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Editorial

On the way from SARS-CoV-sensitive mice to murine COVID-19 model

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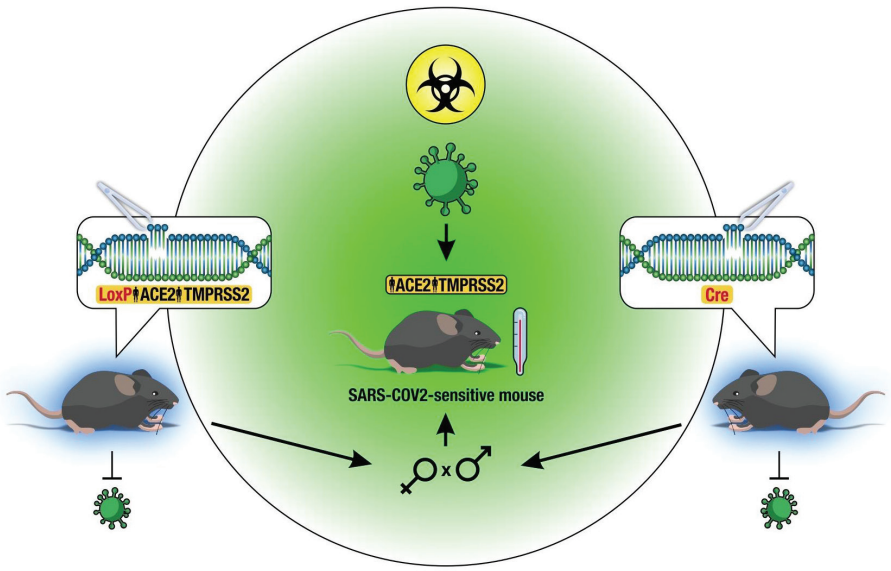
Abstract

The coronavirus disease 2019 (COVID-19) is a master killer which appeared suddenly and which has already claimed more than 200,000 human lives. In this situation, laboratories are in urgent need for a COVID-19 murine model to search for effective antiviral compounds. Here we propose a novel strategy for the development of mice that can be inoculated by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the COVID-19 causative agent. In humans, two proteins – ACE2 and TMPRSS2 – are involved in SARS-CoV-2 cells entry and, thus, we decided to introduce their genes into a murine genome. These genes will be placed with LoxP sites under the murine *Tmprss2* promoter. Such an approach can provide a representative model with the opportunity to control the viral sensitivity of an animal population and tissue specificity of hACE2 and hTMPRSS2 expression.

Graphical abstract



The new COVID-19 model should be based on inducible co-expression of the human ACE2 and TMPRSS2 genes. Activation of ACE2 and TMPRSS2 genes will occur only in the virological laboratory, after crossbreeding with Cre-mice. Before activation, mice will be resistant to SARS-CoV-2 for their biological safety during the pandemic.

Keywords

COVID-19, SARS-CoV-2, ACE2, TMPRSS2, mice, CRISPR/Cas9.

Background

The COVID-19 outbreak is a dramatic, rapidly evolving situation. The search for effective approaches to SARS-CoV-2 infection therapy and prevention has become one of the most important tasks for medicine now and in the foreseeable future. Every day, doctors and scientists receive more and more information about the effectiveness of classic antiviral medications and some off-label-used drugs.

However, these data should also be quickly supplemented by the results of pre-clinical studies that can provide much useful and even crucial information about the most effective drugs. Unfortunately, as most laboratories do not have an accessible SARS-CoV-2-sensitive animal model, this is the main stumbling block for quick *in vivo* screening. The difficulty in obtaining such models in the pandemic condition directed us to develop our own SARS-CoV-2-sensitive mouse model as discussed in this paper.

Virus invasion pathway

To enter the target cells, SARS-CoV and SARS-CoV-2 use their "corona", which is represented by numerous spike (S) proteins. It was shown that the S-protein engages angiotensin-converting enzyme 2 (ACE2) as the entry receptor (Li et al. 2003). During viral infection, the trimeric S protein is cleaved into S1 and S2 subunits (Belouzard et al. 2009; Simmons et al. 2013). Further, the S1 subunit, containing the receptor binding domain, directly binds to the peptidase domain of angiotensin-converting enzyme 2 (ACE2) (Li et al. 2005), whereas S2 is responsible for membrane fusion.

Although ACE2 is present in many types of tissues (Hamming et al. 2004), SARS-CoV is highly pathogenic only in the lungs (To and Lo 2004). Furthermore, type 1 pneumocytes, which poorly express ACE2, are more sensitive to viral invasion in comparison with ACE2-rich type 2 pneumocytes (Matsuyama et al. 2010). The selective nature of tissue damage was explained by the existence of a second molecule, which also contributes to cell contagion. Thus, ACE2 immunoprecipitation captured the transmembrane protease/serine subfamily member 2 (TMPRSS2), a known human airway and alveolar protease (Vaarala et al. 2001). This enzyme was shown as one of the up-regulators of coronavirus infection pathways (Iwata-Yoshikawa et al. 2019). Interaction of ACE2 and TMPRSS2 enhanced the cell entry of SARS-CoV and it correlated with TMPRSS2-mediated proteolysis of both S and ACE2 proteins (Shulla et al. 2011). Recently, Hoffmann et al. (2020) have discovered that serine protease TMPRSS2 is employed by SARS-CoV-2 for S protein priming and the TMPRSS2 inhibitor prevents virus entry.

Homology between human and mouse ACE2 proteins

Human and mouse ACE2 enzymes consist of 805 amino acids with 81.86% interspecies homology. Both hACE and mACE have Collectrin (75.97%) and Peptidase M2 (interdomain similarity = 84.62%) domains and the latter directly interacts with S-protein. It has only recently been identified that amino acids Asp30, His34, Tyr41, Gln42, Lys353, Arg357, Gln24 and Met82 of human ACE2 play a key role in binding with viral S-protein (Yan et al. 2020). Alignment of mouse and human protein ACE2 sequences showed that five of these eight residues differ in human and mice. The similarity between TMPRSS2 cleavage sites (amino acids 697–716) of hACE2 and mACE2 proteins is 78.95% (Fig. 1).

Three regions of SARS-CoV-2 binding site are shown. Five of eight key residues differ between mouse and human aligned sequences (highlighted in red) when three amino acids coincide (highlighted in green). In addition, alignment of mouse and human ACE2 TMPRSS2 cleavage sites is presented below.

Homology between human and mouse TMPRSS2 proteins

Both human and mouse TMPRSS2 proteins consist of 4 domains: Transmembrane; LDL receptor class A; Scavenger receptor cysteine-rich; and Serine protease. Murine TMPRSS2 protein contains 492 amino acids and shares 81.4% similarity and 77.3% identity with the human one. The details of comparison were presented in Vaarala et al. (2001).

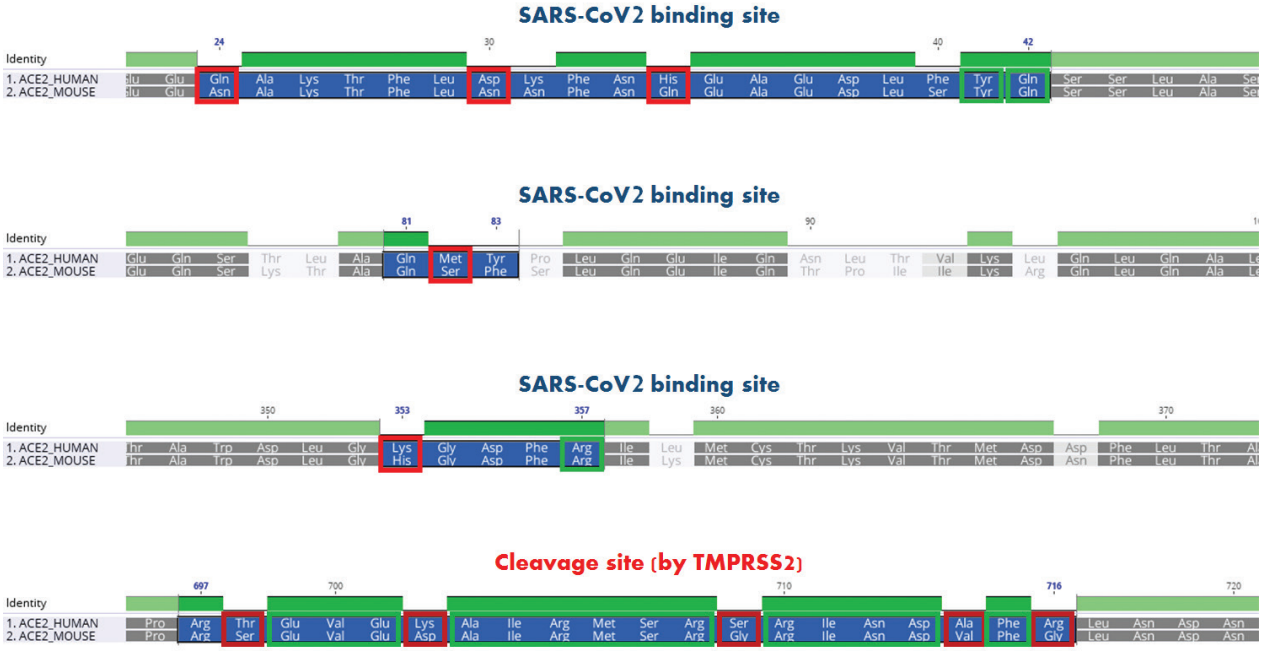


Figure 1. Alignment of human and mouse ACE2 protein sequences.

Mouse models of SARS-CoV infection

Naturally, mice are low-sensitive to SARS-CoV infection, but can be poorly inoculated by the virus. To improve the virus inoculation, a few transgenic lines of mice were created with humanized *ACE2* gene. In the first line, the h*ACE2* gene was introduced under the CAG promoter with CMV-IE enhancer (Tseng et al. 2007). This modification led to a sharp clinical manifestation with an acute wasting syndrome and deaths of the mice within 4 to 8 days after SARS-CoV inoculation. A second line was developed by introducing the h*ACE2* gene regulated by the human cytokeratin 18 (K18) promoter. After the SARS-CoV contagion, these mice demonstrated a clinical picture of encephalitis and pneumonia, resulting in death after 3–5 days of the post-inoculation period (McCray et al. 2007).

The transgenic line, created by the Yang et al. (2007), has the most natural tissue expression profile of ACE because h*ACE* gene was introduced under the mouse's own *Ace2*-promotor. In this line, SARS-CoV replicated more efficiently in the lungs of transgenic mice than in those of wild-type mice. After the SARS-CoV inoculation, the mice had severe pulmonary lesions, including interstitial hyperaemia and haemorrhage, monocytic and lymphocytic infiltration, protein exudation and alveolar epithelial cell proliferation and desquamation.

As SARS-CoV and SARS-CoV-2 have a similar manner of cell contagion, it is believed that the already-created models are also sensitive to COVID-19. Bao et al. (2020) have recently reported that *ACE2*-humanized mice with *ACE2* gene under murine *Ace2* promoter have sensitivity to SARS-CoV-2, but it is lower than that of SARS-CoV. However, these models are not widespread in laboratories and we have not found any publication where *ACE2*-humanised mice were used for preclinical studies of anti-SARS-CoV-2-therapy. Whereas, since a few models have already been developed, we can take the available experience and try to propose our own strategy for the genetic edition.

Novel approach to the creation of SARS-CoV-2-sensitive mice

As can be seen, there is not only ACE2 involved in SARS-CoV-2 invasion. Therefore, first of all, we consider that mice with two humanized ACE2 and TMPRSS2 genes will be more sensitive to viral invasion. This approach will not only make ACE2 more accessible for cleavage (which is important for a viral entry), but will also open up additional possibilities for drugs testing, such as inhibitors of TMPRSS2. Moreover, a high expression of TMPRSS2 in the epithelial cells makes it reasonable to introduce both h*ACE2* and h*TMPRSS2* under the murine *Tmprss2* promoter. We believe that the co-expression of two virus-inviting molecules in the lung epithelium will imitate the events that happen in the human body during SARS-CoV-2 invasion.

In brief, to create this model, we are going to clone *hTMPRSS2* and *hACE* sequences and introduce it into the mouse genome with the use of CRISPR/Cas9 tech-

Table 1. Brief description of the existing SARS-CoV-sensitive transgenic mice.

Promoter	Clinical pattern and pathomorphology		Lethality level		References
	SARS-CoV	SARS-CoV-2	SARS-CoV	SARS-CoV-2	
CAG promoter+CMV-IE enhancer	Acute wasting syndrome. Death within 4 to 8 days of post-inoculation period as result of inflammatory response in the brain (more intensive than in the lungs) Damaged tissues: lungs, kidneys, liver, heart, skeletal muscle, spleen, lymphatic nodes, pancreas, gastrointestinal smooth muscle and ganglia, vascular endothelium, adrenal and central nervous system (CNS)	no data	100% (in mice with high hACE2 expression) 0% (in mice with low hACE2 expression)	no data	Tseng et al. 2007; Yoshikawa et al. 2009
human cytokeratin 18+ alfalfa mosaic virus enhancer	Pneumonia, neuro-inflammation. Infection of the CNS is a major factor contributing to the fatal outcome observed in SARS-CoV-infected mice. Damaged tissues: Lungs, colon, small intestine, kidneys, liver, spleen, heart	no data	100%	no data	McCray et al. 2007; Netland et al. 2008
mouse ACE2 promoter	Gross pulmonary edema, focal haemorrhage, consolidation and lung bullae with no significant histopathological lesions or viral antigens in myocardium, liver, spleen, kidney, cerebrum, intestine and testis. Damaged tissues: Viral antigens were observed in the bronchial epithelial cells, alveolar macrophages and alveolar epithelia.	Interstitial pneumonia with lymphocytes and monocytes infiltration. Accumulation of macrophages in alveolar cavities	0%	0%	Yang et al. 2007; Bao et al. 2020

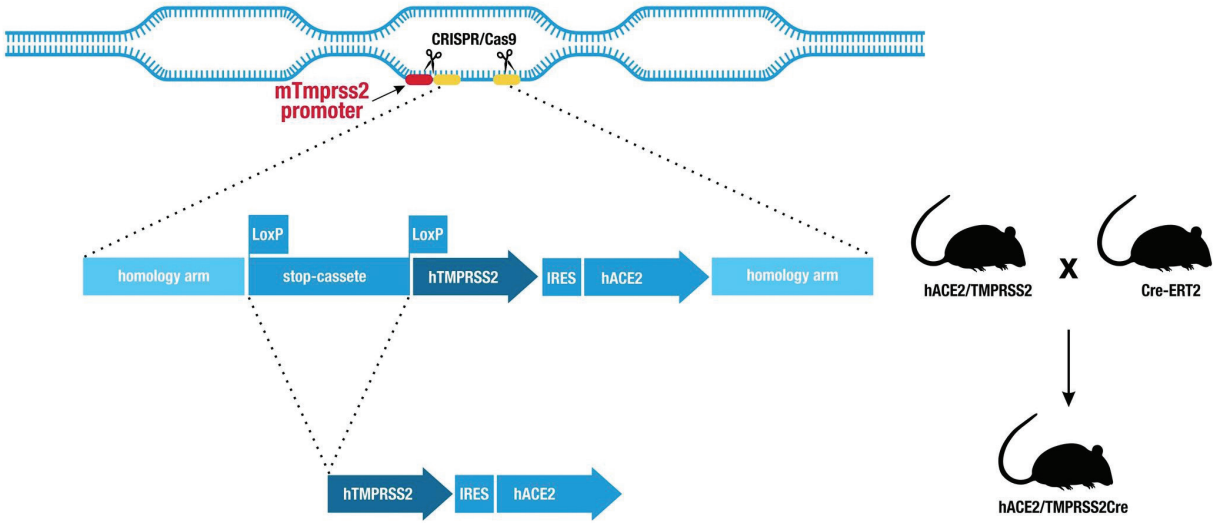


Figure 2. Construction for the development of Cre-regulated h*ACE2*/h*TMPRSS2* mice.

nology. *hTMPRSS2* and *hACE2* will be divided by the IRES element for their equivalent expression. Additionally, to create an inducible h*ACE2*/h*TMPRSS2* expression, we wish to place LoxP sites in front of the *hTMPRSS2* sequence (Fig. 2 for the details). In this manner, it will allow provoking SARS-CoV-2 sensitivity only after breeding with suitable *Cre*-mice within virological laboratories. For example, Cre-ER^{T2} mice express tamoxifen-induced Cre-recombinase in stem cells. Thus, it can cut the stop-cassette between LoxP sites during ontogenesis, unlocking the gates to h*ACE* and h*TMPRSS2* transcription.

Construction consists of homology arms, *hTMPRSS2* sequence, IRES element, *hACE2* sequence and an effective terminator of expression in 3' regions. Homology arms have to direct it next to the murine Tmprss2 promoter with use of CRISPR/Cas9. Transgenic mice can be crossbred with *Cre-ERT2* mice for the excision of the stop-cassette between LoxP-sites and activation of h*ACE2* and h*TMPRSS2* transcription.

Discussion

A previously-unknown coronavirus called SARS-CoV-2 has crossed the species barrier and caused a human infection outbreak, first in Wuhan and then around the world. The rapidly developing epidemic generated interest in mice expressing the human receptors for SARS-CoV-2 entry for further pharmacological screening (Walls et al. 2020).

In the growing avalanche of SARS-CoV-2 pathogenesis details, it is very difficult to focus on specific targets. For example, recent insight into the CD147 as a novel target of SARS-CoV-2 opens up new possibilities for explaining the disease (Wang et al. 2020). Nevertheless, here we describe our strategy for the development of the novel COVID-19 murine model by humanising *ACE2* and *TMPRSS2* genes.

We have formulated the basic requirements for the COVID-19 animal model. First of all, transgenic animals should not be a "laboratory" reservoir of the virus before the start of the pharmacological experiments. Although the SARS and MERS were successfully inoculated in mice, infection did not spread in animals after intranasal administration. In the case of a new SARS-CoV-2 virus, it is impossible to predict its degree of contagion from mice to mice and human population. For the safety of the laboratory staff, we decided to use LoxP-induced expression of humanized genes, as described in (Zvartsev et al. 2018; Deykin et al. 2019; Silaeva et al. 2020).

As shown, the hTMPRSS2 and hACE2 proteins are jointly involved in the pathogenesis of the SARS-CoV-2 invasion. Moreover, the expression profiles of these enzymes in mice and human intersect at lungs, intestine and the male reproductive system epithelium (Vaarala et al. 2001; Sungnak et al. 2020). Therefore, we consider it appropriate to use the mouse *Tmprss2* promoter for the expression of human *hTMPRSS2* and *hACE2* genes in the transgenic line. A modern approach to modification of the mouse genome in this way would be gene editing, based on CRISPR/Cas9. On making a gap after the m*Tmprss2* promoter, a matrix containing homology arms, *hTMPRSS2* sequence, IRES element, *hACE2* sequence and an effective terminator of expression in 3' regions will be used to bridge the gap.

It is very important not only for the COVID-19 murine model to be able to be infected by the SARS-CoV-2, but also for the infection process to be as human-like as possible. The previous models showed effective infection only in high viral load conditions. At the same time, in the models based on the K18 promoter, systemic damage, neuroinflammation and lethargy developed, which is not quite representative of the human clinical picture. In the model, created by Yung et al. (2007), the SARS-CoV-2 infection led to more human-like clinical disorders, but those mice had quite a low level of lethality. The advantages and disadvantages of the existing *ACE2* humanized mice were presented in Gretebeck and Subbarao (2015). We think that reproduction of hTMPRSS2 and hACE2 crosstalk in murine epithelium will be much better because it can intensify lung contagion with no influence on brain damage.

Activation of expression is possible after crossbreeding with mice constitutively or inducibly expressing Cre-recombinase (for example, Cre-ERT2 line). We expect these mice to be a safe and representative COVID-19 model.

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Conflict of interest

The authors declare no conflict of interest.

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