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# ACE2 Up-Regulated Expression and DNA Methylation in Gastric Diseases Associated with *Helicobacter Pylori* Infection: Implications for COVID-19

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## ABSTRACT

**OBJECTIVE:** Coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has broken out globally. As the SARS-CoV-2 receptor, ACE2 determines whether the virus can successfully infect the human body. *Helicobacter pylori* (H. pylori) infection is also a global health problem. We propose that *H. pylori* infection may up-regulate the expression of ACE2.

**DESIGN:** To test this hypothesis, we analyzed the data of gastric diseases associated with *H. pylori* infection, which come from website databases, including STRING, HPA, SMART, CBIOPORTAL, UCSC, GEPIA2, and GEO databases.

**RESULTS:** ACE2 was not expressed in four strains of *H. pylori*. Although the levels of RNA expression of ACE2 were 0.5 transcripts per million (pTPM) in the stomach, it was mainly detected in gastric glandular cells (50%). ACE2 expression was significantly up-graduated in stomach adenocarcinoma (STAD) ($P$<0.01). ACE2 alteration and DNA methylation were observed in STAD. DNA methylation across two CpGs (cg18877734, cg16734967) assayed for the ACE2 gene were higher in the STAD with *H. pylori* infection than those without *H. pylori* infection ($P$=9.20E-06, $P$=5.43E-03, respectively). According to the *H. pylori* infection, ACE2 significantly up-graduated expression was observed in chronic atrophic gastritis compared with gastritis in two gene expression profiles (GEO).

**CONCLUSION:** Up-regulation of ACE2 expression in *H.pylori* infection-associated gastric diseases may increase the body's susceptibility to SARS-CoV-2.

## INTRODUCTION

An outbreak of coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has rapidly spread world widely. ACE2 is the key receptor for SARS-CoV-2 to enter human cells, which is also expressed in the digestive tract, especially intestinal mucosa cells. Several studies have shown that different gastrointestinal symptoms in patients with SARS-CoV-2 infection, such as nausea, vomiting and diarrhea [1-3]. Therefore, it is necessary to pay attention to the possibility of SARS-CoV-2 transmission through the gastrointestinal tract other than the respiratory tract. The high infection rate of

*Helicobacter pylori* (*H. pylori*), especially in developing countries, is still a global health problem, with the highest rate in Africa (79.1%) and the lowest rate in Oceania (24.4%) [4]. Long-term *H. pylori* infection causes diseases including chronic active gastritis, chronic atrophic gastritis-peptic ulcers, B cell lymphoma of mucosa-associated lymphoid tissue, and adenocarcinoma. *H. pylori* produces corresponding pathological results through changes in human genes expression and DNA methylation.

In the event of severe global virus outbreak, identifying the potential risk factors for SARS-CoV-2 infection is imminent, especially the adverse effects of multiple infections with other bacteria or viruses. By analyzing the changes of ACE2 expression in *H. pylori*-associated gastric diseases, we explored the possible impact of *H. pylori* infection on human susceptibility to SARS-CoV-2, and consider reducing the susceptibility and harmfulness of the virus from a new perspective. All data and analysis were based on website databases and online analysis tools.

## MATERIALS AND METHODS

### ACE2 occurrence in different species

ACE2 occurrence in different species was obtained from STRING (https://string-db.org/, Version 11.0). It includes 24584628 proteins from 5090 organisms with 3123056667 interactions.

### RNA and protein expression of ACE2 in human being

From the human protein atlas (HPA) website (https://www.proteinatlas.org/), the information regarding the expression profiles of human genes both on the mRNA and protein level. And the protein expression data is derived from antibody-based protein profiling using immunohistochemistry from 44 normal human tissue types. The consensus normalized expression (NX) levels of RNA expression and the protein expression (score) of ACE2 were obtained. NX levels were created by combining the data from the HPA data, the GTEx portal (https://commonfund.nih.gov/GTEx/), and functional annotation of the FANTOM5 project (https://fantom.gsc.riken.jp/5/).

### DNA methylation

The SMART (http://www.bioinfo-zs.com/smartapp/) App is a user-friendly and easy-to-use web application for comprehensively analyzing the DNA methylation data of TCGA (https://portal.gdc.cancer.gov/) project.

### ACE2 alteration in gastric cancer

Visualization results of ACE2 alteration were obtained from CBIOPORTAL (https://www.cbioportal.org/) for cancer genomics. It includes 440 patients with stomach adenocarcinoma (STAD) from TCGA and PanCancer Atlas (https://www.cell.com/pb-assets/consortium/PanCancerAtlas/PanCani3/index.html).

### *H. pyolri* infection and ACE2 DNA methylation in gastric cancer

Phenotypic analysis for *H. pylori* infection in patients with gastric cancer was performed in website of UCSC Xena platform (https://xenabrowser.net/heatmap/), which can deal public and private cancer genomics data visualization and interpretation.

### Survival analysis of ACE2

Gene (ACE2) was defined a significantly change with adjustment *P*-values (adj.*P*.Val) <0.05 and $\log_2$ fold change ($\log_2$FC) ≥1 (up-regulated) or ≤-1 (down-regulated) .The Kaplan-Meier plotter is an online tool applied to assess the effect of genes on survival using STAD data in GEPIA2 website (http://gepia2.cancer-pku.cn/#index). *P*<0.01 was considered to indicate a statistically significant result.

### *H. pyolri* infection and ACE2 in gastritis

ACE2 expression analyzed in gastritis with or without atrophy was obtained from the GEO database (https://www.ncbi.nlm.nih.gov/geo/). A total of 724 series about *H. pylori* with Homo sapiens were retrieved from the database. After a careful review, two gene expression profiles (GSE27411 and GSE116312) were selected. Among them, GSE27411 was based on the GPL6255 platform (Illumina humanRef-8 v2.0 expression beadchip), and GSE116312 was based on the GPL6244 platform (Affymetrix Human Gene 1.0 ST Array). GEO2R (http://www.ncbi.nlm.nih.gov/geo/geo2r) is an interactive network analysis tool of the GEO database based on soft. ACE2 gene was screened by the tool of GEO2R to select significantly change with adj.*P*.Val <0.05 and $\log_2$FC) ≥1 or ≤-1 in the two sets.

All of the data were freely available online, and no experiment on humans or animals was involved in this study. No ethical review was required.

## RESULTS

There is no ACE2 cooccurrence in four strains of *H. pylori* (SA213A, 26695, J99, SouthAfrica7). ACE2 and neurotensin (NTS) were observed coexpression in human being (RNA coexpression score=0.065). But 19 different genes were observed a coexpression with ACE2 in other organisms. A network analysis showed that at least 8 genes (GHRL, NTS, AGT, CAT, AAMP, CALM3, CALM1, CALM2) had a close interaction with ACE2.

RNA expression of ACE2 was detected in many organs, and enriched in the intestine. Selective membranous expression of protein were observed in renal tubules, intestinal tract, gallbladder, testis, etc, but not been detected in normal stomach tissue. And the NX levels of RNA expression were 0.5 transcripts per million (pTPM), including 4.1 pTPM generated by the HPA and 0.4 pTPM generated by the GTEx. RNA expression was mainly detected in gastric glandular cells compared with other kind of cell types in one sample with the highest pTpM.

The circular plot displayed the chromosomal distribution of all associated CpGs of the ACE2 in STAD data (Figure 3A). About 2.3% sample with both methylation and somatic mutation for

ACE2 (391 patients). The mean methylation (beta-value) among STAD with different copy number variations (CNV) of ACE2 was significantly different using aggregation method (*P*=9.40E-04), including significantly different values in two individual CpGs (cg05748796, cg18458833) (*P*=1.10E-04, *P*=6.70E-03, respectively). Two probes (cg05748796, cg08559914) showed a negative correlation between ACE2 expression and DNA methylation (*P*=2.80E-06, *P*=0.03), and a positive correlation according to one probe (cg05039749, *P*=7.40E-03), with an aggregation difference in total (R=-0.2, *P*=1.40E-04).

ACE2 is altered in 19 (4%) of 440 gastric cancer, including missense mutation (unknown significance), truncating mutation (unknown significance), amplification, and deep deletion. All amplification was observed in male patients. The frequency of somatic mutation was 2.5%, and post translational modifications (PTMs) included three types (Phosphorylation, Acetylation, N-linked Glycosylation). The most frequent mutation was *N338* and *N660Ifs*. In addition to gene amplification and deep deletion, ACE2 mutations were mainly observed in STAD, which distributed across all exons of ACE2 without hot spot mutation site. The alteration type was different in different types of gastric cancer such as signet ring cell carcinoma only with deep deletion. The levels of mRNA expression were showed according to the mutation types and copy number alteration in.

There was no statistical difference between the STAD with *H. pylori* infection and the STAD without *H. pylori* infection according to the copy number segment (*P*=0.9419) or the copy number (gene-level) (*P*=0.7163) of ACE2. However, DNA methylation (Illumina Human Methylation 450) across two CpGs (cg18877734, cg16734967) assayed for the ACE2 gene were higher in the STAD with *H. pylori* infection than those without *H. pylori* infection (*P*=9.20E-06, *P*=5.43E-03, respectively. There was no statistical difference between the STAD with *H. pylori* infection and those without *H. pylori* infection according to RNAseq HTSeq (*P*=0.9105), HTSeq-FPKM (*P*= 0.9561), or HTSeq-FPKM counts (*P*=0.8921) of ACE2 expression.

ACE2 was up-regulated in the tumor tissue (408 cases) than in the normal tissue (211 cases) in the data sets of stomach adenocarcinoma (STAD), matched the TCGA normal and GTEx data (*P*<0.01, $\log_2$FC>1). But there was no statistical difference in the expression of ACE2 in different stages of STAD. According to the median as the cutoff value, the difference in expression of ACE2 did not affect the overall survival, nor did it affect the disease free survival .

ACE2 was up-regulated in 6 samples with both *H. pylori* infection and atrophy compared with 6 samples without *H. pylori* infection (adj.*P*.Val=1.40E-02, $\log_2$FC=3.593). According to the *H. pylori* infection, ACE2 was up-regulated in 6 samples with atrophy compared with 6 sample without atrophy (adj.*P*.Val=5.39E-04, $\log_2$FC=3.596). And ACE2 was also up-regulated in 3 samples with chronic atrophic gastritis compared with 7 sample only with gastritis (adj.*P*.Val=1.47E-03, $\log_2$FC=3.085).

## DISCUSSION

After *H. pylori* infection, there would be certain genes fusion or up-graduated expression, which played a leading role in the outcomes of diseases[5,6]. AECE2 was not expressed in all four strains of *H. pylori*, so the changes of ACE2 in gastric cells was not based on the gene fusion. Compared with other organisms, there was less coexpression with other genes in human being. Although the level of RNA expression was low in normal gastric tissue, half of those were detected in gastric glandular cells. This might affect the susceptibility of gastric epithelial cells to SARS-CoV-2.

*H. pylori* may promote canceration by inducing abnormal methylation of gastric epithelial cells [7-9]. DNA methylation accumulation was associated with molecular irreversibleness and gastric carcinogenesis even after *H. pylori* eradication [10,11]. Except aging and diet, *H. pylori* infection had been identified infection as a main pathogenic factor in the aberrant methylation process in gastric epithelial [12]. Subtypes of gastric cancer showed distinct carcinogenic pathways influenced by *H. pylori* strains [13], and a strong association between gene (CDH1) methylation and *H. pylori*-cagA (+) in intestinal-type gastric cancer [14]. However, the relationship of *H. pylori*, ACE2 expression and DNA methylation was not found. The idea that ACE2 has similar performance in stomach adenocarcinoma caused by *H. pylori* infection is worthy of recognition. In this research, the aggregation beta value of DNA methylation were significant difference according to ACE2 alteration (*P*=9.40E-04). Different types of ACE2 mutations were accompanied by different mRNA expressions, and there were significant differences in ACE2 mutations between different types of STAD in our report. Based on high infection rate of *H. pylori* and high harmfulness of SARS-CoV-2, the difference in ACE2 expression between different types of STAD caused by *H. pylori* infection requires further attention.

Stratifying data by *H. pylori* infection status, a trend for higher median ACE2 copy number in *H. pylori* infection patients. Notably, two of 8 probes detection showed DNA hyper-methylation in *H. pylori* infection patients, which suggested that DNA methylation may be one of the causes due to abnormal expression of ACE2 according to *H. pylori* infection. Further studies are needed to explore other reasons, such as glycosylation and histone modification [14] influence ACE2 expression, considering the factor of *H. pylori* infection.

ACE2 has been proved to be an important regulator in the process of various tumorigenesis in some cancers [17-19]. But ACE2 expression level was not significantly different in different tumor stages or survival time in STAD.

Chronic atrophic gastritis is a very important and common asymptomatic disease because of a potential risk to develop into gastric

cancer in many patients. Although the causes of gastric atrophy included *H. pylori* infection and autoimmune, the latter was relatively rare globally [20,21]. Furthermore, some studies showed that *H. pylori* also played an etiological role for autoimmune gastritis [22, 23]. According to the *H. pylori* infection, ACE2 significantly up-graduated expression was observed in chronic atrophic gastritis compared with gastritis in the two GEO databases.

According to gastric diseases associated with *H. pylori* infection, we firstly summarized the up-regulated ACE2 expression and DNA hyper-methylation. Based on the high prevalence of *H. pylori* and the susceptibility and pathogenicity of the SARS-CoV-2, our research may play an important role in the prevention and treatment of COVID-19. Up to now, the end point of the chronic infection of *H. pylori* and the continued existence of the SARS-CoV-2 cannot be determined. And this might become a new reason for treatment of *H. pylori*. In addition, we hope the problem of co-infection of other bacteria or viruses with SARS-CoV-2 should be concerned. Of course, further experimental design to research this problem was essential, after all, our research conclusions were based on the analysis of network databases.

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