus infection

Yanhua Du, Ronghua Shi, Chaochao Ren, Fubiao Xiao, Fusen Lin, Shaohua Li, Qiong Zhou
Discovery Biology, Biology, WuXi AppTec, 288 Fute Zhong Road, Waigaoqiao Free Trade Zone, Shanghai, China

WuXi Biology

Abstract

Human metapneumovirus (HMPV) is one of the main pathogens causing upper and lower respiratory tract infections in young children, older adults and immunocompromised patients. HPMV infection can lead to bronchiolitis, pneumonia, as well as acute asthma exacerbations. Currently, there is no vaccine or antiviral for the prevention and treatment of HMPV infection.

To expedite the development of drugs and vaccines against HMPV infection, we have established an integrated platform including the conventional cell-based viral infection and neutralizing assays, and a mouse model. In the mouse HPMV infection model, the efficacy of antivirals and vaccines can be evaluated with multiple endpoints, such as lung virus load and pathology. Furthermore, we have also developed non-infectious replicon and cell fusion assays. Our HMPV replicon contains all virus genes except for the envelope glycoproteins and has a GFP reporter. Therefore, the replicon can be used for screening inhibitors acting on intracellular viral targets, such as the polymerase. Furthermore, the replicon can be applied to conduct the study of virus drug resistance, including de novo in vitro selections of drug resistance. HPMV inhibitors have been tested in the replicon and exhibited the comparable inhibitory activities to those observed in the cell-based HPMV infection assay. The cell fusion assay can be used for screening HMPV entry inhibitors.

Results

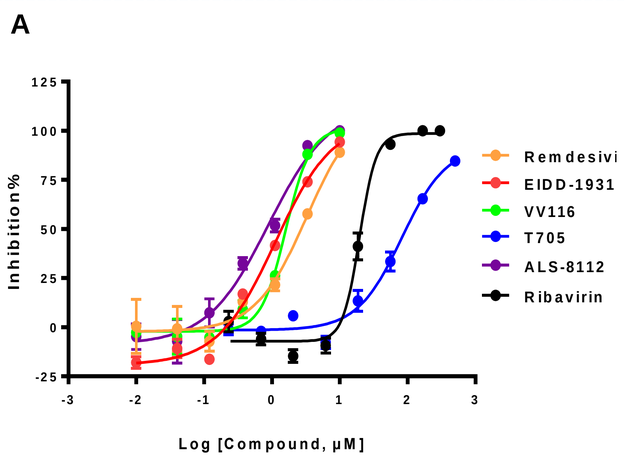


Figure 1. The activity of drugs in the HMPV infection assay. **(A)** Activity of viral polymerase inhibitors in the HMPV cell-based infection assay. **(B)** Activity of an anti-HMPV fusion (F) protein Ab in the HMPV GFP reporter neutralization assay.

Results

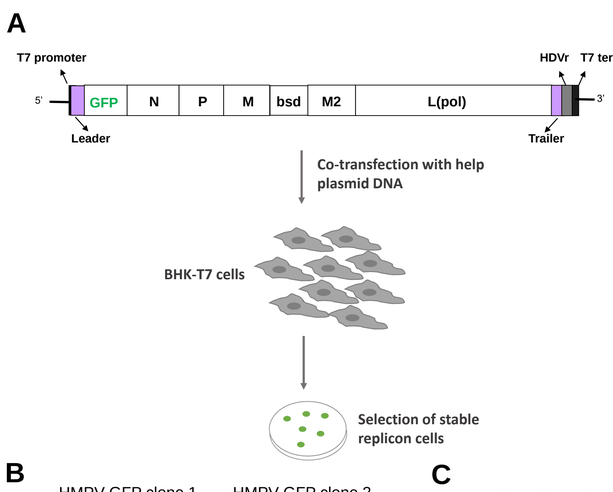
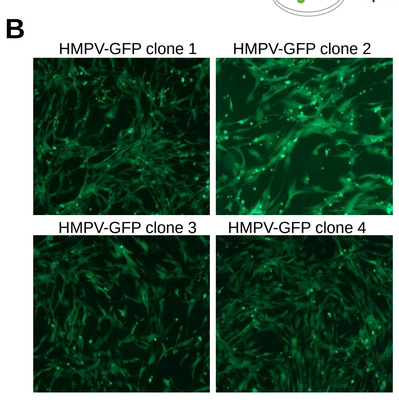
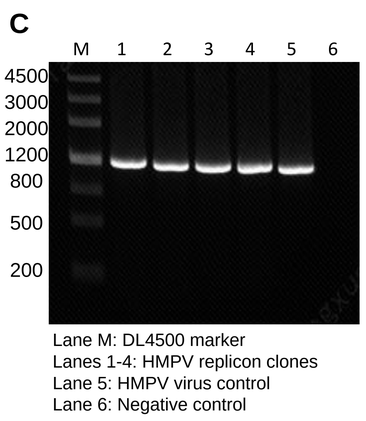
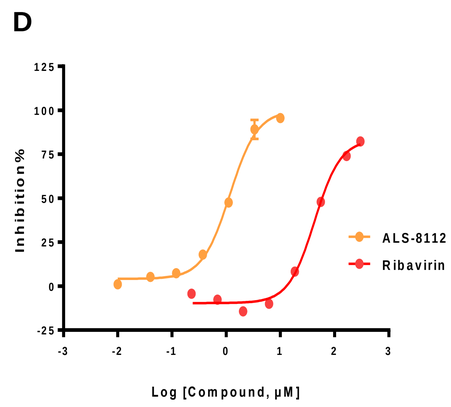


Figure 2. Characterization of HMPV stable replicon cells. **(A)** A schematic of the construction and selection of BHK21-based HMPV stable replicon cells. **(B)** GFP expression of the replicon in the selected stable cell clones. **(C)** Verification of the replicon clones with viral L fragment by RT-PCR. **(D)** The activity of small molecular inhibitors in the HMPV replicon assay.





Lane M: DL4500 marker
Lanes 1-4: HMPV replicon clones
Lane 5: HMPV virus control
Lane 6: Negative control



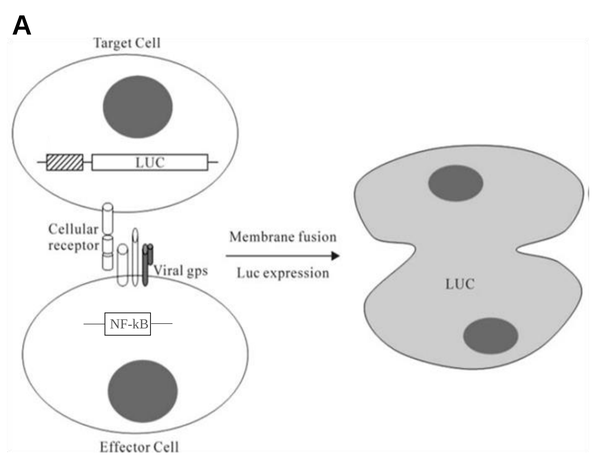
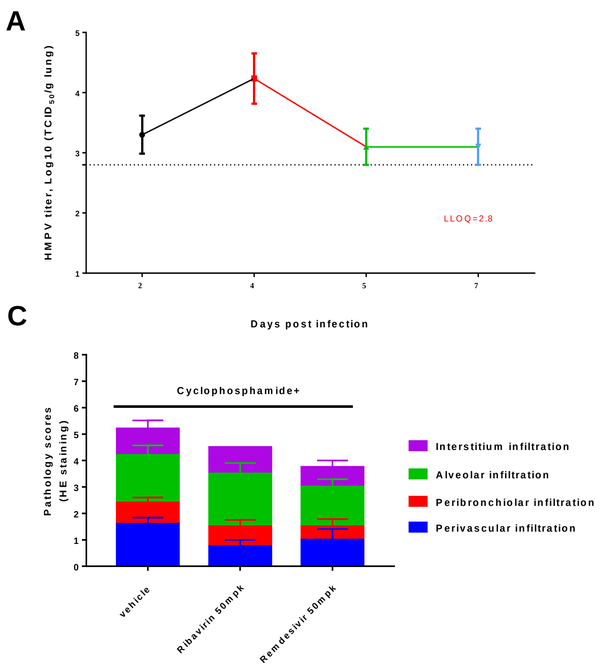


Figure 3. Characterization of HMPV F protein mediated cell fusion reporter assay. **(A)** A schematic of the F protein mediated cell fusion reporter assay. Two stable cell lines were constructed, i.e. effector cell line HEK293-NF-κB transfected with the effector plasmid DNA encoding the activation domain of NF-κB fused with the GAL4 DNA binding domain under the control of HCMV promoter, and target cell line HEK293-Luc transfected with the target plasmid DNA containing the luciferase reporter gene under the control of a promoter containing GAL4 DNA binding sites. The effector and target cells were fused in the present of HMPV F protein, and subsequently luciferase reporter gene was expressed. **(B)** Activity of an anti-HMPV F protein Ab in the HMPV cell-based fusion reporter assay.

Results



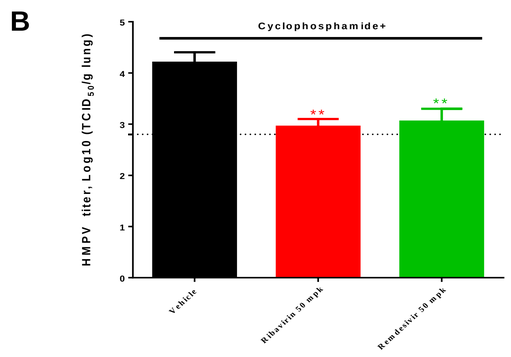


Figure 4. HMPV mouse infection model. **(A)** Balb/c mice (6 to 8 weeks old) were intranasal infected with HMPV. The viral titer dynamic in lungs was titrated at different days post infection. The in vivo efficacy of Ribavirin and Remdesivir (50 mpk, IP, BID, dosed at days 0 to 3) was evaluated in this model with viral load **(B)** and pathology scoring of lungs **(C)** at 4 days post infection.

Summary

We have established a platform including the in vitro assays and the mouse HPMV infection model. We have applied the platform for the evaluation of MPHV inhibitors and vaccines. Our platform can facilitate the discovery of various types of antivirals and vaccines for the treatment and prevention of HPMV infection.

Reference

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药明康德 WuXi AppTec

www.wuxibiology.com
Business Contact: Mahnaz_Arjomand@wuxiapptec.com (US)
Technical Contact: lin_fusen@wuxiapptec.com
Business Contact: dave_madge@wuxiapptec.com (EU and Israel)
Business Contact: sycho@wuxiapptec.com (Korea)
Business Contact: fumio_itoh@wuxiapptec.com (Japan)
Business Contact: xu_longji@wuxiapptec.com