News Release

Title

Association of alcohol intake and female gender with high expression of TMPRSS2 in tongue as potential risk for SARS-CoV-2 infection

Key Points

- ○ACE2 expression in the human tongue was lower than mouse tongue and TMPRSS2 expression was high in the human tongue.
- ○Female gender and alcohol intake were the significant factors to upregulate TMPRSS2 expression in the human tongue.
- ○There is a possibility that TMPRSS2 is more important than ACE2 regarding SARS-CoV-2 infection through mouth.

Summary

COVID-19 is pandemic since 2020 and further information is necessary on the risk factors associated with the infection of SARS-CoV-2. As an entry mechanism, SARSCoV-2 uses angiotensin-converting enzyme 2 (ACE2) as receptor and transmembrane serine protease-2 (TMPRSS2) to activate fusion with host plasma membrane **(Fig.1)**. Because dysgeusia is an early symptom of COVID-19, we here studied the expression of ACE2 and TMPRSS2 in the tongue and the associated tissues of mice and humans with immunohistochemistry and immunoblot analysis. ACE2 expression was low in the human tongue but was observed in the squamous epithelium, perineurium, arterial wall, salivary glands as well as taste buds. In contrast, mice showed high expression. In sharp contrast, TMPRSS2 expression was high in all the cells mentioned above in humans but relatively low in mice except for salivary glands. We then performed semi-quantitation of immunohistochemistry data of human ACE2 and TMPRSS2 and analyzed for age, sex, alcohol intake and smoking habit with logistic regression analysis. We found that alcohol intake and female gender were the significant risk factors for increasing TMPRSS2 expression. In conclusion, TMPRSS2 is an important factor to be considered regarding SARS-CoV-2 entry and amplification in the oral cavity, which is promoted through drinking habit.

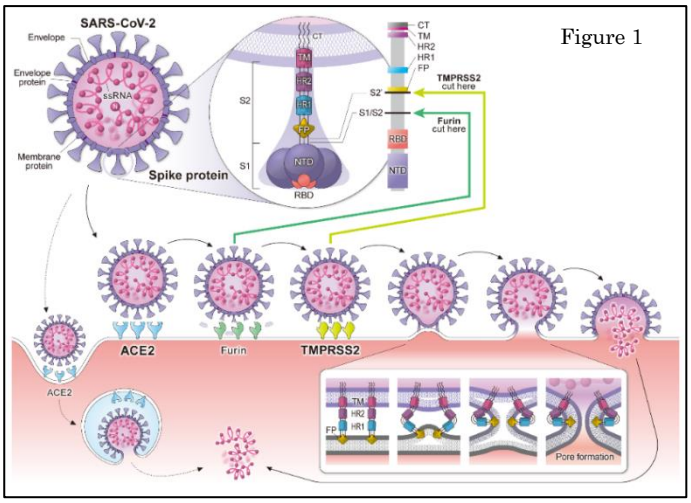

Figure 1: Schematic illustration of the molecular mechanisms of the entry of SARS-CoV-2 to the host cells. Receptor Binding Domain (RBD) of the spike (S) protein 1 of SARS-CoV-2 attaches to ACE2 and Furin cuts between S protein 1 and 2. Fusion Peptide (FP) of the S protein 2 is exposed thereafter based on the function of TMPRSS2, which can attach to the plasma membrane of host cells. Finally, S protein 2 itself alters its structure for the viral envelope to fuse with the plasma membrane of the host cell, which is followed by the release of viral genome into the host cytoplasm. Alternatively, the virus can enter the host cell through the endosomal pathway. CT, cytoplasmic tail; TM, transmembrane domain; HR, heptad repeat; NTD, N-terminal domain.

Research Background

Dysgeusia is a complaint in the 41.5% of COVID-19 patients, based on the systematic review of 20 studies [(1)]. Accordingly, oral mucosa and the associated organs may provide with an important relay base for the SARS-CoV-2 toward lower respiratory infection. Recently, Xu *et al.* reported based on public databases that ACE2 is was highly enriched in epithelial cells of tongue [(2)]. Sakaguchi *et al.* reported that ACE2 is expressed in the stratified squamous epithelium of the dorsal tongue and gingiva and that TMPRSS2 is strongly expressed in stratified squamous epithelium in the keratinized surface layer and detected in the saliva and tongue coating samples via western blot analysis [(3)]. Usami *et al.* showed ACE2 expression in human salivary gland [(4)]. However, their results are contradictory each other and none of them explained the molecular mechanism of dysgeusia.

In the present project, we studied the expression of ACE2 and TMPRSS2 in murine and human tongue and the associated tissue with immunohistochemistry and immunoblot analysis, and further analyzed the results with modifying factors in humans.

Research Results

We studied the expression of ACE2 and TMPRSS2 in the tongue tissue of mice and humans with immunohistochemistry and immunoblot analysis. We found that ACE2 was strongly expressed in the murine tongue, especially plasma membrane of stratified squamous epithelia, perineurium, arterial wall and luminal side of salivary glands **(Fig. 2A)**. In the human tongue, however, the expression of ACE2 in the squamous epithelium, perineurium and salivary glands was lower than those of mice **(Fig. 2B)**. As a positive control, luminal brush border membrane of renal tubular cells was intensely immunostained. Immunoblot analysis revealed similar results to those by immunohistochemical analysis and we could not obtain a specific band with human fresh samples **(Fig. 2C)**. In terms of taste buds in the tongue, the degree of ACE2 immunostaining was almost the same and moderate in mice and humans **(Fig. 2A, B)**.

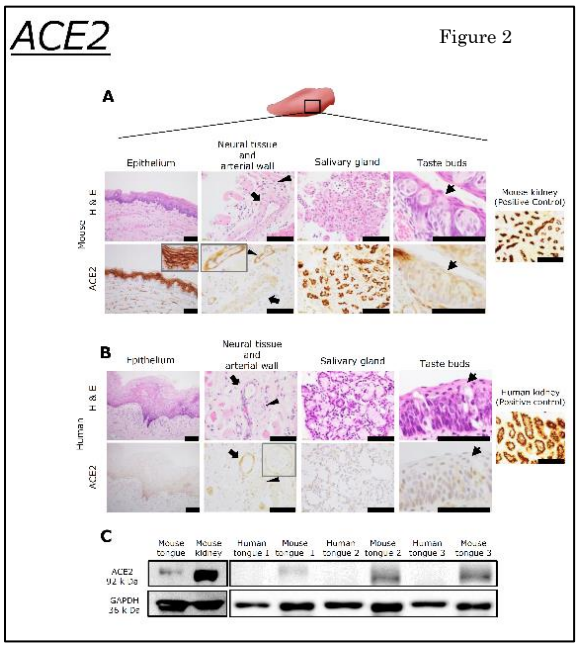

Figure 2: The ACE2 expression in the human and mouse tongue. (A) Mouse samples. Stratified squamous epithelium of dorsal tongue, perineurium of the peripheral nerve and salivary gland reveal high expression (scale bar = 100 μm; arrow head, peripheral nerve; thin arrow, taste buds; thick arrow, arterial wall). (B) Human samples. Stratified squamous epithelium of dorsal tongue, peripeurium of the peripheral nerve and arterial wall, salivary gland and taste buds are relatively low in expression (scale bar = 100 μm; arrow head, peripheral nerve; thin arrow, taste buds; thick arrow, arterial wall). (C) Immunoblotting. ACE2 expression in the mouse tongue (n = 3) was stronger than that of humans (n = 3).

The expression of TMPRSS2 in the mouse tongue was weak in the stratified squamous epithelium, perineurium and vessel wall. However, the immunostaining was strong in the cytoplasm and plasma membrane of salivary glands **(Fig. 3A)**. Remarkably, TMPRSS2 immunostaining was strong in humans in the stratified squamous epithelium, perineurium, vascular wall, salivary glands and taste buds **(Fig. 3B)**. Overall, TMPRSS2 expression was stronger in humans **(Fig. 3A, B)**. As a positive control, cytoplasm and plasma membrane of renal tubular cells were intensely immunostained. Immunoblot analysis revealed similar results to those by immunohistochemical analysis and we clearly obtained a specific band with human samples **(Fig. 3C)**.

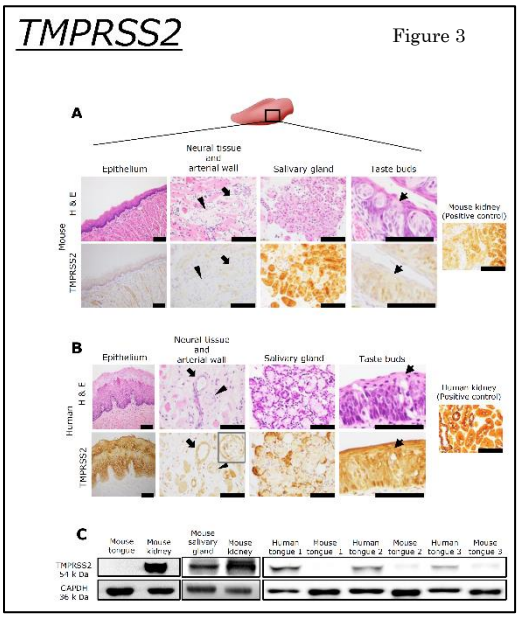

Figure 3: The TMPRSS2 expression in the human and mouse tongue. (A) Mouse samples. Salivary gland revealed high expression (scale bar = 100 μm; arrow head, peripheral nerve; thin arrow, taste buds; thick arrow, arterial wall). (B) Human samples. Stratified squamous epithelium of dorsal tongue, perineurium of the peripheral nerve and arterial wall, salivary gland and taste buds reveal high expression (scale bar = 100 μm; arrow head, peripheral nerve; thin arrow, taste buds; thick arrow, arterial wall). (C) Immunoblotting. TMPRSS2 expression in the human tongue (n = 3) was stronger than that of mice (n = 3).

To identify the upregulating factors of ACE2 and TMPRSS2 expression in the human tongue, we analyzed 38 human samples with the corresponding attributes. In terms of ACE2, the expression level was low, so we scored them as three groups (strong, moderate or weak) by two pathologists independently. Reproducibility of the two pathologists was 70.5% as κ coefficient, which was substantial. There were no significant differences for ACE2 expression among age, gender, smoking and alcohol intake. In contrast, regarding TMPRSS2, we found that female gender and alcohol intake were the significant two factors to upregulate the expression ($p < 0.05$) and that smoking habit (pack-years > 30) showed a tendency to increase TMPRSS2 expression in the tongue squamous epithelia ($p = 0.07$; **Table 1**).

Table 1

ACE2		
Characteristic		*p* Value
Age	< 50	0.95
	≧ 50	
Sex	Male	0.301
	Female	
Smoking	Pack-years< 30	0.969
	Pack-years≧ 30	
Alcohol	Yes	0.929
	No	

TMPRSS2				
Characteristic		Odds Ratio	95%CI	*p* Value
Age	< 50	0.512	0.084-3.103	0.466
	≧ 50			
Sex	Male	13.878	1.662-115.914	0.015*
	Female			
Smoking	Pack-years< 30	8.017	0.820-78.371	0.074
	Pack-years≧ 30			
Alcohol	**Yes**	7.048	1.005-49.426	0.049*
	No			

Table 1. Logistic regression analysis with attributes in humans.
(A) ACE2 and TMPRSS2 grade and clinical features of patients. We enrolled 38 patients as a retrospective study and 3 as a prospective study (total 41 patients).
(B) Logistic regression analysis.
Female gender and alcohol intake were the upregulating factors of TMPRSS2 in the tongue (*$p < 0.05$).
Smoking habit showed a tendency to increase TMPRSS2 expression in the tongue epithelium ($p = 0.07$).

Reference

(1) Ibekwe TS, Fasunla AJ, Orimadegun AE. Systematic Review and Meta-analysis of Smell and Taste Disorders in COVID-19. *OTO Open* 2020; **4**: 2473974X20957975.

(2) Xu H, Zhong L, Deng J, *et al*. High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral mucosa. *Int J Oral Sci* 2020; **12**: 8.

(3) Sakaguchi W, Kubota N, Shimizu T, *et al*. Existence of SARS-CoV-2 Entry Molecules in the Oral Cavity. *Int J Mol Sci* 2020; **21**.

(4) Usami Y, Hirose K, Okumura M, Toyosawa S, Sakai T. Brief communication: Immunohistochemical detection of ACE2 in human salivary gland. *Oral Sci Int* 2020. DOI: 10.1002/osi2.1085.

Publication

Kotaro Sato, Koki Fujii, Noriyuki Yamamoto, Norihisa Ichimura, Satoshi Yamaguchi, Hirohisa Yamada, Hideharu Hibi and Shinya Toyokuni. Association of alcohol intake and female gender with high expression of TMPRSS2 in tongue as potential risk for SARS-CoV-2 infection. JCBN 2022 September 71; 2: 129-135. DOI: 10.3164/jcbn.21-172.

Japanese ver.
https://www.med.nagoya-u.ac.jp/medical_J/research/pdf/Jou_220913.pdf