

The potential effect of the angiotensin-converting enzyme 2 (ACE2) receptor of 2019-nCoV on lung adenocarcinoma patients

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Research

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45 **Abstract**

46 **Background:** The 2019-nCoV epidemic is the public health emergency that has had the greatest
47 impact on the world. Our study aimed to better understand the underlying mechanisms and
48 function of angiotensin-converting enzyme 2 (ACE2) receptor of 2019-nCoV on lung
49 adenocarcinoma patients (LUAD), and provide a theoretical basis for early diagnosis, prognosis
50 and targeted therapy of 2019-nCoV.

51 **Methods:** This study focuses on the expression level, functions, mutation rate, and copy number
52 variations (CNVs) of ACE2 in LUAD using an extensive bioinformatics data mining process. The
53 interaction between ACE2 expression and clinical-pathological parameters of patients with LUAD
54 was investigated using UALCAN. Also, the essential biological features, single nucleotide
55 variations (SNVs), CNVs, and pathway activities of genes interacting with ACE2 in these cancers
56 were further analyzed.

57 **Results:** We found that ACE2 expression in LUAD patients increased with age, but it was not
58 related to cancer status, patient's race, patient's gender, or patient's smoking habits. Moreover,
59 our results showed that compared to that in normal tissues, ACE2 was highly expressed in colon
60 adenocarcinoma (COAD), kidney renal papillary cell carcinoma (KIRP), pancreatic
61 adenocarcinoma (PAAD), rectum adenocarcinoma (READ), and stomach adenocarcinoma (STAD).
62 However, there is no significant difference in the expression of ACE2 in patients of different ages.

63 **Conclusions:** These findings demonstrate the importance of ACE2 in LUAD, and provide insights
64 into the regulatory mechanisms and function of ACE2.

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67 **Keyword:** 2019-nCoV, ACE2, expression, lung adenocarcinoma, clinical-pathological parameters

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89 **Background**

90 During the Spring Festival of 2020, the outbreak of pneumococcal infection caused by the
91 2019 coronavirus disease (2019-nCoV) spread rapidly in China and many countries. Subsequently,
92 the virus was found to spread through interpersonal communication was found when a group of
93 2019-nCov patients with a Wuhan travel record of patients appeared to travel more cities in
94 China [1-3]. On January 21, 2020, The World Health Organization (WHO) announced that
95 2019-nCoV could be maintained through interpersonal communication [WHO.
96 <https://www.who.int/emergencies/diseases/novel-coronavirus-2019>]. By July 19, the National
97 Health Commission had received reports of 83,682 confirmed cases and 4,634 deaths in 31
98 provincial-level regions and the Xinjiang Production and Construction Corps on the Chinese
99 mainland, and in all 78,799 patients had been cured and discharged from hospital
100 (http://en.nhc.gov.cn/2020-03/11/c_77599.htm). The epidemic spreading all over China has
101 brought strong shock. In the face of the new coronavirus epidemic, the whole country has made
102 concerted efforts to treat patients and prevent its spread. With the accumulation and analysis of
103 more cases, people's understanding of 2019-nCoV is gradually deepening. To date, there is still no
104 specific medicine for 2019-nCoV, and it is urgent to conduct in-depth research on its pathogenic
105 mechanisms to promote the development of effective control measures. To prevent further
106 spread of the epidemic, a nationwide campaign was launched.

107 2019-nCoV infection of the human body requires binding to receptors expressed by host cells,
108 and although the virus can invade the human body in many different ways, invading the lungs
109 through the respiratory tract and causing severe pneumonia is still the main mode. Previous
110 studies demonstrated that angiotensin-converting enzyme 2 (ACE2) is a receptor for SARS
111 coronavirus (SARS-CoV) and the novel coronavirus 2019-nCoV/SARS-CoV-2 [4, 5]. As reported,
112 ACE2 is expressed in the lung, heart, kidney, and intestine [6]. Zhang H et al. revealed that ACE2
113 was not only the entry receptor of the virus, but also had a protective effect on lung injury [7].
114 Moreover, Hofmann, H. et al. revealed a positive correlation between ACE2 expression and
115 SARS-CoV infection in vitro [8]. Recent work has indicated that Asian males may have higher
116 expression levels of ACE2 [9]. However, Chen, Y. et al. found that there was no significant
117 difference in the expression of ACE2 in Asians compared with other races, but the expression was
118 positively correlated with age [10]. Whether the expression of ACE2 is higher in patients with
119 lung adenocarcinoma (LUAD) remains to be studied. Therefore, more attention should be paid to
120 the expression of ACE2 in patients with LUAD. In addition, the relationship between ACE2
121 expression and clinical and pathological parameters, such as individual cancer status, patient's
122 race, patient's gender, patient's age, patient's smoking habits, node metastasis status, and
123 histological subtypes, is unclear. It is necessary to perform a more accurate analysis of the
124 expression of ACE2, which was the aim of the current study.

125 This study focused on the correlation between ACE2 expression and clinicopathological
126 parameters, and we described the functions, mutation rates, copy number variation (CNVs), and
127 genomic alterations of ACE2 in LUAD. We next statistically analyzed the proteins interacting with
128 ACE2 and Gene Ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG)
129 pathway analysis. Moreover, we investigated ACE2 expression in other cancers, including colon
130 adenocarcinoma (COAD), kidney renal papillary cell carcinoma (KIRP), pancreatic
131 adenocarcinoma (PAAD), rectum adenocarcinoma (READ), and stomach adenocarcinoma (STAD).
132 We then discussed the essential biological features, single nucleotide variations (SNVs), CNVs,

133 and pathway activities of the genes interacting with ACE2 in these cancers. The findings of this
134 study will help enhance the understanding of the potentially positive role of ACE2 in LUAD and
135 provide a theoretical basis for the early diagnosis, prognosis, and targeted therapy of 2019-nCoV.

136

137 **Methods**

138 **Analysis of the mutation rate and CNVs distribution of ACE2 analysis in LUAD**

139 DriverDBv3 (<http://driverdb.tms.cmu.edu.tw/>) is a cancer database that incorporates somatic
140 mutation, methylation, copy number variation, and clinical data in addition to annotation bases.
141 This database can help researchers visualize the relationships between cancers and driver genes
142 [11]. The mutation squares indicate the number of mutation tools that identify this gene as a
143 mutation driver. As the number of tools goes from low to high, the blue color goes from light to
144 deep. The CNVs squares indicate the CNVs gain or loss of a gene. Red represents gain (1) and the
145 green represents a loss (-1). In this study, we used the DriverDBv3 tool to determine the mutation
146 rate and CNVs distribution of ACE2 and their correlations with LUAD.

147

148 **Analysis of the protein network of ACE2**

149 To better understand the function of related proteins and understand their regulatory
150 mechanisms more clearly. We predicted the protein-protein interactions of ACE2 via the STRING
151 database (<https://string-db.org/>), which is a system that searches for known and predicted
152 protein-protein interactions [12]. The interactions include both direct physical interactions
153 between proteins and indirect functional correlations between proteins. Besides, we analyzed
154 the GO enrichment (biological process, molecular function, and cellular component) and KEGG
155 pathways of the genes interacting with ACE2 with the Enrichr online database [13].

156

157 **ACE2 expression and clinical-pathological parameter analysis in LUAD**

158 We investigated the interaction between ACE2 expression and the clinicopathological
159 parameters of patients with LUAD using UALCAN (<http://ualcan.path.uab.edu/index.html>), which
160 is a comprehensive, user-friendly, and interactive web resource for analyzing gene expression
161 data using The Cancer Genome Atlas (TCGA) level 3 RNA-seq data and clinical data from 31
162 cancer types [14]. It provides gene expression and clinicopathological parameter information. In
163 the study, we entered the target gene ACE2 on the homepage of the website, selected LUAD, and
164 obtained the differential expression of ACE2 in pathological parameters (individual cancer status,
165 patient's race, patient's gender, patient's age, patient's smoking habits, node metastasis status,
166 and histological subtypes).

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168 **Immunohistochemical staining**

169 This study was performed on archived tissues from 20 diagnosed cases of lung
170 adenocarcinoma patients who underwent surgery in Shenzhen Second People's Hospital. All the
171 patients signed the informed consent form. This study was approved by the Ethics Committee of
172 Shenzhen Second People's Hospital in accordance with the principles of the Declaration of
173 Helsinki. The tissue samples were fixed in 4% paraformaldehyde and embedded in paraffin.
174 Anti-ACE2 (1: 200, Affinity Biosciences) were used as primary antibodies. MXB was used to detect
175 secondary antibodies. The expression density of TACC3 in lung adenocarcinoma tissue was
176 quantitated by scoring staining intensity, including negative (-) and weak (+) staining, moderate

177 (++) and strong (+++) staining, respectively [15, 16].

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179 **ACE2 expression in other cancers**

180 We analyzed the expression of ACE2 in other cancers, including COAD, KIRP, PAAD, READ, and
181 STAD compared to normal tissues using Gene Expression Profiling Interactive Analysis (GEPIA)
182 database (<http://gepia.cancer-pku.cn/>), an interactive web application based on the gene
183 expression analysis of 9,736 tumors and 8,587 healthy tissue samples from the TCGA and
184 Genotype-Tissue Expression (GTEx) databases [17]. The correlation between ACE2 expression and
185 patient's age was further analyzed using the UALCAN database.

186

187 **Gene Set Cancer analysis in LUAD**

188 GSCALite (<http://bioinfo.life.hust.edu.cn/web/GSCALite/>) is a very useful and important
189 platform for gene set analysis in cancer [18]. In this GSCALite, we integrated the cancer genomics
190 data from TCGA. The alterations in the DNA or RNA of cancer-related genes may contribute to
191 cancer initiation, progression, diagnosis, prognosis, and therapy. We analyzed the gene set for
192 SNVs: statistics, distribution, and types; CNVs: statistics of deletion/amplification of
193 hetero/homozygous CNVs; and cancer pathway activity: the activity of 10 cancer-related
194 pathways.

195

196 **Results**

197 **Genomic alterations of ACE2 in LUAD**

198 We then used the DriverDBv3 tool to determine the mutation rate and CNVs distribution of
199 ACE2 and their correlations with LUAD. Data on the distribution of variants may help to further
200 study the role of ACE2 in acute lung injury and lung function [19]. Fig. 1 A shows the mutation
201 rate of ACE2 and its protein positions in LUAD. We found that the ACE2 protein has the highest
202 mutation rate at positions 201-242. In contrast, the mutation rate of the ACE2 protein at 483-523
203 was the lowest. Additionally, a moderate mutation frequency occurs at the protein sites of 0-40,
204 80-121, 282-322, 644-684, and 765-805. The CNVs squares indicate the CNVs gain or loss of ACE2
205 in LUAD (Fig. 1B). We found the CNVs distribution mainly included gain, loss, none, and normal,
206 and was positively correlated with ACE2 expression in LUAD (cor = 0.149, p = 0.00075). Among
207 them, ACE2 expression was higher in copy number loss than in copy number gain.

208

209 **ACE2 protein network analyses**

210 Data from STRING were applied to determine the proteins interacting with ACE2 and the
211 results are shown in Fig. 2. The following ten proteins were found to interact with ACE2:
212 Angiotensinogen (AGT), Renin (REN), Neprilysin (MME), Dipeptidyl peptidase 4 (DPP4), Lysosomal
213 Pro-X carboxypeptidase (PRCP), Meprin A subunit alpha (MEP1A), Type-1 angiotensin II receptor
214 (AGTR1), Meprin A subunit beta (MEP1B), Xaa-Pro aminopeptidase 2 (XPNPEP2), and Type-2
215 angiotensin II receptor (AGTR2), and their correlation scores were 0.991, 0.950, 0.950, 0.942,
216 0.924, 0.915, 0.904, 0.880, 0.876, and 0.858, respectively.

217

218 **The enrichment analyses of ACE2**

219 To further explore the regulators of ACE2 in LUAD, we next statistically analyzed the significant
220 GO enrichment terms and KEGG pathway of the identified genes via the Enrichr online database

221 (Fig. 3, Table S2). The biological processes of these proteins were mainly involved in the
222 regulation of systemic arterial blood pressure by renin-angiotensin (GO: 0003081), angiotensin
223 maturation (GO: 0002003), and regulation of angiotensin levels in the blood (GO: 0002002).
224 Regarding molecular functions, these proteins were mainly involved in the dipeptidyl-peptidase
225 activity (GO: 0008239), aminopeptidase activity (GO: 0004177), and exopeptidase activity (GO:
226 0008238). The cell component analysis of these proteins showed that they were significantly
227 enriched in invadopodium (GO: 0071437), azurophil granule membrane (GO: 0035577), and
228 ficolin-1-rich granule membrane (GO: 0101003). Moreover, KEGG pathway analysis showed
229 enrichment in the renin-angiotensin system, protein digestion and absorption, and renin
230 secretion.

231

232 **Relationships between ACE2 expression and clinical-pathological parameters of patients with** 233 **LUAD**

234 The goal of our study was to gain insights into the interaction between ACE2 expression and
235 the clinical-pathological parameters of patients with LUAD (Table S1). To accomplish this, we first
236 investigated ACE2 expression based on sample types, As shown in Fig. 4 A, the expression of
237 ACE2 in primary samples was significantly higher than that in normal tissues ($p = 2.16E-8$).

238 An analysis of individual cancer status showed that stage 1, stage 2, and stage 3 cancer tissues
239 had significantly higher expression than that in normal tissues (normal-vs-stage 1: $p = 1.81E-10$;
240 normal-vs-stage 2: $p = 1.26E-03$; normal-vs-stage 3: $p = 2.48E-03$), However, there was no
241 significant difference between stage 4 and normal tissues ($p > 0.05$) (Fig. 4 B).

242 In comparing the patient's race (Fig. 4 C), we found that Caucasians and Asians with cancer had
243 significantly higher ACE2 expression than normal control individuals (normal-vs-Caucasian: $p =$
244 $1.55E-05$; normal-vs-Asian: $p = 4.57E-02$). However, there was no significant difference in the
245 expression of ACE2 among Caucasians, Asians, and African Americans ($p > 0.05$).

246 In addition, we analyzed the relationship between ACE2 expression and patient's age (Fig. 4 D).
247 Notably, we found that the expression of ACE2 in patients aged 61-80 years was significantly
248 higher than that in patients aged 21-40 years ($p = 7.23E-04$), the expression of ACE2 in patients
249 aged 81-100 years was significantly higher than that in patients aged 21-40 years ($p = 3.68E-02$),
250 and the expression of ACE2 in patients aged 61-80 years was significantly higher than that in
251 patients aged 41-60 years ($p = 1.60E-03$). The results help explain why older people are more
252 susceptible to SARS-CoV2.

253 Next, we investigated whether there was a difference between the expression of ACE2 and the
254 patients' gender. As shown in Fig. 4 E, ACE2 expression was higher in both male and female
255 cancer patients than that in the normal group (normal-vs-male: $p = 6.17E-04$; normal-vs-female:
256 $p = 1.45E-09$), but no significant difference was found between ACE2 expression and sexes
257 (male-vs-female: $p > 0.05$).

258 Smoking is the most important risk factor for lung cancer [20]. The relationship between the
259 expression of ACE2 and smoking in LUAD remains to be studied. Here, we focused on ACE2
260 expression according to patient's smoking habits (Fig. 4 F), including non-smoker, smoker,
261 reformed smoker 1 (< 15 years), and reformed smoker 1 (> 15 years). Regarding the smoking
262 habits of LUAD patients, the expression levels of ACE2 in patients with all conditions were higher
263 than those in normal controls, but we found that there were no significant differences between
264 patients' smoking habits and the expression of ACE2 ($p > 0.01$). Therefore, we speculate that the

265 expression of ACE2 may not be related to the smoking habits of patients.

266 Subsequently, it is worth noting the relationship between ACE2 expression and node
267 metastasis status (N0: no regional lymph node metastasis; N1: metastases in 1 to 3 axillary lymph
268 nodes; N2: metastases in 4 to 9 axillary lymph nodes; and N3: metastases in 10 or more axillary
269 lymph nodes). As shown in Fig. 4 G, we found that there was no significant difference between
270 ACE2 expression and node metastasis status ($p > 0.05$).

271 Based on histological subtypes, the data showed that ACE2 expression was highest in lung
272 clear cell adenocarcinoma (Clear Cell), but there was no significant difference compared with the
273 normal group ($p > 0.05$). We found high ACE2 expression in the lung adenocarcinoma-not
274 otherwise specified (NOS) and lung adenocarcinoma mixed subtype compared to normal controls
275 in this cancer, with greater statistically significant (normal-vs-NOS: $p = 4.38E-10$; normal-vs-mixed:
276 $p = 2.46E-03$) (Fig. 4 H). We also found that the expression of ACE2 was higher in lung mucinous
277 adenocarcinoma compared to NOS in LUAD tumors ($p = 7.67E-09$).

278 Taken together, ACE2 expression in the lung increased with age, we further analyzed the
279 expression of ACE2 in patient's age by immunohistochemistry were shown in Fig. 5. The
280 expression level of ACE2 was quantified by scoring the intensity of staining, including negative (-)
281 and weak (+) staining, moderate (++) and strong (+++) staining. We found that ACE2 mainly
282 localized in the plasma membrane and cytoplasm. ACE2 showed moderate expression in patients
283 aged 81-100 years samples, and patients aged 61-80 years samples showed weak expression.
284 However, the expression of ACE2 in patients aged 21-40 years and 41-60 years was negative.

285

286 **ACE2 expression in other cancers**

287 To study the expression of ACE2 in other cancers and whether it is related to the patient's age.
288 We examined the difference in ACE2 expression between tumor and adjacent normal tissues by
289 using GEPIA (Fig. 6 A). We found that ACE2 was also highly expressed in COAD, KIRP, PAAD, READ,
290 and STAD compared to normal tissues. These results suggested that the transcription level of
291 ACE2 was cancer type-specific ($p < 0.05$). However, there was no significant difference in the
292 expression of ACE2 among patients aged 21-40 years, 41-60 years, 61-80 years, and 81-100 years
293 (Fig. 6 B).

294

295 **SNVs, CNVs, and pathway activity of hub proteins in LUAD**

296 To further understand the SNVs, CNV, and pathway activity of these proteins, we performed
297 the analysis with GSCALite (Fig. 7 A-C). The SNVs module presented the SNVs frequency and
298 variant types of these genes in LUAD. We found that the SNVs frequencies of MME, XPNPEP2,
299 DPP4, AGTR1, and ACE2 were in the top five, and are 19 %, 16 %, 14 %, 12 %, and 11 %,
300 respectively. Among them, the variant types of ACE2 were missense mutations. In the CNVs
301 module, the main copy number variants of ACE2 include heterozygous amplification and
302 heterozygous deletion.

303 We then determined the pathway activity of these genes (Fig. 7 D-F). The pathways involved
304 are apoptosis, cell cycle, DNA damage response, EMT, hormone AR, hormone ER, PI3K/AKT,
305 RAS/MAPK, RTK (receptor tyrosine kinase), and TSC/mTOR. The results showed that RTK was
306 activated by DPP4 and ACE2. The EMT pathway was mainly activated by AGTR1, DPP4, MME, and
307 XPNPEP2. However, the cell cycle was mainly inhibited by AGTR1, DPP4, and PRCP. Besides, we
308 found that the Hormone AR pathway was mainly inhibited by DPP4, AGTR1, XPNPEP2, MME,

309 MEP1A, and ACE2.

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311

312 **Discussion**

313 At present, the 2019-nCoV epidemic is the public health emergency that has had the greatest
314 impact on the world and has received great attention from the international community. Facing
315 the “encounter” of the epidemic, China responded positively, acted quickly, and took effective
316 measures to resolutely curb the spread of the epidemic, which was highly appraised by the
317 international community. Given that 2019-nCoV pneumonia has become a new infectious disease
318 transmitted from person to person, while working hard to comply with national instructions, we
319 must work harder to understand the 2019-nCoV virus, and we should have a deeper
320 understanding of how viruses invade the human body. A recent study showed that the ACE2
321 protein had a strong binding affinity with the spike protein of SARS-CoV-2 [21]. However, whether
322 the expression of ACE2 is higher in patients with LUAD and whether it is related to the clinical and
323 pathological parameters of patients is yet to be confirmed. In this study, we described the
324 correlation between ACE2 expression and pathological parameters in LUAD and determined the
325 proteins interacting with ACE2. Next, we analyzed SNVs, CNVs, and pathway activities of the hub
326 genes in other cancers.

327 We statistically analyzed the proteins interacting with ACE2 and performed GO enrichment and
328 KEGG pathway analysis. Among the ACE2 binding proteins, we found that AGT, REN, and MME
329 had the highest correlation with ACE2 by STRING. Previous studies have suggested that AGT is
330 abnormally methylated in gastric cancer and is associated with prognosis [22]. AGT was shown to
331 inhibit vascular cell growth and angiogenesis [23]. MME, a zinc-metalloendopeptidase, has
332 important roles in the physiology and pathology of many diseases such as cancer [24]. The
333 biological processes of these proteins were mainly involved in the regulation of systemic arterial
334 blood pressure by renin-angiotensin, angiotensin maturation, and regulation of angiotensin levels
335 in the blood. Moreover, these proteins in this network are involved in different pathways, and
336 KEGG pathway analysis showed enrichment in the renin-angiotensin system, protein digestion
337 and absorption, and renin secretion. These data analysis results show that ACE2 is related to the
338 regulation of systemic arterial blood pressure, providing a direction for further research.

339 Based on sample types, we found that the expression of ACE2 in primary samples was
340 significantly higher than that in normal tissues. Notably, there was no significant difference
341 between ACE2 expression and patient’s smoking habits. Therefore, we speculate that the
342 expression of ACE2 may not be related to the smoking habits of patients. In addition, our data
343 showed that ACE2 expression was not related to the cancer stage or the patient’s gender, node
344 metastasis status, or histological subtypes. However, ACE2 expression in the lung increased with
345 age, but there was no significant difference in the expression of ACE2 among Caucasians, Asians,
346 and African Americans. This finding is consistent with the report from [10]. Furthermore, the
347 representative immunohistochemical staining patterns for ACE2 were further verification, and
348 high ACE2 expression was found in patients with advanced LUAD. The results help explain why
349 older people are more susceptible to 2019-nCoV.

350 Further studies are required to investigate ACE2 expression in other cancers (COAD, KIRP,
351 PAAD, READ, and STAD). Jia X et al. revealed that the expression of ACE2 in cervical squamous cell
352 carcinoma and endometrial adenocarcinoma, kidney renal clear cell carcinoma, KIRP, and PAAD

353 was higher than that in surrounding tissues [25]. The present study not only provided evidence of
354 high ACE2 expression in KIRP and PAAD but also discovered markedly increased levels of ACE2 in
355 COAD, READ, and STAD. Therefore, we suspect that patients with these cancers may be more
356 susceptible to 2019-nCoV, and they are the key protection targets in epidemic prevention work.
357 However, there was no significant difference in the expression of ACE2 among patients aged
358 21-40 years, 41-60 years, 61-80 years, and 81-100 years. Further large-scale studies are needed
359 to verify these findings. Special attention should be given to cancer patients clinically, noting that
360 they may have a longer course of the illness or a higher risk of severe illness.

361 Subsequently, the RTK pathway was activated by ACE2. RTKs can be expressed in many cell
362 types, including cells in the tumor microenvironment [26]. As a key regulator of cancer
363 development, the RTK pathway plays an important role in the proliferation, invasion,
364 angiogenesis, and metastasis of cancer [27]. These results suggest that ACE2 plays an important
365 role in tumorigenesis and development. In addition, we found that PI3K/AKT and RAS/MAPK
366 pathways activated by ACE2. Besides, we found that the EMT pathway was inhibited by ACE2, but
367 was activated by AGT, AGTR1, DPP4, MME, PRCP, and XPNPEP2. The deregulation of the cell cycle
368 is a fundamental process that underlies cancer proliferation [28]. We found that some genes
369 were mainly inhibited in the cell cycle, especially ACE2, AGTR1, and DPP4. Although we identified
370 a potential correlation between ACE2 expression and these pathways, whether they are involved
371 in regulating ACE2 expression is worthy of future study.

372 373 **Conclusion**

374 Our study aimed to better understand the underlying mechanisms and function of ACE2 with
375 the utilization of extensive databases. Our results demonstrate the importance of ACE2 in LUAD
376 and provide insights into the regulatory mechanisms and functions of ACE2. We hope that these
377 findings provide useful information on the treatment and prevention of 2019-nCoV.

378 379 380 **Abbreviations**

381 ACE2: angiotensin-converting enzyme 2; LUAD: lung adenocarcinoma; CNVs: copy number
382 variations; SNVs: single nucleotide variations; COAD: colon adenocarcinoma; KIRP: kidney renal
383 papillary cell carcinoma; PAAD: pancreatic adenocarcinoma; READ: rectum adenocarcinoma;
384 STAD: stomach adenocarcinoma; COVID-19: 2019 coronavirus disease; WHO: The World Health
385 Organization; SARS-CoV: SARS coronavirus; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of
386 Genes and Genomes; TCGA: The Cancer Genome Atlas; GEPIA: Gene Expression Profiling
387 Interactive Analysis; GTEx: Genotype-Tissue Expression; AGT: Angiotensinogen; REN: Renin; MME:
388 Neprilysin; DPP4: Dipeptidyl peptidase 4; PRCP: Lysosomal Pro-X carboxypeptidase; MEP1A:
389 Meprin A subunit alpha; AGTR1: Type-1 angiotensin II receptor; MEP1B: Meprin A subunit beta;
390 XPNPEP2: Xaa-Pro aminopeptidase 2; AGTR2: Type-2 angiotensin II receptor; NOS: not otherwise
391 specified; RTK: receptor tyrosine kinase.

392 393 **Ethics approval and consent to participate**

394 This study was approved by the Ethics Committee of Shenzhen Second People's Hospital in
395 accordance with the principles of the Declaration of Helsinki. Informed consent from patients for
396 their medical data to be used in the study were obtained.

397 **Consent for publication**

398 All authors have seen and agreed to publish.

399

400 **Availability of data and materials**

401 The authors are grateful to freely available from WHO.
402 (<https://www.who.int/emergencies/diseases/novel-coronavirus-2019>),
403 (<http://driverdb.tms.cmu.edu.tw/>), STRING database (<https://string-db.org/>), UALCAN
404 (<http://ualcan.path.uab.edu/index.html>), GEPIA database (<http://gepia.cancer-pku.cn/>), GSCALite
405 (<http://bioinfo.life.hust.edu.cn/web/GSCALite/>).

406

407 **Competing interests**

408 The authors declare that they have no competing interests.

409

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416

417 **Authors' contributions**

418 Q H and Z L designed the work and wrote the manuscript. L S provided patient samples. Z L, S C
419 and J L performed the statistical analysis, Q H participated in the discussion and language editing.
420 N X reviewed the manuscript. All authors read and approved the final manuscript.

421

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424

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432

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534 **Figures**

535 **Fig. 1 The mutation rate and CNV distribution of ACE2 analysis in LUAD.** A: the mutation rate of
536 ACE2 and its protein positions for LUAD. The mutation squares indicate the number of mutation
537 tools that identify this gene as a mutation driver. B: The CNV squares indicated CNV gain or loss
538 of ACE2 in LUAD. The red represents gain (1) and the green represents a loss (-1).

539

540 **Fig. 2 Identification of proteins known and predicted to interact with ACE2 (STRING).**

541

542 **Fig. 3 GO enrichment and KEGG pathway analysis via the Enrichr online database.** A: biological
543 process; B: molecular function; C: cellular component; D: KEGG pathway analysis.

544

545 **Fig. 4 The correlation between ACE2 mRNA expression level and clinicopathological parameters**
546 **of breast cancer using UALCAN.** A: sample type (normal/primary tumor), B: cancer stage (stages
547 1, 2, 3, and 4), C: patient's race (Caucasian, African American, and Asian), D: patient's age (21-40;
548 41-60; 61-80; 81-100), D: patients' gender (Male-vs-Female), E: patient's smoking habits
549 (Non-smoker, Smoker, Reformed smoker 1 (< 15 years), and Reformed smoker 1 (> 15 years), F:
550 node metastasis status (N0: No regional lymph node metastasis; N1: Metastases in 1 to 3 axillary
551 lymph nodes; N2: Metastases in 4 to 9 axillary lymph nodes; and N3: Metastases in 10 or more
552 axillary lymph nodes), G: histological subtypes (NOS: Lung Adenocarcinoma-Not Otherwise
553 Specified; Mixed: Lung Adenocarcinoma Mixed subtype; ClearCell: Lung Clear Cell
554 Adenocarcinoma; LBC-Nonmucinous: Lung Bronchioloalveolar Carcinoma Non mucinous;
555 SolidPatternPredominant: Lung Solid Pattern Predominant Adenocarcinoma; Acinar: Lung Acinar
556 Adenocarcinoma; LBC-Mucinous: Lung Bronchioloalveolar Carcinoma Mucinous; Mucinous:)
557 Mucinous (Colloid) Carcinoma; Papillary: Lung Papillary Adenocarcinoma; Mucinous: Lung
558 Mucinous Adenocarcinoma; Micropapillary: Lung Micropapillary Adenocarcinoma; SignetRing:
559 Lung Signet Ring Adenocarcinoma).

560

561 **Fig. 5 Levels of ACE2 in patients with LUAD by Immunohistochemical analysis.** A: ACE2
562 expression (-) in patients aged 21-40 years samples. B: ACE2 expression (+) in patients aged 41-60
563 years samples. C: ACE2 expression (++) in patients aged 61-80 years samples. D: ACE2 expression
564 (+++) in patients aged 81-100 years samples. The expression density of ACE2 in LUAD tissue was
565 quantitated by scoring staining intensity, including negative (-) and weak (+) staining, moderate
566 (++) and strong (+++) staining, respectively.

567

568 **Fig. 6 ACE2 expression in other cancers.** A: Human ACE2 expression levels in different tumor
569 types from the TCGA database were determined using GEPIA. ACE2 was highly expressed in colon
570 adenocarcinoma (COAD), kidney renal papillary cell carcinoma (KIRP), pancreatic
571 adenocarcinoma (PAAD), rectum adenocarcinoma (READ), and stomach adenocarcinoma (STAD)
572 compared to normal tissues. P-value Significant Codes: $0 \leq *** < 0.001 \leq ** < 0.01 \leq * < 0.05$. B:

573 The correlation between ACE2 mRNA expression level and patient's age (21-40; 41-60; 61-80;
574 81-100) in COAD, KIRP, PAAD, READ, and STAD.

575

576 **Fig. 7 SNVs, CNVs, and pathway activity analysis in LUAD.** A-C: SNVs, CNVs, and pathway activity
577 analysis of the genes interacting with ACE2 by GSCALite. D-F: Pathway activity (proptosis, cell
578 cycle, DNA damage response, EMT, hormone AR, hormone ER, PI3K/AKT, RAS/MAPK, RTK,
579 TSC/mTOR) analysis.

580

581 **Tables**

582

583 Table S1. Relationship between ACE2 expression and clinicopathological parameters of patients
584 with LUAD

585

586 Table S2. The GO functional enrichment and KEGG pathway analyses of ACE2

Figures

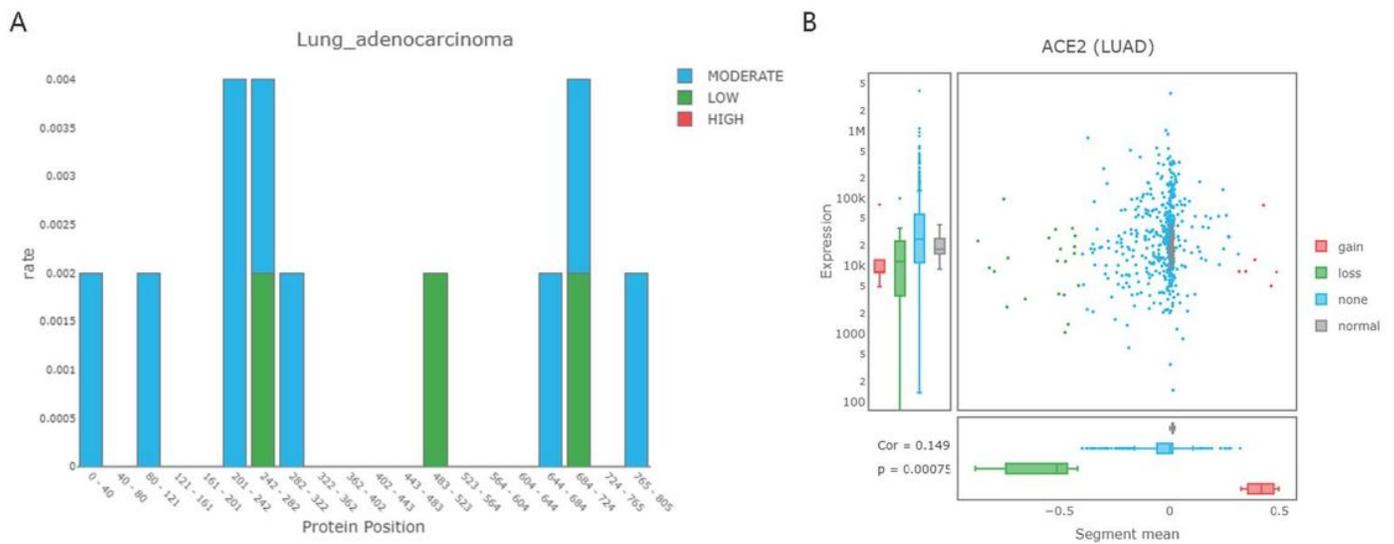


Figure 1

The mutation rate and CNV distribution of ACE2 analysis in LUAD. A: the mutation rate of ACE2 and its protein positions for LUAD. The mutation squares indicate the number of mutation tools that identify this gene as a mutation driver. B: The CNV squares indicated CNV gain or loss of ACE2 in LUAD. The red represents gain (1) and the green represents a loss (-1).

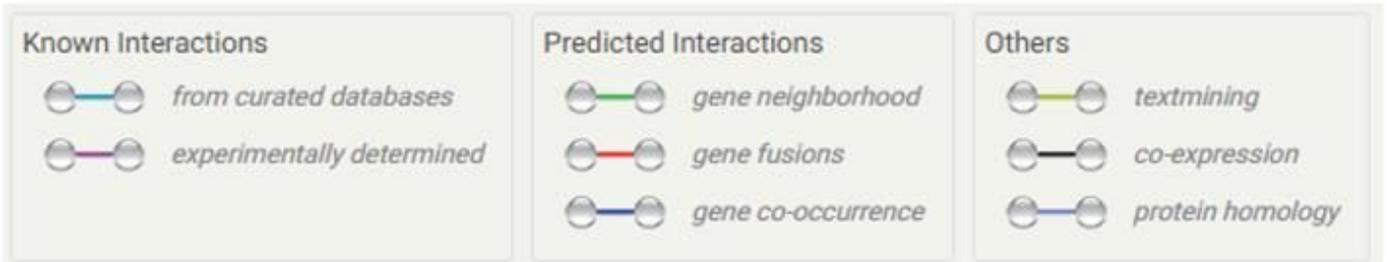
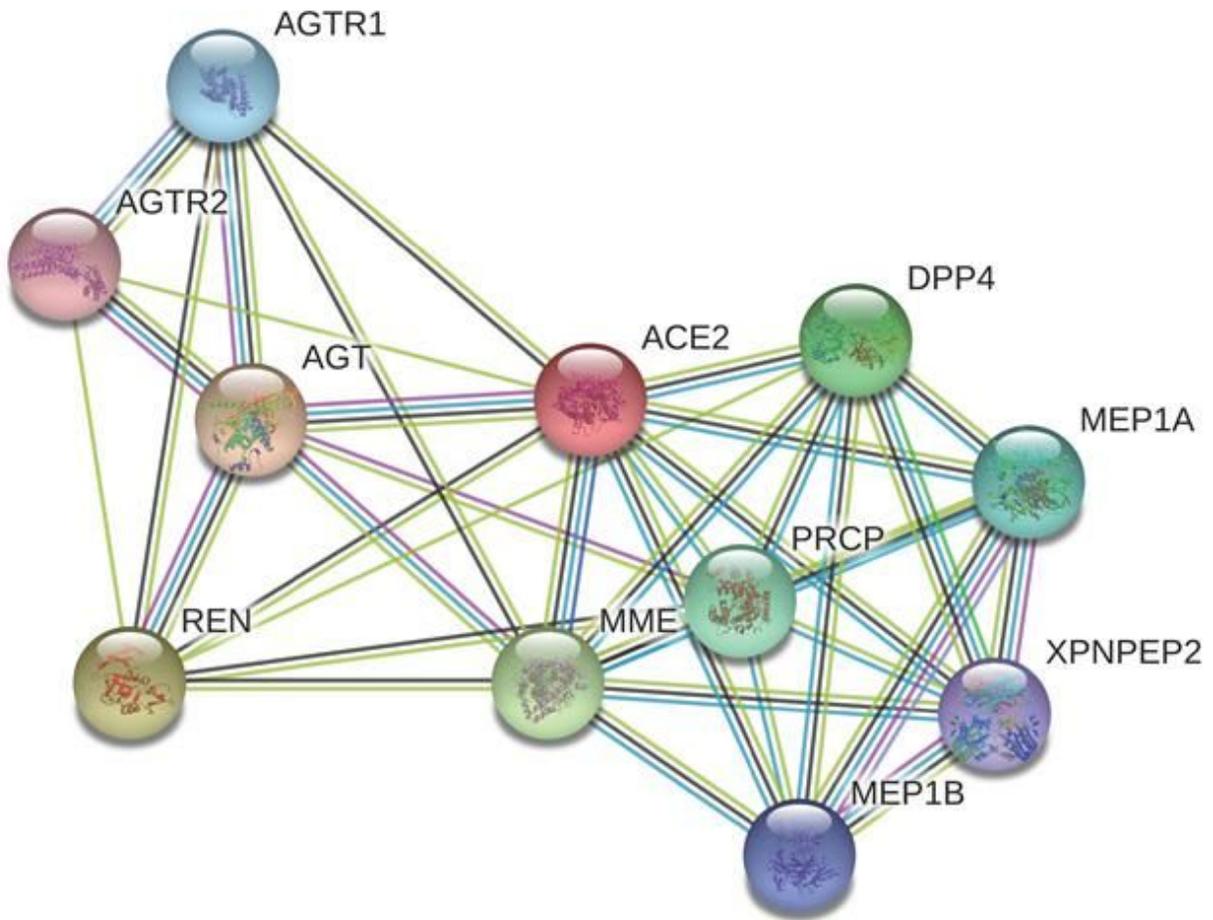


Figure 2

Identification of proteins known and predicted to interact with ACE2 (STRING).

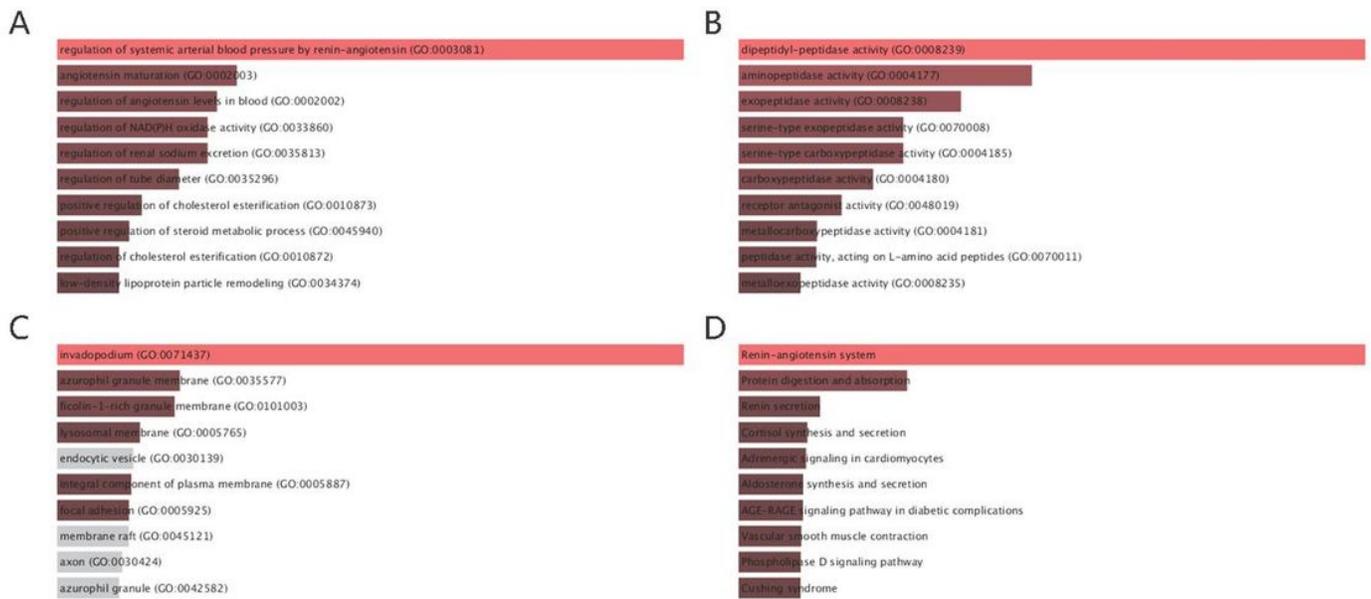


Figure 3

GO enrichment and KEGG pathway analysis via the Enrichr online database. A: biological process; B: molecular function; C: cellular component; D: KEGG pathway analysis.

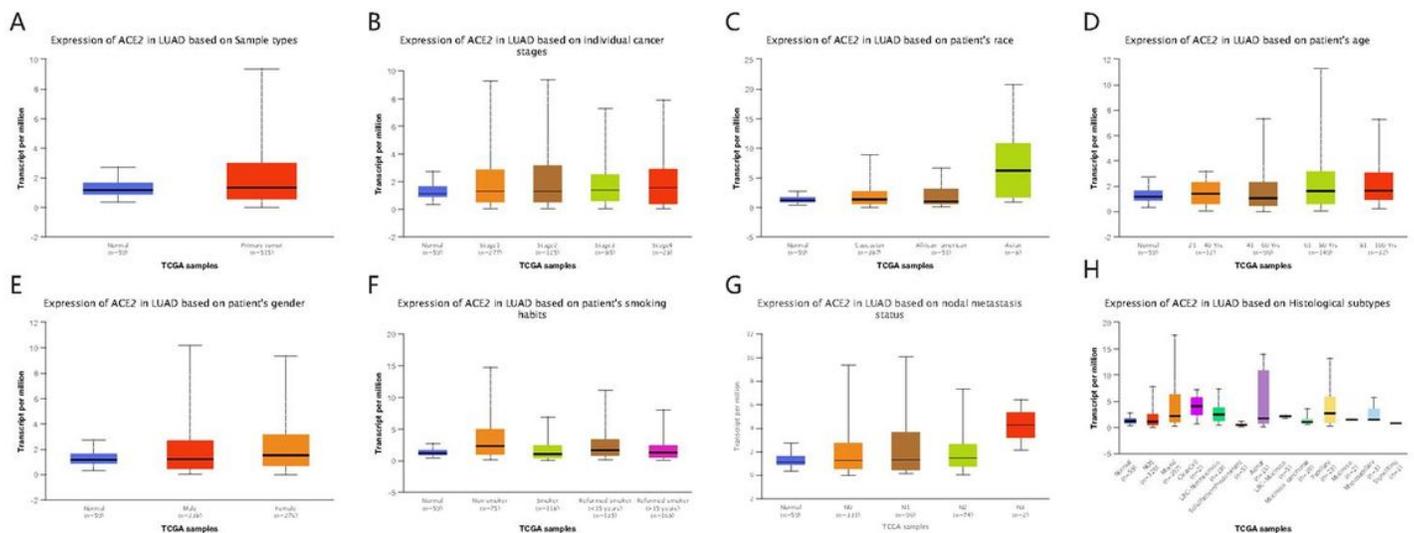


Figure 4

The correlation between ACE2 mRNA expression level and clinicopathological parameters of breast cancer using UALCAN. A: sample type (normal/primary tumor), B: cancer stage (stages 1, 2, 3, and 4), C: patient's race (Caucasian, African American, and Asian), D: patient's age (21-40; 41-60; 61-80; 81-100), E: patients' gender (Male-vs-Female), F: patient's smoking habits (Non-smoker, Smoker, Reformed smoker 1 (< 15 years), and Reformed smoker 1 (> 15 years), G: node metastasis status (N0: No regional lymph node

metastasis; N1: Metastases in 1 to 3 axillary lymph nodes; N2: Metastases in 4 to 9 axillary lymph nodes; and N3: Metastases in 10 or more axillary lymph nodes), G: histological subtypes (NOS: Lung Adenocarcinoma-Not Otherwise Specified; Mixed: Lung Adenocarcinoma Mixed subtype; ClearCell: Lung Clear Cell Adenocarcinoma; LBC-Nonmucinous: Lung Bronchioloalveolar Carcinoma Non mucinous; SolidPatternPredominant: Lung Solid Pattern Predominant Adenocarcinoma; Acinar: Lung Acinar Adenocarcinoma; LBC-Mucinous: Lung Bronchioloalveolar Carcinoma Mucinous; Mucinous:) Mucinous (Colloid) Carcinoma; Papillary: Lung Papillary Adenocarcinoma; Mucinous: Lung Mucinous Adenocarcinoma; Micropapillary: Lung Micropapillary Adenocarcinoma; SignetRing: Lung Signet Ring Adenocarcinoma).

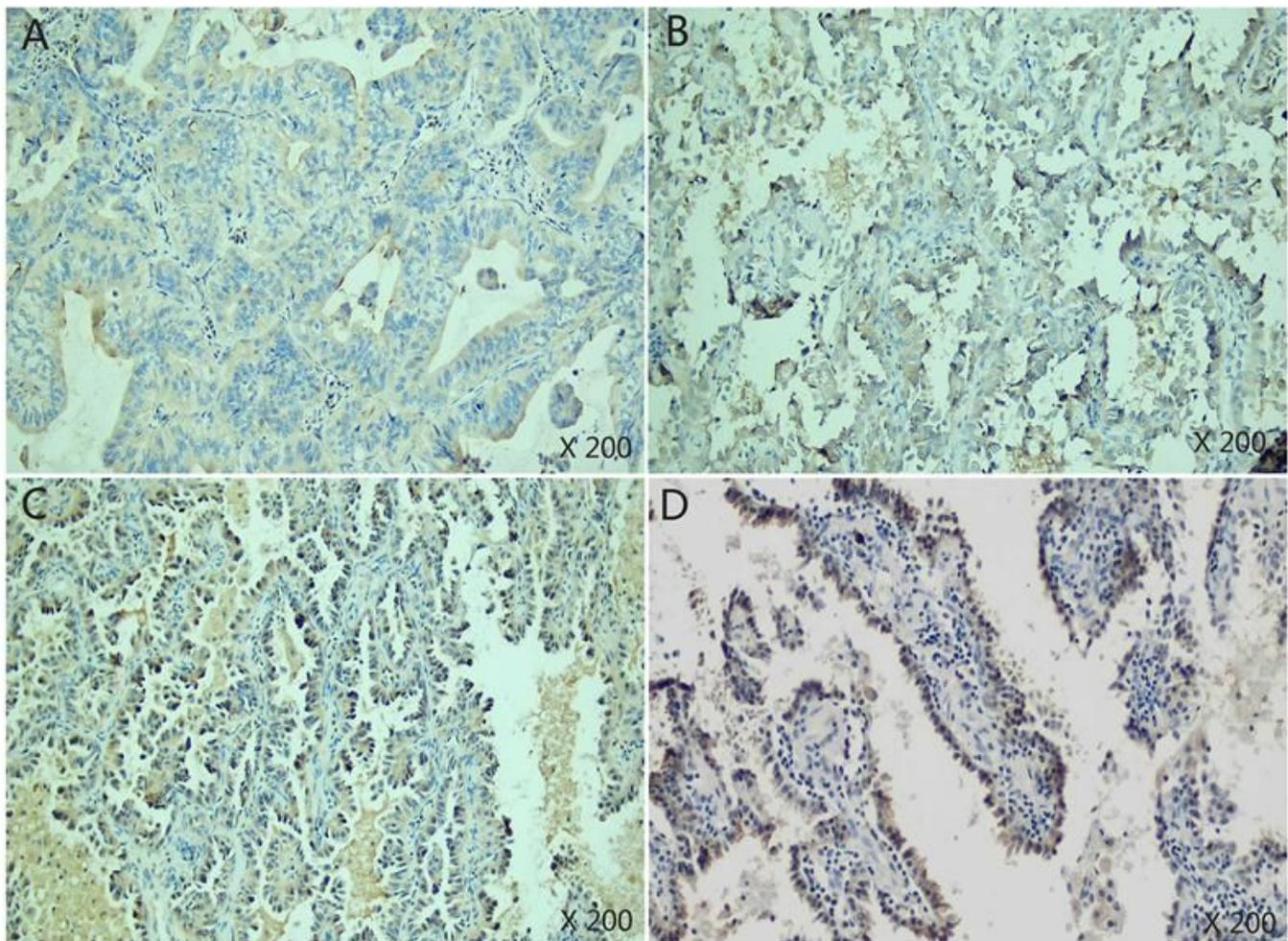


Figure 5

Levels of ACE2 in patients with LUAD by Immunohistochemical analysis. A: ACE2 expression (-) in patients aged 21-40 years samples. B: ACE2 expression (+) in patients aged 41-60 years samples. C: ACE2 expression (++) in patients aged 61-80 years samples. D: ACE2 expression (+++) in patients aged 81-100 years samples. The expression density of ACE2 in LUAD tissue was quantitated by scoring

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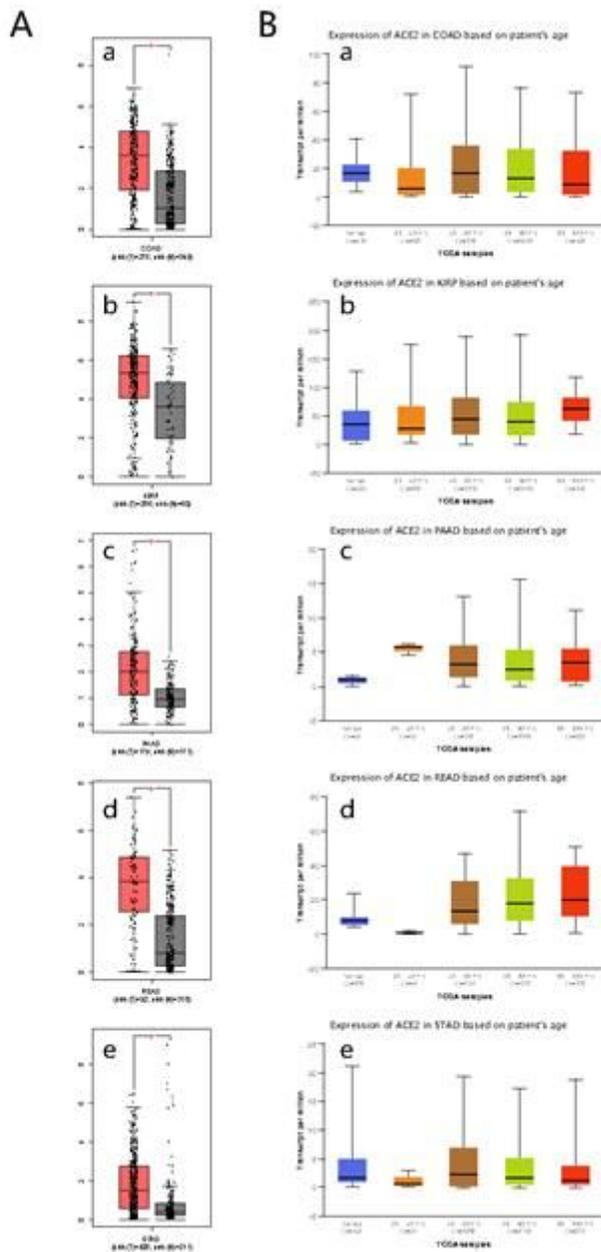


Figure 6

ACE2 expression in other cancers. A: Human ACE2 expression levels in different tumor types from the TCGA database were determined using GEPIA. ACE2 was highly expressed in colon adenocarcinoma (COAD), kidney renal papillary cell carcinoma (KIPR), pancreatic adenocarcinoma (PAAD), rectum adenocarcinoma (READ), and stomach adenocarcinoma (STAD) compared to normal tissues. P-value Significant Codes: $0 \leq *** < 0.001 \leq ** < 0.01 \leq * < 0.05$. B: The correlation between ACE2 mRNA expression level and patient's age (21-40; 41-60; 61-80; 81-100) in COAD, KIPR, PAAD, READ, and STAD.

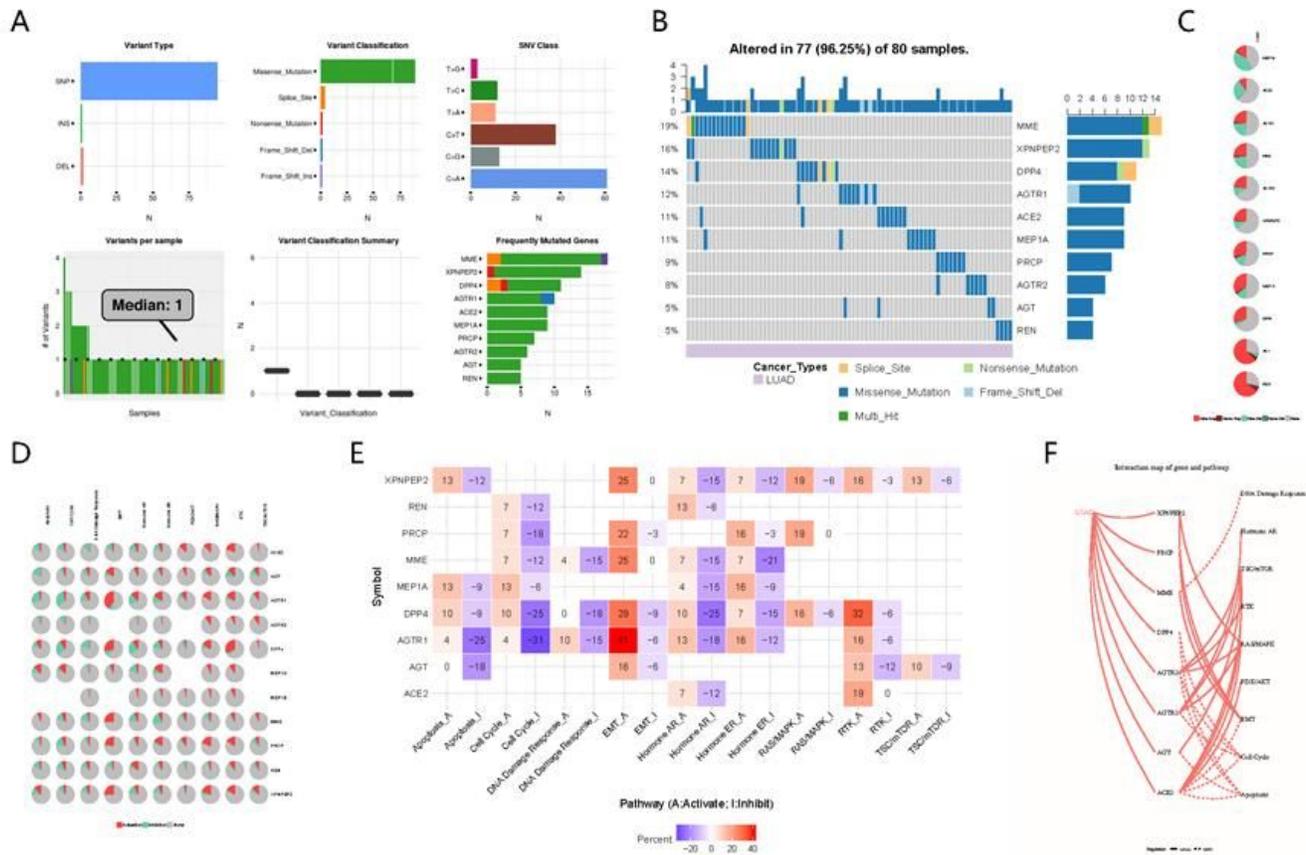


Figure 7

SNVs, CNVs, and pathway activity analysis in LUAD. A-C: SNVs, CNVs, and pathway activity analysis of the genes interacting with ACE2 by GSCALite. D-F: Pathway activity (proptosis, cell cycle, DNA damage response, EMT, hormone AR, hormone ER, PI3K/AKT, RAS/MAPK, RTK, TSC/mTOR) analysis.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [TableS1.docx](#)
- [TableS2.docx](#)