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The accessible promoter-mediated supplementary effect of ACE2 provides new insight into the tissue tropism of SARS- CoV-2

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Abstract

Background: Angiotensin-converting enzyme 2 (ACE2) has been confirmed to be a receptor for the newly discovered severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). However, cell surface ACE2 expression is reported to be inconsistent with clinical tissue tropism of SARS-CoV-2, which complicates understanding of the pathogenesis of 2019 novel coronavirus disease (COVID-19). The consumption of ACE2 by internalization and shedding processes may explain this discordance.

Results: To understand the discordance between ACE2 expression and the tissue tropism of SARS-CoV-2, we examined the chromatin accessibility of ACE2 promoter

in hundreds of tissues and cell lines using public DNase-seq and assay for transposase-accessible chromatin with high throughput sequencing (ATAC-seq) data. We find that ACE2 promoter is only accessible in three tissues including lung, large intestine and placenta. Also, we examined tumors tissues and ACE2 promoter is observed accessible in five tumors with reported SARS-CoV-2 susceptibility. We confirmed the susceptibility by performing SARS-CoV-2 pseudovirus infection in several cell lines.

Conclusions: We propose that open chromatin at the promoter mediates the ACE2 supplementary effect and ensures the entry of SARS-CoV-2. This hypothesis provides a new view and potential clues for further investigation of COVID-19 pathogenesis.

Introduction

The emerging novel betacoronavirus, known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has caused major outbreaks in many parts of the world and threatens public health [1]. The World Health Organization has declared that 2019 novel coronavirus disease (COVID-19), caused by SARS-CoV-2, has created a public health emergency of international concern. Similarly to SARS-CoV [2, 3], SARS-CoV-2 employs angiotensin-converting enzyme 2 (ACE2) as a receptor for cellular entry [4]. However, previous studies have documented generally low ACE2 expression in multiple tissues, including lung and airway epithelium [5-7]. We also confirmed this observation using genotype-tissue expression [8] RNA sequencing (RNA-seq) data (Fig. S1 and Table S1). The discordance between ACE2 expression and SARS-CoV-2 tropism complicates the understanding of the infection, spread, and clearance of this

virus. One possible explanation is that ACE2 expression is under-detected due to technical dropout effects, especially in single-cell RNA sequencing (scRNA-seq) [5].

The state of chromatin dictates fundamental cellular processes, including gene expression [9]. Accessible chromatin has long been known to mark regulatory sequences and to interact with transcription factors to execute transcriptional programs instructing cell fate determination and development [10, 11]. The activation of genes generally correlates positively with increased promoter accessibility [12]. Thus, the examination of chromatin accessibility will help to reveal the transcriptional activity and cell state. Here, by investigating the chromatin accessibility of the ACE2 gene in multiple datasets from different tissues and cell lines, we show that the ACE2 promoter is accessible in limited tissues and cells, and that this tissue-specific pattern is quite accordant with clinical findings. We propose that open chromatin at the promoter mediates the ACE2 supplementary effect and further induces SARS-CoV-2 tropism.

Methods

SARS-CoV-2 pseudovirus infection assays

Cells were seeded at a density of 1×10^4 /well in 96-well plates 16 h before infection. Cells in each well were then infected with SARS-CoV-2 pseudovirus expressing luciferase at a multiplicity of infection of 10. The culture medium was changed 12 h post-infection, and luciferase activity was detected at 72 h post-infection using the Steady-Lumi™ II Firefly Luciferase Assay Kit (Beyotime). Cell was purchased

from *ATCC* (*ATCC* number: CCL-10)

Results

The ACE2 promoter is accessible in limited human tissues, including lung tissue

As previously reported, ATAC-seq and DNase-seq approach as been recognized as the best measures of chromatin accessibility [13]. To investigate the chromatin accessibility of ACE2, we developed a pipeline by simply mapping ATAC-seq peaks and DNase I hypersensitivity sites (DHSs) to bin-based genomic regions. Briefly, genomic region chrX: 15576000–15622500 (Gencode V19), covering the ACE2 gene locus, was split by 500 bp/bin, and accessible bins were defined as those overlapping with ATAC-seq peaks and DHSs by at least one base pair. We first examined ACE2 accessibility in 33 human tissue types (Table S2). A non-coding region (chrX: 1558300–1558400) was accessible in almost all tissues except those from the brain and thymus, indicating the importance of chromatin regulation by this region. In contrast, the promoter region of ACE2 (chrX: 15619500–15620000) was accessible only in lung, large intestine, and placenta tissue. These observations are consistent with the tissue tropism observed in previous studies. First, as a respiratory virus, SARS-CoV-2 targets primarily the lung. Second, unexpected diarrhea was reported in patients with COVID-19 [14], and most affected patients exhibited positivity in their stool, which persisted in some patients even after the viral RNA load had decreased to an undetectable level in the respiratory tract [15]. Third, ACE2 expression is noticeable in certain placental/decidual cell types without transmembrane serine protease 2 (TMPRSS2) [5]. In addition, there are clinical

reports suggesting the occurrence of vertical SARS-CoV-2 transmission, e.g. a neonate born to a mother with COVID-19 had elevated antibody levels and abnormal cytokine test results 2 hours after birth [16], an infant whose mother had been infected with SARS-CoV-2 showed positivity at 36 h after birth [17].

Appropriate activities of gene promoters are essential for coordinated transcription within a cell [13]. Chromatin accessibility at the promoter represents potency in terms of the ability to execute transcriptional programs. Thus, this specificity of chromatin accessibility at the ACE2 promoter site provides important hints about the tissue tropism of SARS-CoV-2 infection.

The ACE2 promoter is specifically accessible in SARS-CoV-2-susceptible tumors

In contrast to normal tissues, cancer tissues are highly heterogeneous, with each tumor type exhibiting distinct clinical features and associated with different patient outcomes and therapeutic responses [11]. The examination of chromatin accessibility enables the identification of cancer-specific DNA regulatory elements and the classification of tumor subtypes [11]. Due to the systemic immunosuppressive state caused by malignancy and anticancer treatments, patients with cancer are more susceptible to COVID-19 infection [18-20]. Thus, chromatin accessibility at the ACE2 promoter site in cancer cells needs to be investigated. We investigated ATAC-seq data for 23 types of tumor tissue from The Cancer Genome Atlas project [21]. The promoter of ACE2 was

accessible in five (22%) tumor tissue samples (lung adenocarcinoma, colon adenocarcinoma, adrenocortical carcinoma, breast invasive carcinoma, and thyroid carcinoma; Fig. 2A and Table S3); this proportion was much higher than that in normal tissues (5/33 [15%]; Fig. 1). The accessibility of the ACE2 promoter in these five tumor types is consistent with a recent clinical report, which stated that 18 of 1590 individuals with COVID-19 had histories of cancer, including 5 with lung cancer, 3 with breast cancer, 3 with colorectal carcinoma, 1 with adrenal neoplasm, and 1 with papillary thyroid microcarcinoma histories [19]. We also examined DHS data for 63 cancer cell lines from the Encyclopedia of DNA Elements project [22] (Table S4). Similarly, the percentage of cancer cell lines (10/63 [16%]; Fig. 2B) with ACE2 promoter accessibility was much higher than that in normal cell lines (11/126 [9%]; Fig. 2C and Table S5). Furthermore, we conducted a SARS-CoV-2 pseudovirus infection experiment to examine susceptibility to this virus. Four cancer cell lines (HepG2 and Caco-2 with DNase-seq peaks in the ACE2 promoter, K562 and Jurkat without) and the normal cell line BHK21 (background) were infected with HIV-based pseudovirus bearing the SARS-CoV-2 Spike protein. Luciferase activity in these cells was detected at 72 h post infection. As expected, we observed that the HepG2 and Caco-2 cell lines were susceptible to the SARS-CoV-2 pseudovirus, and, K562 and Jurkat were unsusceptible (Fig. 2D).

A model of the accessible promoter-mediated supplementary effect of ACE2

ACE2 has been reported to be the surface cellular receptor for SARS-CoV and SARS-CoV-2 cell entry [23, 24]. However, as endocytosis is essential for the establishment of virus entry, ACE2 is internalized together with SARS-CoV upon infection [2, 25-27]. In addition, ACE2 undergoes ectodomain shedding, making it subject to juxtamembrane cleavage events [28, 29]. Thus, ACE2 expression on cell surfaces is dynamic, with new ACE2 supplementing diminished ACE2 cell surface expression. Here, we propose an accessible promoter-based model to show the potential supplementary mechanism of ACE2 cell surface expression, using the lung as an example (Fig. 3). Accessible chromatin at the ACE2 promoter site provides templates for transcription factor binding, and transcription factors then recruit cofactors and Pol II for transcription initiation. Thus, accessibility of the ACE2 promoter is required for continuous transcription to compensate cell surface ACE2, which is consumed by internalization and shedding processes.

Discussion

A fundamental phenomenon in SARS-CoV-2 infection is the discordance between virus tropism and ACE2 receptor expression, as measured by RNA-seq or scRNA-seq. Here, we report on a systematic survey of accessible chromatin of ACE2 in hundreds of tissues and cell lines. Our results reveal that the promoter of ACE2 has limited accessibility, restricted to SARS-CoV-2-susceptible tissues and cell lines. Due

to the contribution of accessible chromatin to the establishment of transcriptional programs, open chromatin at the ACE2 promoter site ensures the compensation of cell surface ACE2. This result provides new insight into the tissue tropism of SARS-CoV-2. Further investigations with additional experiments are warranted to fully confirm this hypothesis.

Supplemental information

Supplemental Information can be found online at the website.

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Author contributions

X. B., H. C. and H. L. conceived the project. Q.J., G.D., J.W., X.T., Y.D., F.L., Y.S., H.T. and M.L. conducted the investigations and data mining. X.W. conducted the pseudovirus infection experiment. Q.J., G.D. and J.W. wrote the paper.

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173 **Ethics approval and consent to participate**

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179 **Competing interests**

180 The authors declare that they have no competing interests

181 **References**

- 182 1. Lee Elizabeth C. WNI, Grabowski M Kate., Gurley Emily S., Lessler
183 Justin.(2020). The engines of SARS-CoV-2 spread. : **The engines of SARS-**

- 184   **CoV-2 spread.** *Science (New York, NY)*, **370**(6515):406-407.
- 185   2.   Li W, Moore MJ, Vasilieva N, Sui J, Wong SK, Berne MA, Somasundaran M,
186   Sullivan JL, Luzuriaga K, Greenough TC *et al*: **Angiotensin-converting**
187   **enzyme 2 is a functional receptor for the SARS coronavirus.** *Nature* 2003.
- 188   3.   Matsuyama S, Nagata N, Shirato K, Kawase M, Takeda M, Taguchi F: **Efficient**
189   **Activation of the Severe Acute Respiratory Syndrome Coronavirus Spike**
190   **Protein by the Transmembrane Protease TMPRSS2.** *Journal of Virology*
191   2010, **84**(24):12658-12664.
- 192   4.   Clausen TM, Sandoval DR, Spliid CB, Pihl J, Perrett HR, Painter CD,
193   Narayanan A, Majowicz SA, Kwong EM, McVicar RN *et al*: **SARS-CoV-2**
194   **Infection Depends on Cellular Heparan Sulfate and ACE2.** *Cell* 2020,
195   **183**(4):1043-1057 e1015.
- 196   5.   Sungnak W, Huang N, Bécavin C, Berg M, Queen R, Litvinukova M, Talavera-
197   López C, Maatz H, Reichart D, Sampaziotis F *et al*: **SARS-CoV-2 entry factors**
198   **are highly expressed in nasal epithelial cells together with innate immune**
199   **genes.** *Nature Medicine* 2020.
- 200   6.   Zou X, Chen K, Zou J, Han P, Hao J, Han Z: **Single-cell RNA-seq data analysis**
201   **on the receptor ACE2 expression reveals the potential risk of different**
202   **human organs vulnerable to 2019-nCoV infection.** *Frontiers of medicine*
203   2020.
- 204   7.   Qi F, Qian S, Zhang S, Zhang Z: **Single cell RNA sequencing of 13 human**

205   **tissues identify cell types and receptors of human coronaviruses.**
206   *Biochemical and biophysical research communications* 2020, **526**(1):135-140.

207   8.   Mele M, Ferreira PG, Reverter F, DeLuca DS, Monlong J, Sammeth M, Young
208   TR, Goldmann JM, Pervouchine DD, Sullivan TJ *et al*: **Human genomics. The**
209   **human transcriptome across tissues and individuals.** *Science (New York, NY)*
210   2015, **348**(6235):660-665.

211   9.   Kouzarides T: **Chromatin Modifications and Their Function.** *Cell* 2007,
212   **128**(4):0-705.

213   10.   Rivera CM, Ren B: **Mapping Human Epigenomes.** *Cell* 2013, **155**(1):39-55.

214   11.   Corces MR, Granja JM, Shams S, Louie BH, Seoane JA, Zhou W, Silva TC,
215   Groeneveld C, Wong CK, Cho SW *et al*: **The chromatin accessibility**
216   **landscape of primary human cancers.** *Science (New York, NY)* 2018,
217   **362**(6413):eaav1898.

218   12.   Fenouil R, Cauchy P, Koch F, Descostes N, Cabeza JZ, Innocenti C, Ferrier P,
219   Spicuglia S, Gut M, Gut I: **CpG islands and GC content dictate nucleosome**
220   **depletion in a transcription-independent manner at mammalian promoters.**
221   *Genome Research* 2012, **22**(12):2399-2408.

222   13.   Andersson R, Sandelin A: **Determinants of enhancer and promoter activities**
223   **of regulatory elements.** *Nature reviews Genetics* 2020, **21**(2):71-87.

224   14.   Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, Liu L, Shan H, Lei CL, Hui
225   DSC *et al*: **Clinical Characteristics of Coronavirus Disease 2019 in China.**

226 *The New England journal of medicine* 2020.

227 15. Xiao F, Tang M, Zheng X, Li C, He J, Hong Z, Huang S, Zhang Z, Lin X, Fang
228 *Z et al: Evidence for gastrointestinal infection of SARS-CoV-2. medRxiv*
229 2020.

230 16. Dong L, Tian J, He S, Zhu C, Wang J, Liu C, Yang J: **Possible Vertical**
231 **Transmission of SARS-CoV-2 From an Infected Mother to Her Newborn.**
232 *Jama* 2020.

233 17. Chen H, Guo J, Wang C, Luo F, Yu X, Zhang W, Li J, Zhao D, Xu D, Gong Q
234 *et al: Clinical characteristics and intrauterine vertical transmission*
235 **potential of COVID-19 infection in nine pregnant women: a retrospective**
236 **review of medical records.** *Lancet (London, England)* 2020, **395**(10226):809-
237 815.

238 18. Sepkowitz KA: **Nosocomial infections in patients with cancer.** *Lancet*
239 *Oncology* 2009, **10**(6):589-597.

240 19. Liang W, Guan W, Chen R, Wang W, Li J, Xu K, Li C, Ai Q, Lu W, Liang H *et*
241 *al: Cancer patients in SARS-CoV-2 infection: a nationwide analysis in*
242 **China.** *The Lancet Oncology* 2020, **21**(3):335-337.

243 20. Jie-Yao, Li, Xiu-Fang, Duan, Li-Ping, Wang, Yu-Jie, Xu, Lan, Huang: **Selective**
244 **depletion of regulatory T cell subsets by docetaxel treatment in patients**
245 **with nonsmall cell lung cancer.** *Journal of Immunology Research* 2014.

246 21. Weinstein JN, Collisson EA, Mills GB, Shaw KR, Ozenberger BA, Ellrott K,

247 Shmulevich I, Sander C, Stuart JM: **The Cancer Genome Atlas Pan-Cancer**
248 **analysis project.** *Nature genetics* 2013, **45**(10):1113-1120.

249 22. Dunham I, Kundaje A, Aldred SF, Collins PJ, Davis CA, Doyle F, Epstein CB,
250 Fietze S, Harrow J, Kaul R *et al*: **An integrated encyclopedia of DNA**
251 **elements in the human genome.** *Nature* 2012, **489**(7414):57-74.

252 23. Wang Q, Zhang Y, Wu L, Niu S, Song C, Zhang Z, Lu G, Qiao C, Hu Y, Yuen
253 KY *et al*: **Structural and Functional Basis of SARS-CoV-2 Entry by Using**
254 **Human ACE2.** *Cell* 2020.

255 24. Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S,
256 Schiergens TS, Herrler G, Wu N-H, Nitsche A *et al*: **SARS-CoV-2 Cell Entry**
257 **Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven**
258 **Protease Inhibitor.** *Cell* 2020.

259 25. Holmes KV: **Human Coronavirus HCoV-229E Enters Susceptible Cells via**
260 **the Endocytic Pathway.** *Oxygen Transport to Tissue XXXIII* 2001, **494**:193-
261 198.

262 26. Inoue Y, Tanaka N, Tanaka Y, Inoue S, Morita K, Zhuang M, Hattori T,
263 Sugamura K: **Clathrin-dependent entry of severe acute respiratory**
264 **syndrome coronavirus into target cells expressing ACE2 with the**
265 **cytoplasmic tail deleted.** *J Virol* 2007, **81**(16):8722-8729.

266 27. Wang H, Yang P, Liu K, Guo F, Zhang Y, Zhang G, Jiang C: **SARS coronavirus**
267 **entry into host cells through a novel clathrin- and caveolae-independent**

268   **endocytic pathway.** *Cell research* 2008, **18**(2):290-301.

269   28.   Jia HP, Look DC, Tan P, Shi L, Hickey M, Gakhar L, Chappell MC, Wohlford-
270   Lenane C, McCray PB, Jr.: **Ectodomain shedding of angiotensin converting**
271   **enzyme 2 in human airway epithelia.** *American journal of physiology Lung*
272   *cellular and molecular physiology* 2009, **297**(1):L84-96.

273   29.   Lambert DW, Yarski M, Warner FJ, Thornhill P, Parkin ET, Smith AI, Hooper
274   NM, Turner AJ: **Tumor necrosis factor-alpha convertase (ADAM17)**
275   **mediates regulated ectodomain shedding of the severe-acute respiratory**
276   **syndrome-coronavirus (SARS-CoV) receptor, angiotensin-converting**
277   **enzyme-2 (ACE2).** *The Journal of biological chemistry* 2005, **280**(34):30113-
278   30119.

279

Figures

Figure 1

Landscape of open chromatin for the ACE2 gene in tissues Genomic region chrX:15576000622500(human genome: hg19), covering the ACE2 gene locus ,was split by 500bp/bin. ACE2 accessible regions were identified as genomic bins overlapping with DH Speaks for each tissue. Accessible regions at the ACE2 promoter site were specifically open in tissue from the lung, large intestine ,and placenta (black boxes).

Figure 2

Open chromatin for the ACE2 promoter in different tissues and cell lines. (A) DNase-seq peaks detected in the ACE2 promoter for tumor tissues obtained from TCGA project. (B, C) DNase-seq peaks detected in the ACE2 promoter for cancer cell lines (B) and normal cell lines (C) obtained from the ENCODE project. (D) The HepG2 and Caco-2 cell lines with DNase-seq peaks in the ACE2 promoter were susceptible to SARS-CoV-2 pseudovirus. Susceptibility was defined by luciferase activity exceeding three times that in unsusceptible BHK21 cells (dashed line).

Accessible promoter mediated compensatory effect of *ACE2* provides new insights into the tissue tropism of SARS-CoV-2

Figure 3

Hypothetical model in which open chromatin at the promoter mediates ACE2 supplementation and ensures the entry of SARS-CoV-2

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