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Potent neutralization of 2019 novel coronavirus by recombinant ACE2-Ig

Changhai Lei[1], Wenyan Fu[2], Kewen Qian[3], Tian Li[1,3], Sheng Zhang[4], Min Ding[5], and Shi Hu[1, 3]*

[1] Department of Biophysics, College of Basic Medical Sciences, Second Military Medical University, Shanghai 200433, China.

[2]Department of Assisted Reproduction, Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, 200011 Shanghai, China.

[3] Team SMMU-China of International Genetically Engineered Machine (iGEM) competitions, Department of Biophysics, Second Military Medical University, Shanghai 200433, China.

[4] Department of Critical Care Medicine, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, 200011 Shanghai, China.

[5] Pharchoice Therapeutics Inc., Shanghai 201406, China

*Corresponding Author: Shi Hu, Second Military Medical University, 800 Xiangyin Road, Shanghai, China, 200433. Phone: +86-02181870925; E-mail: hus@smmu.edu.cn.

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Abstract

A novel coronavirus, designated as 2019-nCoV, emerged in Wuhan, China, at the end of 2019. As of Feb 1, 2020, at least 11,844 cases had been diagnosed in China, however, there is no specific antiviral treatment or vaccine currently. Very recently report have suggest that novel CoV uses the same cell entry receptor, ACE2, as SARS-CoV. In this report, we generated a new recombinant protein by connecting the extracellular domain of human ACE2 to the Fc region of the human immunoglobulin IgG1. An ACE2 mutant with low catalytic activity was also used in the study. The fusion proteins were then characterized. Both fusion proteins has high affinity binding to the receptor-binding domain (RBD) of SARS-CoV and 2019-nCoV and exerted desired pharmacological properties. Moreover, fusion proteins potently neutralized SARS-CoV and 2019-nCoV *in vitro*. As these fusion proteins exhibit cross-reactivity against coronavirus and could have potential applications for diagnosis, prophylaxis, and treatment of 2019-nCoV.

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A novel coronavirus, designated as 2019-nCoV, emerged in Wuhan, China, at the end of 2019. After initial reports of a SARS-like virus emerging in Wuhan, it appears that 2019-nCoV may be less pathogenic than MERS-CoV and SARS-CoV. Spike (S) proteins of coronaviruses, including the coronavirus that causes severe acute respiratory syndrome (SARS) and the 2019-nCoV, associate with cellular receptors to mediate infection of their target cells. The metallopeptidase, angiotensin-converting enzyme 2 (ACE2), has been identified as a functional receptor for SARS-CoV[1] and a potent receptor for 2019-nCoV[2]. ACE2 is a carboxypeptidase that potently degrades angiotensin II to angiotensin 1–7, plying key role in the renin-angiotensin system (RAS)[3]. RAS is a hormonal cascade that plays a central role in the homeostatic control of cardiorenal actions.

Earlier studies reported that, recombinant ACE2 (rACE2) protects against severe acute lung injury and acute Ang II-induced hypertension[4-6]. Recombinant ACE2 (rACE2) was also reported to attenuate Ang II-induced heart hypertrophy, cardiac dysfunction, and adverse myocardial remodeling in murine models[7], as well as renal oxidative stress, inflammation, and fibrosis.[8,9] However, pharmacokinetic studies revealed that rACE2 exhibits fast clearance rates with a reported half-life of only hours in humans and mice[10,11]. Recently, long-acting rACE2 by fusing ACE2 with an Fc fragment (rACE2-Fc) shows ong-lasting effects that attenuate hypertension and shows organ protection in both acute and chronic models of angiotensin II-dependent hypertension in mice[12].

Based on the receptor function of ACE2 for coronavirus, we hypothesis that ACE2 fusion protein may have neutralization Potential for coronavirus, especially the 2019-nCoV. To investigate the therapeutic potential of ACE2, we constructed and generated a fusion protein (ACE2-Ig) consisting of the extracellular domain of human ACE2 linked to the Fc domain of human IgG1 (Fig. 1). An ACE2 variant (HH/NN) in which two active-site histidines (residues 374 and 378) have been altered to asparagines was also used in our study to reduce the catalytic activity. The fusion protein contain the ACE2 variant were termed as mACE2-Ig. The expression and purification methods were described in our previous reports[13]. The affinities of the fusion protein for SARS-CoV RBD and 2019-nCoV RBD were determined with BIAcore binding assays (Fig. S1).

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Compared with the Fc fusion protein TIGIT-Ig reported in our pervious report, the ACE2-Ig and mACE2-Ig had similar denaturation temperature and thus exhibited IgG-like stability. The lowest concentration (< 2%) of high molecular weight and low molecular weight products was observed after 1 week of storage at 40 °C at a 1 mg/ml concentration. Mice were treated separately with a single intravenous dose of fusion proteins to measure their pharmacokinetic (PK) parameters, and the serum concentrations of fusion proteins were determined by ELISA. The results showed that the main PK parameters of ACE2-Ig, mACE2-Ig and TIGIT-Ig were very similar in mice and demonstrated the high stability of the fusion proteins. The experimental data are summarized in Table S1.

After we identified that ACE2 fusion proteins binds with high affinity to the RBD, we next sought to test the inhibitory activity of ACE2 fusion proteins against 2019-nCoV and compare it with that against SARS-CoV, we used viruses pseudotyped with the S glycoprotein of SARS-CoV and 2019-nCoV. Our data shows that Both SARS-CoV and 2019-nCoV viruses were potently neutralized by ACE2-Ig and mACE2-Ig. The IC50 of SARS-CoV and 2019-nCoV viruses neutralized by ACE2-Ig were 0.8 and 0.1 μg/ml, respectively. And The IC50 of the two viruses neutralized by mACE2-Ig were 0.9 and 0.08 μg/ml, respectively (Fig. 2). No evidence of neutralization was observed for the TIGIT-Ig. We next using cell fusion assay to further characterize the in vitro neutralization effect of the fusion proteins (Fig .3). ACE2-Ig potently inhibited the SARS CoV-S protein-mediated fusion with an IC50 of 0.85 μg/ml, and the 2019 nCoV-S protein-mediated fusion with an IC50 of 0.65 μg/ml. Under the same experimental conditions, another fusion protein mACE2-Ig exhibited an IC50 of 0.76 μg/ml and 0.48μg/ml, for the SARS CoV and 2019 nCoV, respectively. The control TIGIT-Fc did not show any inhibitory effect in this assay. These data suggest that both ACE2-Ig and mACE2-Ig exhibit potent inhibitory activity against SARS CoV and 2019 nCOV.

ACE2 is an important drug targets for the treatment of cardiovascular and kidney diseases. As it is already know that ACE2 is a key player in the coronavirus infection, no study as we know reported to use recombinant ACE2 protein as therapeutic candidate for coronavirus. The short half-life of ACE2 is an important barrier to its practical use. The technique of using recombinant Fc fusion to extend plasma residence time of

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pharmacological agents has been used most notably in applications to improve in vivo efficacy of existing drugs. For example, new long-acting forms of both recombinant coagulation factors rFVIII-Fc and rFIX-Fc were recently approved for clinical treatments of hemophilia A and B that require less frequent infusions[14,15]. It should be noted that, unlike the blood resident enzymes of coagulation factors, full-length endogenous ACE2 is a transmembrane protein anchored to the cell surface and that ACE2 activities are, in fact, present at very low levels in systemic circulation[16-18]. One safety concern of the ACE2 fusion protein is that they may have cardiovascular side-effect. Interestingly, one recently report shows that treatment of murine ACE2 in mice show no evidence of side-effect[12]. Moreover, our preliminary studies showed that the neutralization effect remained efficient when two active-site histidines of ACE2 were modified to asparagine.

Taken together, our results demonstrate potential ACE2 based therapeutics against 2019-nCoV which could be used alone or in combination, and they elucidate the molecular mechanisms of their potent and broad neutralizing activity. These ACE2 fusion proteins could be also used for diagnosis and as research reagents in the development of vaccines and inhibitors.

Methods

Generation of fusion proteins

The sequences of the extracellular domains (ECDs) of ACE2 (aa: 1-740) were ligated to the Fc segment of humian IgG1, to construct a recombinant plasmid. Mutations were generated by Integrated DNA Technologies. The FreeStyle 293 expression system (Invitrogen) was used in our study, and the recombinant protein was obtained according to the methods used in a previous study[19] and then immediately purified using protein A Sepharose and the harvested cell culture supernatant. TIGIT-Ig were described in our pervious report[13]. The concentration and purity of the fusion protein were determined by measuring the UV absorbance at a wavelength of 280 nm and by polyacrylamide gel electrophoresis, respectively.

Affinity measurement

CoV RBDs were prepared as previously described[1]. We immobilized an anti-human Fc polyclonal antibody on a CM5 chip (~150 RU) using standard amine-coupling chemistry and then injected CoV RBDs (12.5 nM~200 nM) using a previously reported method to capture the fusion proteins. The binding response was corrected by subtracting the RU

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from the blank flow cells. We used the surface plasmon resonance (SPR) method with a BIAcore-2000 to measure the monovalent binding affinity of the fusion protein and performed kinetic analysis using a 1:1 L model that simultaneously fit ka and

Pharmacokinetics

We used female BALB/c mice to determine the pharmacokinetic profile of the fusion protein. Eight-week-old mice were administered the fusion protein at a dose of 1 mg/kg body weight by tail vein injection. Mice were divided into 15 groups, corresponding to day 1 to day 15. Blood was collected from the septum in heparin-containing tubes and then centrifuged to remove blood cells and to obtain plasma samples. The serum concentration of the fusion protein was determined by competitive ELISA.

Cell Fusion Inhibition Assay.

For assessment of the neutralization activity of the fusion proteins, a quantitative cell fusion assay based on β-galactosidase (β-gal) as a reporter gene was used as described previously[20]. The fusion proteins were preincubated with 293T cells transfected with the indicated CoV S glycoprotein gene at room temperature for 10 min, then mixed with 293T cells transfected with ACE2 at 1:1 ratio and incubated at 37°C for 4 h. Cells were then lysed, and the β-gal activity was measured. The protein concentrations during fusion were used for calculation of the IC50 defined as the concentration at which the β-gal activity was reduced by 50%.

Pseudovirus Neutralization Assay.

Pseudoviruses containing the S glycoprotein from various virus, and a defective HIV-1 genome that expresses luciferase as a reporter protein, were prepared, and the assays performed as described previously[21-24].

Conflicts of interest

Y.L., and M.D. declare they are employees of Pharchoice Therapeutics Inc. (Shanghai). M.D. is a shareholder at Pharchoice Therapeutics Inc. (Shanghai). The other authors declare no competing interests.

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Figure Legends

Figure 1. Schematic of ACE2-Ig.

Figure 2. ACE2-Ig potently neutralizes viruses pseudotyped with S glycoproteins from the SARS CoV and 2019 nCoV. HIVs pseudotyped with the S glycoprotein from CoVs were incubated with different fusion proteins for 1 h before infection. Luciferase activities in target cells were measured, and the percent neutralization was calculated.

Figure 3. Inhibition of cell fusion by ACE2-Ig. Potent inhibition of cell fusion mediated by the SARS-CoV (left) or 2019 nCoV (right) spike (S) glycoprotein. Cells expressing diffferent S glycoprotein were incubated with indicated fusion proteins and mixed with ACE2-expressing cells. The activity of the reporter gene, β-gal, was measured as a correlate of fusion. The curves represent the best fit to the experimental data and were used for calculation of the IC50. Bars indicate the S.E.

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Figure 1

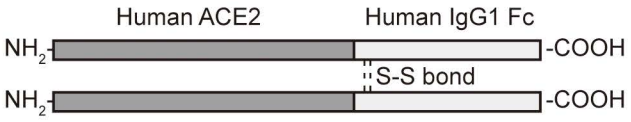

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Figure 2

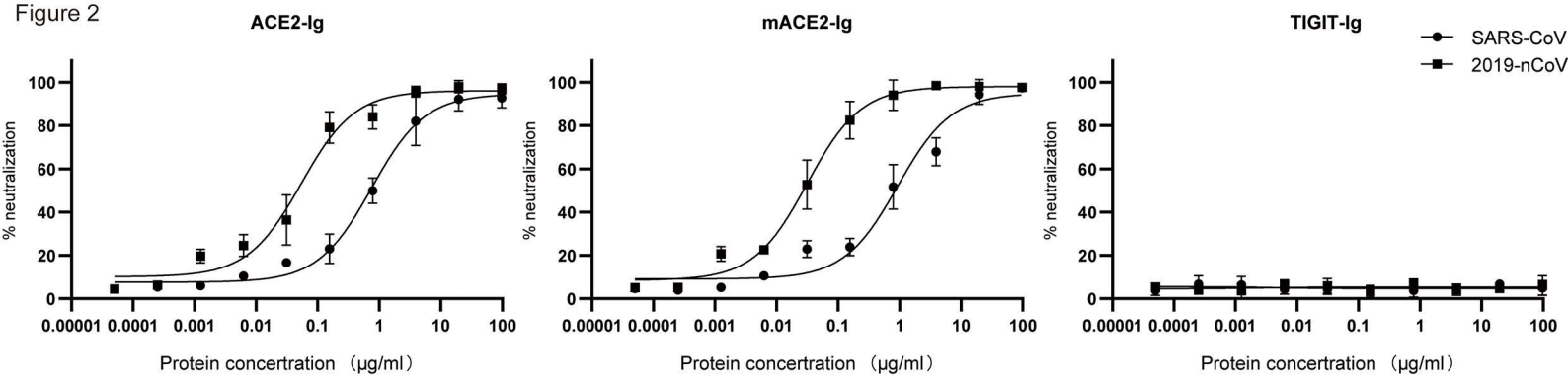

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Figure 3