A pan-coronavirus fusion inhibitor targeting the HR1 domain of human coronavirus spike

With the support by the National Natural Science Foundation of China and the Ministry of Science, the research team led by Dr. Lu Lu (陆路) and Dr. Jiang ShiBo (姜世勃) at Fudan University, in collaboration with the Lab of Antibody Structure research team at ShanghaiTech University, developed a pan-coronavirus (pan-CoV) fusion inhibitor EK1, which was published in *Science Advances* (2019, 5(4): eaav4580).

The continuously emerging highly pathogenic human coronaviruses (HCoVs) remain a significant threat to human health, as illustrated by the life-threatening outbreaks of SARS-CoV and MERS-CoV in this century. To date, there are still no effective treatments or vaccines available against HCoVs infections. Besides, zoonotic CoVs exhibit high genomic variability and harbor great potential to cross the species barrier and infect humans, which poses further challenges for broad-spectrum anti-HCoVs drug development and design. Recently, the World Health Organization (WHO) proposed the concept of defense against "Disease X", which includes the future lethal transmission of zoonotic CoVs to humans (http://www.who.int/blueprint/priority-diseases/en/). Hence, the development of a broad-spectrum and highly-efficient anti-HCoV inhibitor holds great significance for the prevention and control of current and emerging CoVs pandemics. To this end, identifying conserved druggable targets in HCoVs is a key question to address in the field.

In this work, the joint research team first established cell-cell fusion systems for multiple existing HCoVs. They then performed cross-screening using these systems and finally identified a polypeptide that has pan-HCoV inhibitory activity. This polypeptide, which was derived from the HCoV-OC43 HR2 region (OC43-HR2P), was further optimized into peptide EK1 with better solubility and higher inhibitory activity. In both pseudovirus and live virus infection assays, the polypeptide EK1 exhibited promising potency and breadth in inhibiting infection by multiple α-HCoVs and β-HCoVs. Administration of EK1 via the nasal route demonstrated highly protective effects in both HCoV-OC43 and MERS-CoV infected mouse models. Moreover, EK1 manifests satisfactory safety profiles and low immunogenicity *in vivo*, suggesting it possesses promising clinical potential to be further developed into a broad-spectrum antiviral agent against current and emerging HCoVs.

To unravel the molecular basis for the pan-CoV inhibitory effect of the EK1 peptide, the joint research team further determined co-crystal structures of EK1 in complex with the HR1 regions from multiple HCoVs. The results clearly show that EK1 can act broadly in the HR1 regions of both α-HCoVs and β-HCoVs through forming extensive and highly-conserved hydrophobic and hydrophilic interactions, thereby antagonizing the formation of the autologous 3HR1-3HR2 six helical bundle that is pivotal for the host entry of HCoVs. In summary, the structural study not only revealed that EK1 exhibits good conformational and surface charge plasticity to accommodate variation among the HR1s from different HCoVs, but more importantly explained the conserved molecular basis for the HR1-EK1 interaction, further indicating that HR1 region could indeed serve as a promising target site for the development of pan-CoV inhibitors.

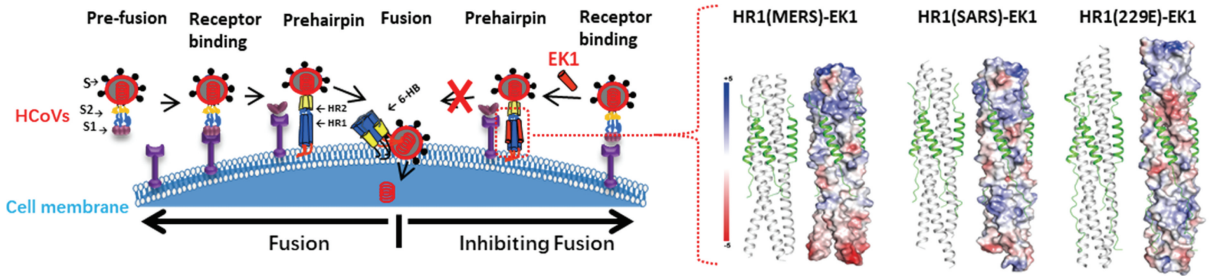

Figure The broad-spectrum antiviral mechanism of EK1.