ACE2 Expression and its Prognostic Significance in Head and Neck Squamous Cell Carcinoma

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Abstract

Background and objective: Angiotensin-converting enzyme 2 (ACE2), a membrane structural glycoprotein that acts as a key receptor in the process of SARS-CoV-2 infection, has been identified as an oncogene in some tumor types. However, few studies have explored the role of the ACE2 gene in head and neck squamous cell carcinoma (HNSCC). The purpose of this study was to investigate the potential relationship between ACE2 and HNSCC and explore early markers and molecular targets for the treatment of HNSCC.

Methods: Integrative bioinformatics analyses were applied to uncover the potential role of ACE2 in HNSCC development and tumor-associated immunology.

Results: The results showed that ACE2 was highly expressed in HNSCC and significantly correlated with clinical features such as sex. In addition, ACE2 may be a potential prognostic marker for HNSCC, as it was correlated with shorter recurrence-free survival (RFS) according to the Kaplan-Meier method. The PPI network revealed that STAT1 is the gene most closely related to ACE2 and that the NOD-like receptor signaling pathway was the most relevant pathway. Moreover, ACE2 expression was closely associated with the immune-infiltrating levels of CD8 + T cells, myeloid dendritic cells, and neutrophils.

Conclusions: The viral entry molecule ACE2 plays an important role in the tumorigenesis and cancer-immune interactions of HNSCC, suggesting that it is a novel molecular target and a new immune checkpoint in the diagnosis and treatment of HNSCC.

Introduction

As a homolog of the angiotensin-converting enzyme (ACE), ACE2 can negatively regulate the activated renin angiotensin system by degrading Ang II to heptapeptide angiotensin 1–7 (1). Some studies have shown that the RAS system is involved in the important pathological process of head and neck squamous cell carcinoma. For example, Rho/Ras CO activation, PLC epsilon-ca2 + signal transduction, and Raf/ERK are necessary for the development of head and neck squamous cell carcinoma(2). In terms of invasion and metastasis, Ang can promote the invasion and migration of head and neck squamous cell carcinoma (HNSCC) cells(3). In drug resistance, activation of RAS signaling leads to continuous extracellular signal-regulated kinase 1/2 signaling, resulting in a resistance to cetuximab(4). In immune infiltration, KRAS mutations in the RAS family increase TGF-1 levels in head and neck squamous cell carcinoma patients, while anti-tumor drugs used for its treatment need to have anticancer effects that overcome the anti-tumor immunosuppression induced by TGF-1 (5). However, the role of the novel coronavirus pneumonia receptor ACE2 is still undetermined in HNSCC.

In general, squamous cell carcinoma of the head and neck is a malignant tumor covered by squamous epithelium that occurs in the mouth, pharynx, nose, and throat(6). In terms of human malignant tumors, HNSCC has the sixth highest incidence, and its mortality rate has remained high (7). Despite the rapid development of medical technology in recent decades, the five-year survival rate of patients with HNSCC

has not been greatly improved (8). Since head and neck squamous cell carcinoma is highly invasive and complex to treat, it is urgent to find early markers and molecular targets for the treatment of HNSCC (9).

Previous studies have shown that NOTCH1, TP53, TP63, and CDKN2A are the malignant tumor genes of HNSCC, and that dysregulation of these genes is the driving factor of HNSCC cancer (10–13). However, few studies have explored the role of the ACE2 gene in HNSCC. Based on the corresponding bioinformatics websites, we found that ACE2 has the potential to be a molecular biomarker in HNSCC based on its expression level, methylation level, survival analysis, and corresponding immune invasion results. To find out whether ACE2 can play a crucial role in the occurrence, development, and evolution of HNSCC, as has been demonstrated previously by various HNSCC malignant genes, experiments that are more adequate are still necessary.

Materials And Methods

Use of different public biology websites for data collection and reanalysis

The relevant ACE2 bioinformatics data obtained from different bioinformatics network resources are summarized in Table S1(Table S1).

As a database for tumor-related gene research, the Oncomine database integrates RNA and DNA SEQ data from GEO, TCGA, and published literature, and has become the largest cancer gene chip database and integrated data mining platform in the world (14). UALCAN is an effective online cancer data analysis and mining website, which is mainly based on the relevant cancer data in the TCGA database, such as biomarker identification, expression profile analysis, survival analysis, and so on (15). CancerRNA-Seq Nexus, also known as the CRN database, is a website for the direct analysis of tumor transcriptome data. Its main data sources are the geo database and TCGA database, including 40 types of tumors, 89 tumor sequencing datasets, 325 phenotypic sets and 12167 samples (16). Through the above public bioinformatics platform, we can understand the expression level of ACE2 in human HNSCC. The Kaplan Meier plotter is a tool used to evaluate the effect of genes on the survival rates of cancer samples. It provides tools such as overall survival rates and post-progression survival rates, which can help to evaluate the prognosis of the disease(17). MethSurv is a methylation biomarker that is primarily used to explore the survival rates of cancer patients. It includes 7,358 methylated data points from 25 different human cancers as well as their corresponding survival analyses (18). cBioPortal is a multi-dimensional data network resource platform for cancer genomes, which can visually analyze gene changes in cancer research samples and explore the relationship between gene changes and clinical practice (19). Therefore, we used it to screen the ACE2 co-expression genes in human HNSCC. These co-expressed genes were put into the string database to obtain the protein-protein interaction (PPI) network of these co-expressed genes(20). Then, a detailed visual analysis was carried out using Cytoscape software (version 3.7.2) and the Kobas website (21, 22). Next, we used a web-based gene set analysis kit (Webgestalt) to

perform a gene ontology (GO) enrichment analysis and a KEGG pathway analysis (23). At the same time, a pathview web was used to organize the analyzed data into graphs(24).

Tumor IMmune Estimation Resource (TIMER)is a component analysis software for tumor-infiltrating immune cells. It can associate tumor-infiltrating immune cells with their relevant gene expression, gene mutation, somatic copy number variation, and other data, and can predict the immune cell composition of each tumor sample (25).

Statistical analysis

The difference of mRNA expression between cancer and non cancer tissues was analyzed by Student t test. Chi square test and a generalized linear model analysis were used to analyze the relationship between the expression of ACE2 and clinicopathological characteristics of head and neck squamous cell carcinoma. If $P < 0.05$, the results are considered to be statistically significant. All the above methods were calculated by SPSS (SPSS 23.0, IBM Analytics).

Results

ACE2 was highly expressed in HNSCC

We obtained and analyzed the expression of ACE2 in human HNSCC from the data from the different databases. First, two HNSCC groups named Toruner Head-Neck and Ye Head-Neck were downloaded from the Oncomine public bioinformatics website. After data processing, we found that the expression of ACE2 was upregulated in two HNSCC groups compared to normal tissues, and there was a significant difference found (Fig. 1A, B). In addition, from the TCGA database, we found that the expression of ACE2 in the HPV-negative group GSE40774 was significantly increased. These data indicate that ACE2 and HPV infection may opposite effects in HNSCC, and their specific effects and mechanisms still require further experimental study (Fig. 1C). Finally, we further verified the expression level of ACE2 in HNSCC from the UALCAN database, which proved that the expression of ACE2 was upregulated in HNSCC (Fig. 1D). When examining the methylation level of ACE2 in the tissues of patients with HNSCC compared to normal tissues, we can clearly see from the UALCAN that ACE2 methylation levels were downregulated in grade 1–3 HNSCC (Fig. 2A). However, in the N stage, the methylation levels of ACE2 in patients with stage N0-2 HNSCC were significantly different from the levels seen in healthy patients, and the differences were statistically significant ($P < 0.05$) (Fig. 2B). In addition, the expression of ACE2 mRNA in HNSCC was further determined using the cancer RNA-seq Nexus (CRN) database (Table 1).

Table 1
The expression of ACE2 in TCGA Head and neck squamous carcinoma (HNSC) RNA-seq dataset were analyzed by the Cancer RNASeq Nexus

	ACE2 (Transcript ID: uc004cxa.1)		
Colon adenocarcinoma subset pair	Average expression in cancer	Average expression in normal	Cancer versus Normal *P*-value
Head and neck squamous carcinoma––Stage I versus Normal (adjacent normal)	2.60	0.79	$P < 0.05$
Head and neck squamous carcinoma––Stage II versus Normal (adjacent normal)	2.03	0.79	
Head and neck squamous carcinoma––Stage III versus Normal (adjacent normal)	1.70	0.79	
Head and neck squamous carcinoma––Stage IVA versus Normal (adjacent normal)	2.34	0.79	
Head and neck squamous carcinoma––Stage IVB versus Normal (adjacent normal)	2.09	0.79	

Note: The Cancer RNASeq Nexus (CRN, http://syslab4.nchu.edu.tw/CRN) is an open resource for intuitive data exploration, providing coding-transcript/lncRNA expression profiles that was contained alternative splicing to support researchers generating new hypotheses in cancer research and personalized medicine.

Correlation Between Ace2 Expression And Clinical Features Of Hnscc

In order to explore the correlation between ACE2 expression and the clinicopathological characteristics of HNSCC patients, we downloaded the relevant clinical data of HNSCC patients from the TCGA database. Following the data analysis and statistical analysis, we found that high expression of ACE2 in HNSCC was significantly correlated with gender, N stage, and HNSCC grade (P = 0.008, P = 0.014, P = 0.034, respectively) (Table 2). Based on the univariate analysis, the clinical features with P < 0.24 were included in the multivariate analysis. The results showed that high expression level of ACE2 was only related to gender, but not to N stage, M stage, and HNSCC grade (Table 3). In summary, we have reason to believe that ACE2 may play a role in the occurrence and development of HNSCC.

Table 2
Relationship between the expression levels of ACE2 and clinicpathological parameters in head and neck squamous carcinoma

Parameter		Number	ACE2 mRNA expression		*P* value
			Low(n = 333)	High(n = 163)	
Age	<=60	241	165	76	0.541
	> 60	255	168	87	
Gender	Male	363	256	107	0.008*
	Female	133	77	56	
T stage	T1 + T2 + Tx	186	123	63	0.711
	T3 + T4	310	210	100	
N stage	N0 + Nx	259	161	98	0.014*
	N1 + N2 + N3	237	172	65	
M stage	Mx + M0	491	331	160	0.202
	M1	5	2	3	
Pathologic stage	Stage +	195	125	70	0.247
Grade	Stage	301	208	93	0.034*
	Gx + G1 + G2	375	242	133	
	G3 + G4	121	91	30	

Table 3
Generalized linear model analysis of ACE2 and clinic pathological charateristics

Source	**Type Sum of Squares**	**df**	**Mean Square**	**F**	***P* value**
Gender (Male vs Female)	14.860	1	14.860	5.50	0.0195*
N (N0 + Nx vs N1 + N2 + N3)	23.140	7	3.306	1.22	0.2888
M (Mx + M0 vs M1)	13.556	2	6.778	2.51	0.0828
Grade (Gx + G1 + G2 vs G3 + G4)	10.284	4	2.571	0.95	0.4344

Ace2 May Be A Potential Prognostic Marker For Hnscc

Based on the above results, we speculated that the expression level of ACE2 might affect the survival rate of patients with HNSCC. To test our hypothesis, we first evaluated the impact of ACE2 expression on patient survival indices using Kaplan Meier plotter tools, and confirmed that high and enriched expression

of ACE2 in Th1 cells and Th2 cells was significantly associated with shorter recurrence-free survival values (RFS) (P = 0.039, 0.021, and 0.024, respectively) (Fig. 3A, 3B, and 3C). Finally, relevant data were downloaded from the Methsurv database, and it was found that the high expression of ACE2 was correlated with the short survival time of patients with HNSCC (P < 0.05) (Fig. 3D and 3E). Based on the above findings, we concluded that ACE2 can be used as a potential biomarker for the prognosis of patients with HNSCC.

Analysis of the co-expression network and enrichment pathway of the ACE2 gene

We used the cBioportal database to analyze the biological function of ACE2. First, all co-expressed genes were downloaded from the database, and then these genes were screened with an absolute value of correlation 0.33, and P < 0.05; 217 differentially expressed genes (Table 3S) were obtained. The PPI network was constructed using the string public information website, and then the corresponding co-expression network map was constructed using Cytoscape software. In the network diagram, we can see that STAT1 is the gene most closely related to ACE2 (Fig. 4A). At the same time, the KEGG enrichment pathway of these co-expressed genes was studied using the KOBAS biological website (Table 4S). We found that the NOD-like receptor signaling pathway was the most relevant pathway (Fig. 4B). Finally, we used the WebGestalt website to carry out a GO biological analysis on the 217 different co-expressed genes and identified the molecular functions, cellular components and the biological processes of ACE2 biology. The main processes were found to be protein binding, membranes, and biological regulation, respectively (Fig. 4C).

Expression of the ACE2 gene is associated with immune infiltration in HNSCC

TIMER provides high-throughput data on immune cell infiltration for the study of ACE2 in relation to immune responses in the tumor microenvironment (TME). In the data on immune invasion in HNSCC, we can clearly obtain the following results: in B cells, CD8 + T cells, myeloid dendritic cells, and neutrophils, the expression of ACE2 was positively correlated with the level of immune cell infiltration (P < 0.05) (Fig. 5). We found that among the invasive immune cell signal markers of HNSCC, M1 macrophage cell immune gene signal markers such as PTGS2 (r = 0.101, P = 2.51e-02) and NOS2 (r = 0.092, P = 4.11e-02) were correlated with the expression of ACE2 in HNSCC. Meanwhile, other immune cell signal markers were not correlated with the expression level of ACE2 in HNSCC (Fig. 6). These results suggest that ACE2 expression plays a potentially important role in the immune infiltration of HNSCC.

Discussion

In this study, we explored the potential relationship between angiotensin converting enzyme 2 (ACE2) and the development of head and neck squamous cell carcinoma (HNSCC) in humans. On the one hand, we used public datasets to analyze the expression level of ACE2 in HNSCC. On the other hand, we constructed a co-expression network of ACE2 and screened several important co-expressed genes and signaling pathways that may significant for the tumor's progression. Then, the GEO and TCGA databases

were analyzed. According to the results, ACE2 expression was significantly upregulated in HNSCC. In addition, ACE2 significantly affects the HNSCC survival time and tumor-associated immune response.

Angiotensin converting enzyme (ACE2) is a structural membrane glycoprotein that participates in the renin-angiotensin reaction; it also an important member of the renin-angiotensin system (RAS). It is known during SARS-CoV-2 infection, the v2irus invades a key receptor in the body. After entering the cell, the virus can induce heterogeneous protein synthesis and the release of inflammatory factors, promoting vasoconstriction, an inflammatory response, hypertension, oxidation, and fibrosis, resulting in multiple organ dysfunction(26). The entry of the coronavirus into the cells depends on the binding of viral spike proteins to the cell receptor and the initiation of S proteins by host cell proteases. Hoffmann *et al*. (27)demonstrated that SARS-CoV-2 uses ACE2 as a SARS-CoV receptor to enter cells, and the virus also uses the serine protease TMPRSS2 for S protein priming. Sacconi *et al*. (28) found that the expression of TMPRSS2 in HNSCC cells was significantly lower than in normal tissues, while ACE2 was slightly upregulated in female patients; however, the overall expression levels of ACE2 were comparable to normal tissues. ACE2 hydrolyzes angiotensin I (Ang I) to produce angiotensin II (Ang II). It has been proven that Ang II can promote the invasion and migration of HNSCC cells in an autocrine manner or by triggering stromal tumor-paracrine interactions (3). Narayan *et al*. (29) performed RT-PCR and a protein analysis on thyroid cancer and normal tissues, respectively, using ACE- and ACE2 specific primers or antibodies. The results showed that the expression of ACE2 in thyroid carcinoma was significantly increased, and the higher the degree of differentiation, the higher the ratio of ACE2/ACE. In addition, Carlos *et al*. (30)found that ACE2 is significantly associated with epithelial malignancies and can be used as a therapeutic target for malignant epithelial tumors, especially oral squamous cell carcinoma (OSCC).

Through a previous bioinformatics analysis, it was found that ACE2 mainly plays a role in regulating STAT1, and also has a role in bioregulatory and metabolic pathways. STAT1 is an important component of the IFNγ/ STAT1 signaling pathway, which is involved in many cell life activities such as cell growth inhibition and apoptosis promotion. Jiang *et al*. (31) showed that myotubularin-related protein 2 (MTMR2) can promote the invasion and metastasis of gastric cancer cells by inhibiting the IFNγ/ STAT1 pathway. It has also been shown that the IFNγ/STAT1 pathway promotes tumor cell survival and induces an adaptive immune resistance via CD4 + T cell loss and PD-L1 upregulation (32). Ryan *et al*. (33)found that in mouse HNSCC, STAT1 could promote a T cell immune response and inhibit myeloid-derived suppressor cell aggregation, thus mediating an anti-tumor immune response. Aldo keto reductase family 1 member C1 (AKR1C1) was positively correlated with cisplatin resistance and was a poor prognostic factor for HNSCC. A transcriptomic analysis by Chang *et al*. (34)showed that STAT1 and STAT3 could activate an AKR1C1-induced cisplatin resistance, which could be overcome by treatment with ruxolitinib. Metabolic pathways play an important role in regulating tumorigenesis and the development of many tumors. Tumor cells possess new metabolic pathways that enable them to increase the uptake efficiency of nutrients through metabolic reprogramming, in order to meet their requirements for growth and invasion (35). Sur *et al*. (36) found that oral cancer cells induced the generation of mitochondrial reactive oxygen species (ROS) and inhibited cell apoptosis by altering glycolysis and lipid metabolism pathways. Common reprogramming metabolic pathways include the IKB1-AMP kinase (AMPK) signaling

pathways(37). Chen *et al*.(38)showed that nuclear AMPK recruits PMK2 and β-Catenin by interacting with them, which plays an important role in promoting the cell migration of thyroid cancer. In addition, metabolic pathways are also involved in maintaining tumor stemness. Liu *et al*. (39)demonstrated that activation of the HSP27/hK2 pathway caused cancer stem cells (CSCs) to exhibit reprogrammed metabolic features and enhanced stem cell phenotypes, such as increased ALDH activity, chemoresistance, and tumor formation. However, the specific relationship between ACE2 and metabolic pathways in tumorigenesis, as well as its role in the pathogenesis, development, and prognosis of HNSCC remains to be further explored.

The tumor microenvironment (TME) and tumor-related immune responses have always been the focus of tumor research, and they have also directed new therapy regimens (40). HNSCC cells can evade the host's immune system by manipulating their own immunogenicity, producing immunosuppressive mediators, and promoting the creation of immunoregulatory cell types (41). Mandal *et al*. (42) comprehensively described the immune landscape of HNSCC using the transcriptome data of 280 tumor profiles depicted by The Cancer Genome Atlas (TCGA), and found that both HPV + and HPV-HNSCC tumors were among the most highly immune-infiltrated cancer types. HNSCC has a high level of Treg/CD8 + T cell and NK cell infiltration, which is statistically correlated with its prognosis. ACE2 is an important molecule that participates in TME regulation. Zhang *et al*. (43) used TCGA to explore the relationship between pan-cancer ACE2 expression and several factors including anti-tumor immunity, immunotherapy responses, the carcinogenic pathway, tumor progression phenotypes, and clinical outcomes. It was found that ACE2 upregulation was associated with increased antitumor immune signatures and increased PD-L1 expression, as well as favorable anti-PD-1/PD-L1/CTLA-4 immunotherapy responses. ACE2 may provide a reference for prognosis following tumor immunotherapy. Previous studies have shown that the expression of ACE2 is negatively correlated with immune cell infiltrates, such as neutrophils and macrophages (44, 45). Cheng *et al*. (46) showed that overexpression of ACE2 could inhibit the synthesis of vascular endothelial growth factor in TME and it inhibited tumor invasion and inflammation. However, by using the GSE30589 database, Yang *et al*. (47)found that the expression level of ACE2 was positively correlated with the level of immune infiltration of macrophages, B cells, CD4 + T cells, neutrophils, and dendritic cells in uterine corpus endometrial carcinoma (UCEC). We also found that the expression of ACE2 was positively correlated with the level of immune infiltration of B cells, CD8 + T cells, neutrophils, and dendritic cells. Therefore, the mechanism by which ACE2 participates in the HNSCC immune response warrants further study.

Conclusions

We can conclude that ACE2 expression plays an important role in the tumor immune response in HNSCC, suggesting that ACE2 can be a novel molecular target and a new immune checkpoint in tumor immune escape and tolerance.

ACE2 is a potential biomarker of HNSCC and is involved in the formation of the TME. However, whether ACE2 promotes or inhibits the immune escape of HNSCC tumor cells remains unknown. Further analysis

utilizing the public HNSCC data is needed to provide clearer correlation networks and lay a foundation for the development of novel targeted drugs.

Abbreviations

HNSCC: head and neck squamous cell carcinoma cells ; ACE2：Angiotensin converting enzyme 2; ACE： Angiotensin converting enzyme; AngI：Angiotensin I; AngII：Angiotensin II; RAS: Renin-angiotensin system; CRN: cancer RNA-seq Nexus; TCGA: The Cancer Genome Atlas; PPI: protein protein interaction; GO: Gene ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; RFS: recurrence-free survival values; OSCC: oral squamous cell carcinoma; SARS-CoV-2：Severe Acute Respiratory Syndrome coronavirus 2; AKR1C1: Aldo keto reductase family 1 member C1; TIMER: Tumor Immune Estimation Resource; HPA: Human Protein Atlas; STAT: Signal transducer and activator of transcription; ROS: reactive oxygen species; AMPK: AMP kinase; CSCs: cancer stem cells; TME: Tumor microenvironment; TAMs: Tumor-associated macrophages; TFF: Trifoliate factor; PD-1: Programmed death 1; UCEC: uterine corpus endometrial carcinoma; MSI-H: Microsatellite instability high; MSS: Microsatellite stable; MSI-L: Microsatellite instability low; VEGF: Vascular endothelial growth factor.

Declarations

Authors' contributions

Hui NIE, Yutong WANG, Xia HUANG and Zhiming LIAO performed the literature research, wrote and edited the manuscript. Jianhua ZHOU and Chunlin OU provided expert comments, edited and revised the manuscript. Both authors have read and approved the final manuscript.

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Availability of data and materials

The data sets during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no conflict of interest.

References

1. Jia H. Pulmonary Angiotensin-Converting Enzyme 2 (ACE2) and Inflammatory Lung Disease. Shock. 2016;46(3):239–48.
2. Bourguignon LY, Gilad E, Brightman A, Diedrich F, Singleton P. Hyaluronan-CD44 interaction with leukemia-associated RhoGEF and epidermal growth factor receptor promotes Rho/Ras co-activation, phospholipase C epsilon-Ca2 + signaling, and cytoskeleton modification in head and neck squamous cell carcinoma cells. J Biol Chem. 2006;281(20):14026–40.
3. Hinsley EE, de Oliveira CE, Hunt S, Coletta RD, Lambert DW. Angiotensin 1–7 inhibits angiotensin II-stimulated head and neck cancer progression. Eur J Oral Sci. 2017;125(4):247–57.
4. Rampias T, Giagini A, Siolos S, Matsuzaki H, Sasaki C, Scorilas A, et al. RAS/PI3K crosstalk and cetuximab resistance in head and neck squamous cell carcinoma. Clin Cancer Res. 2014;20(11):2933–46.
5. Weidhaas JB, Harris J, Schaue D, Chen AM, Chin R, Axelrod R, et al. The KRAS-Variant and Cetuximab Response in Head and Neck Squamous Cell Cancer: A Secondary Analysis of a Randomized Clinical Trial. JAMA Oncol. 2017;3(4):483–91.
6. Leemans CR, Snijders PJF, Brakenhoff RH. The molecular landscape of head and neck cancer. Nat Rev Cancer. 2018;18(5):269–82.
7. Agrawal N, Frederick MJ, Pickering CR, Bettegowda C, Chang K, Li RJ, et al. Exome sequencing of head and neck squamous cell carcinoma reveals inactivating mutations in NOTCH1. Science. 2011;333(6046):1154–7.
8. Crooker K, Aliani R, Ananth M, Arnold L, Anant S, Thomas SM. A Review of Promising Natural Chemopreventive Agents for Head and Neck Cancer. Cancer Prev Res (Phila). 2018;11(8):441–50.
9. Alsahafi E, Begg K, Amelio I, Raulf N, Lucarelli P, Sauter T, et al. Clinical update on head and neck cancer: molecular biology and ongoing challenges. Cell Death Dis. 2019;10(8):540.
10. Stransky N, Egloff AM, Tward AD, Kostic AD, Cibulskis K, Sivachenko A, et al. The mutational landscape of head and neck squamous cell carcinoma. Science. 2011;333(6046):1157–60.
11. Tanaka N, Zhao M, Tang L, Patel AA, Xi Q, Van HT, et al. Gain-of-function mutant p53 promotes the oncogenic potential of head and neck squamous cell carcinoma cells by targeting the transcription factors FOXO3a and FOXM1. Oncogene. 2018;37(10):1279–92.
12. Lakshmanachetty S, Balaiya V, High WA, Koster MI. Loss of TP63 Promotes the Metastasis of Head and Neck Squamous Cell Carcinoma by Activating MAPK and STAT3 Signaling. Mol Cancer Res. 2019;17(6):1279–93.

13. Akervall J, Bockmuhl U, Petersen I, Yang K, Carey TE, Kurnit DM. The gene ratios c-MYC:cyclin-dependent kinase (CDK)N2A and CCND1:CDKN2A correlate with poor prognosis in squamous cell carcinoma of the head and neck. Clin Cancer Res. 2003;9(5):1750–5.
14. Rhodes DR, Yu J, Shanker K, Deshpande N, Varambally R, Ghosh D, et al. ONCOMINE: a cancer microarray database and integrated data-mining platform. Neoplasia. 2004;6(1):1–6.
15. Chandrashekar DS, Bashel B, Balasubramanya SAH, Creighton CJ, Ponce-Rodriguez I, Chakravarthi B, et al. UALCAN: A Portal for Facilitating Tumor Subgroup Gene Expression and Survival Analyses. Neoplasia. 2017;19(8):649–58.
16. Li JR, Sun CH, Li W, Chao RF, Huang CC, Zhou XJ, et al. Cancer RNA-Seq Nexus: a database of phenotype-specific transcriptome profiling in cancer cells. Nucleic Acids Res. 2016;44(D1):D944-51.
17. Pan JH, Zhou H, Cooper L, Huang JL, Zhu SB, Zhao XX, et al. LAYN Is a Prognostic Biomarker and Correlated With Immune Infiltrates in Gastric and Colon Cancers. Front Immunol. 2019;10:6.
18. Modhukur V, Iljasenko T, Metsalu T, Lokk K, Laisk-Podar T, Vilo J. MethSurv: a web tool to perform multivariable survival analysis using DNA methylation data. Epigenomics. 2018;10(3):277–88.
19. Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov. 2012;2(5):401–4.
20. Szklarczyk D, Morris JH, Cook H, Kuhn M, Wyder S, Simonovic M, et al. The STRING database in 2017: quality-controlled protein-protein association networks, made broadly accessible. Nucleic Acids Res. 2017;45(D1):D362-D8.
21. Otasek D, Morris JH, Boucas J, Pico AR, Demchak B. Cytoscape Automation: empowering workflow-based network analysis. Genome Biol. 2019;20(1):185.
22. Xie C, Mao X, Huang J, Ding Y, Wu J, Dong S, et al. KOBAS 2.0: a web server for annotation and identification of enriched pathways and diseases. Nucleic Acids Res. 2011;39:W316-22. (Web Server issue).
23. Zhang B, Kirov S, Snoddy J. WebGestalt: an integrated system for exploring gene sets in various biological contexts. Nucleic Acids Res. 2005;33(Web Server issue):W741-8.
24. Luo W, Pant G, Bhavnasi YK, Blanchard SG Jr, Brouwer C. Pathview Web: user friendly pathway visualization and data integration. Nucleic Acids Res. 2017;45(W1):W501-W8.
25. Li T, Fan J, Wang B, Traugh N, Chen Q, Liu JS, et al. TIMER: A Web Server for Comprehensive Analysis of Tumor-Infiltrating Immune Cells. Cancer Res. 2017;77(21):e108-e10.
26. Gavriatopoulou M, Korompoki E, Fotiou D, Ntanasis-Stathopoulos I, Psaltopoulou T, Kastritis E, et al. Organ-specific manifestations of COVID-19 infection. Clin Exp Med. 2020.
27. Hoffmann M, Kleine-Weber H, Schroeder S, Kruger N, Herrler T, Erichsen S, et al. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. Cell. 2020;181(2):271–80. e8.

28. Sacconi A, Donzelli S, Pulito C, Ferrero S, Spinella F, Morrone A, et al. TMPRSS2, a SARS-CoV-2 internalization protease is downregulated in head and neck cancer patients. J Exp Clin Cancer Res. 2020;39(1):200.
29. Narayan SS, Lorenz K, Ukkat J, Hoang-Vu C, Trojanowicz B. Angiotensin converting enzymes ACE and ACE2 in thyroid cancer progression. Neoplasma. 2020;67(2):402–9.
30. de Carvalho Fraga CA, Farias LC, Jones KM, Batista de Paula AM, Guimaraes ALS. Angiotensin-Converting Enzymes (ACE and ACE2) as Potential Targets for Malignant Epithelial Neoplasia: Review and Bioinformatics Analyses Focused in Oral Squamous Cell Carcinoma. Protein Pept Lett. 2017;24(9):784–92.
31. Jiang L, Liu JY, Shi Y, Tang B, He T, Liu JJ, et al. MTMR2 promotes invasion and metastasis of gastric cancer via inactivating IFNgamma/STAT1 signaling. J Exp Clin Cancer Res. 2019;38(1):206.
32. Liu C, Gao AC. IFNgamma, a Double-Edged Sword in Cancer Immunity and Metastasis. Cancer Res. 2019;79(6):1032–3.
33. Ryan N, Anderson K, Volpedo G, Hamza O, Varikuti S, Satoskar AR, et al. STAT1 inhibits T-cell exhaustion and myeloid derived suppressor cell accumulation to promote antitumor immune responses in head and neck squamous cell carcinoma. Int J Cancer. 2020;146(6):1717–29.
34. Chang WM, Chang YC, Yang YC, Lin SK, Chang PM, Hsiao M. AKR1C1 controls cisplatin-resistance in head and neck squamous cell carcinoma through cross-talk with the STAT1/3 signaling pathway. J Exp Clin Cancer Res. 2019;38(1):245.
35. Biswas SK. Metabolic Reprogramming of Immune Cells in Cancer Progression. Immunity. 2015;43(3):435–49.
36. Sur S, Nakanishi H, Flaveny C, Ippolito JE, McHowat J, Ford DA, et al. Inhibition of the key metabolic pathways, glycolysis and lipogenesis, of oral cancer by bitter melon extract. Cell Commun Signal. 2019;17(1):131.
37. Boroughs LK, DeBerardinis RJ. Metabolic pathways promoting cancer cell survival and growth. Nat Cell Biol. 2015;17(4):351–9.
38. Chen J, Zhou Q, Feng J, Zheng W, Du J, Meng X, et al. Activation of AMPK promotes thyroid cancer cell migration through its interaction with PKM2 and beta-catenin. Life Sci. 2019;239:116877.
39. Liu CC, Chou KT, Hsu JW, Lin JH, Hsu TW, Yen DH, et al. High metabolic rate and stem cell characteristics of esophageal cancer stem-like cells depend on the Hsp27-AKT-HK2 pathway. Int J Cancer. 2019;145(8):2144–56.
40. Royce GH, Brown-Borg HM, Deepa SS. The potential role of necroptosis in inflammaging and aging. Geroscience. 2019;41(6):795–811.
41. Ferris RL. Immunology and Immunotherapy of Head and Neck Cancer. J Clin Oncol. 2015;33(29):3293–304.
42. Mandal R, Senbabaoglu Y, Desrichard A, Havel JJ, Dalin MG, Riaz N, et al. The head and neck cancer immune landscape and its immunotherapeutic implications. JCI Insight. 2016;1(17):e89829.

43. Zhang Z, Li L, Li M, Wang X. The SARS-CoV-2 host cell receptor ACE2 correlates positively with immunotherapy response and is a potential protective factor for cancer progression. Comput Struct Biotechnol J. 2020;18:2438–44.
44. Sodhi CP, Wohlford-Lenane C, Yamaguchi Y, Prindle T, Fulton WB, Wang S, et al. Attenuation of pulmonary ACE2 activity impairs inactivation of des-Arg(9) bradykinin/BKB1R axis and facilitates LPS-induced neutrophil infiltration. Am J Physiol Lung Cell Mol Physiol. 2018;314(1):L17–31.
45. Bae EH, Fang F, Williams VR, Konvalinka A, Zhou X, Patel VB, et al. Murine recombinant angiotensin-converting enzyme 2 attenuates kidney injury in experimental Alport syndrome. Kidney Int. 2017;91(6):1347–61.
46. Cheng Q, Zhou L, Zhou J, Wan H, Li Q, Feng Y. ACE2 overexpression inhibits acquired platinum resistance-induced tumor angiogenesis in NSCLC. Oncol Rep. 2016;36(3):1403–10.
47. Yang J, Li H, Hu S, Zhou Y. ACE2 correlated with immune infiltration serves as a prognostic biomarker in endometrial carcinoma and renal papillary cell carcinoma: implication for COVID-19. Aging. 2020;12(8):6518–35.

Figures

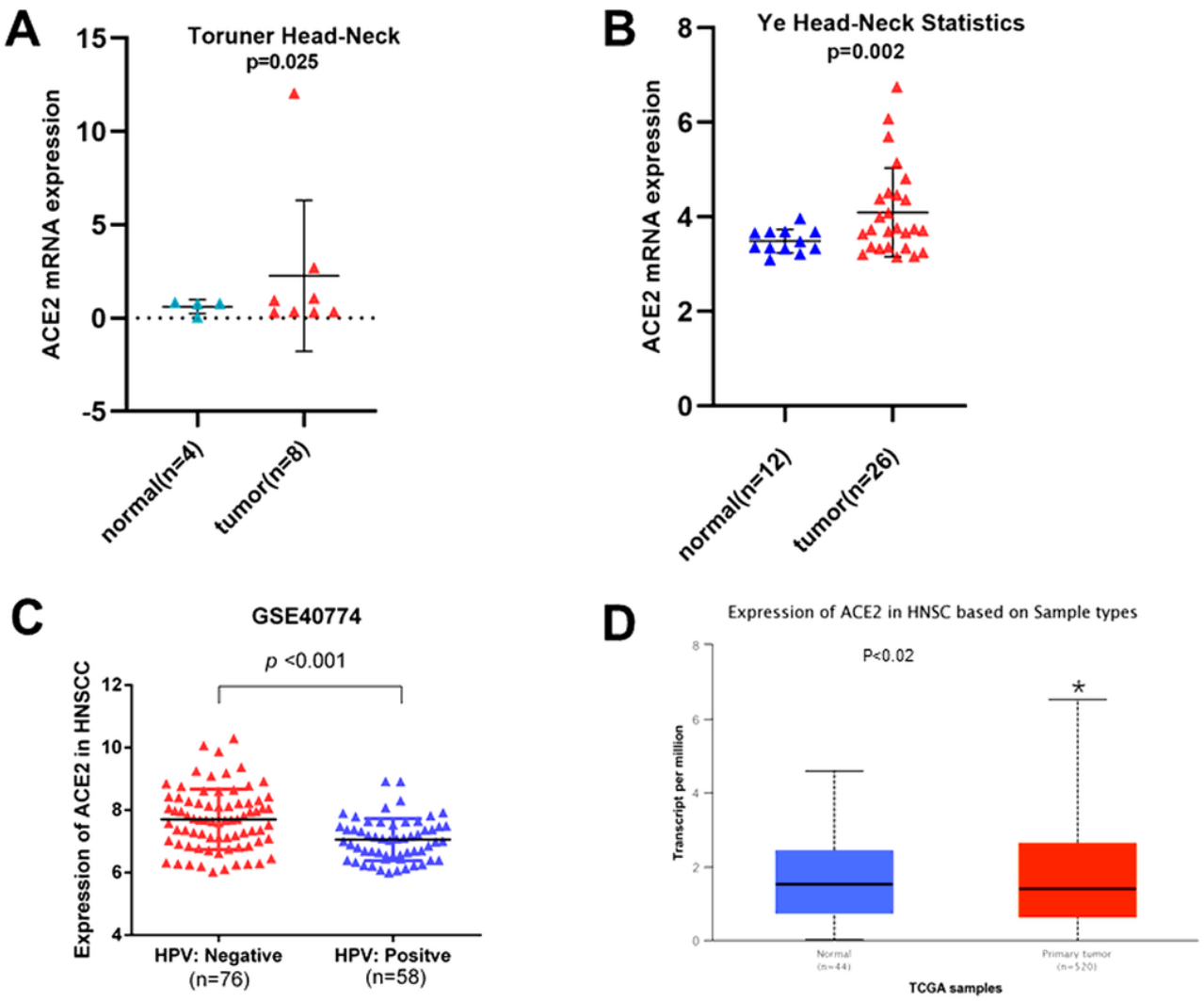

Figure 1

Expression of ACE2 in human HNSCC A: The expression levels of ACE2 mRNA in colon cancer in Tourner Head-Neck data sets was downloaded from Oncomine database. B: The expression levels of ACE2 mRNA in colon cancer in Ye Head-Neck data sets were downloaded from Oncomine database. C: The correlation between the expression of ACE2 and HPV infection in HNSCC was analyzed from the TCGA database. D: The expression level of ACE2 protein in HNSCC was downloaded from the UALCAN database.

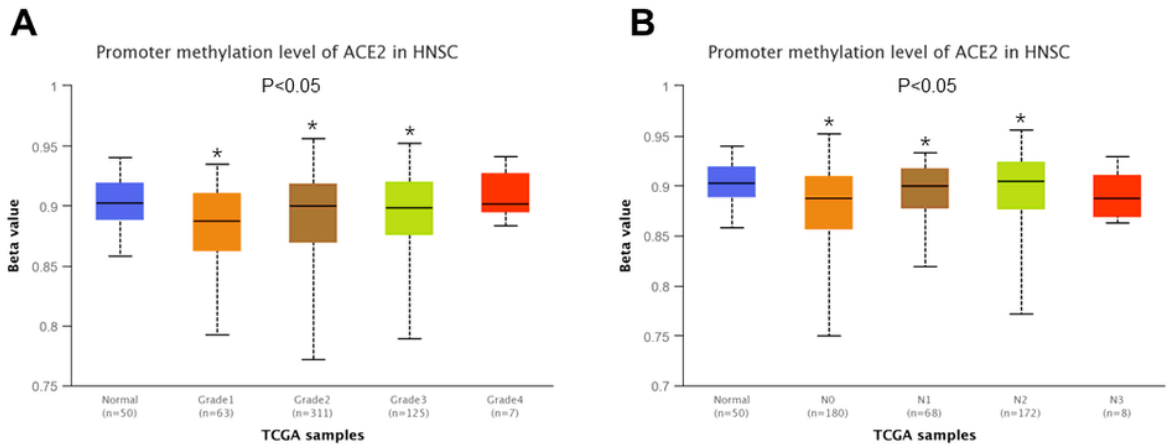

Figure 2

The methylation level of ACE2 in HNSCC in different grades and stages A: The association between the methylation level of ACE2 and the grades of HNSCC was analyzed in UALCAN database. (Among them, * represents significant difference, i.e, P < 0.05). B :The association between the methylation level of ACE2 and the N stages of HNSCC was analyzed in UALCAN database. (Among them, * represents significant difference, i.e, P < 0.05). Please refer to Table S2(Table S2) for detailed data.

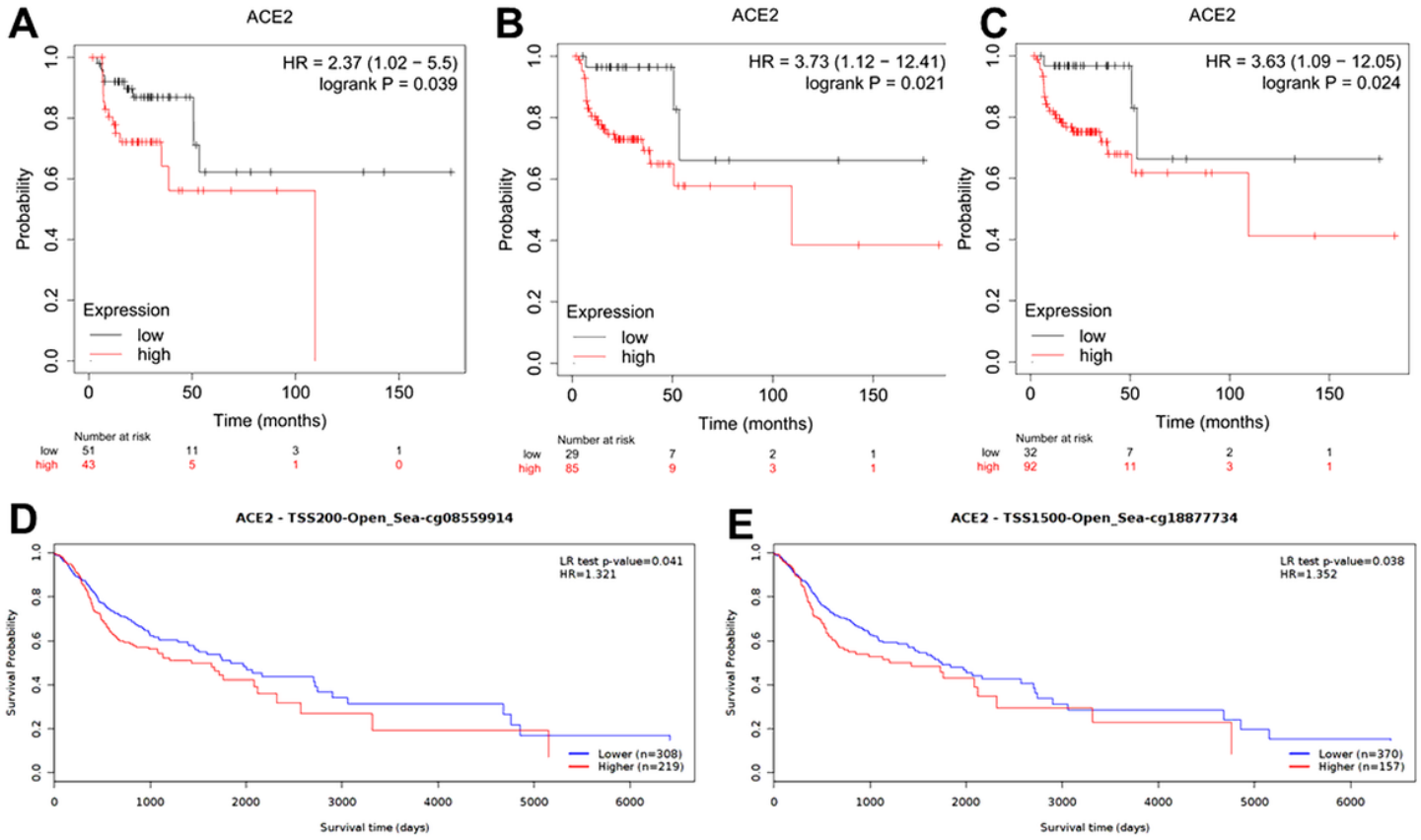

Figure 3

The expression of ACE2 may effect the prognosis of HNSCC A: Relationship between the expression of ACE2 in Th1 cells and the recurrence free survival (RFS) of HNSCC was analyzed by Kaplan Meier plotter tools. B: Relationship between the expression of ACE2 in Th2 cells and the recurrence free survival (RFS) of HNSCC was analyzed by Kaplan Meier plotter tools. C: Relationship between the expression of ACE2 in both Th1 cells and Th1cells and the recurrence free survival (RFS) in HNSCC was analyzed by Kaplan Meier plotter tools. D: Relationship between methylated ACE2 and the recurrence free survival (RFS) in HNSCC was analyzed by MethSurv database. (With probe cg08559914 used). E: Relationship between methylated ACE2 and the recurrence free survival (RFS) in HNSCC was analyzed by MethSurv database. (With probe cg18877734 used)

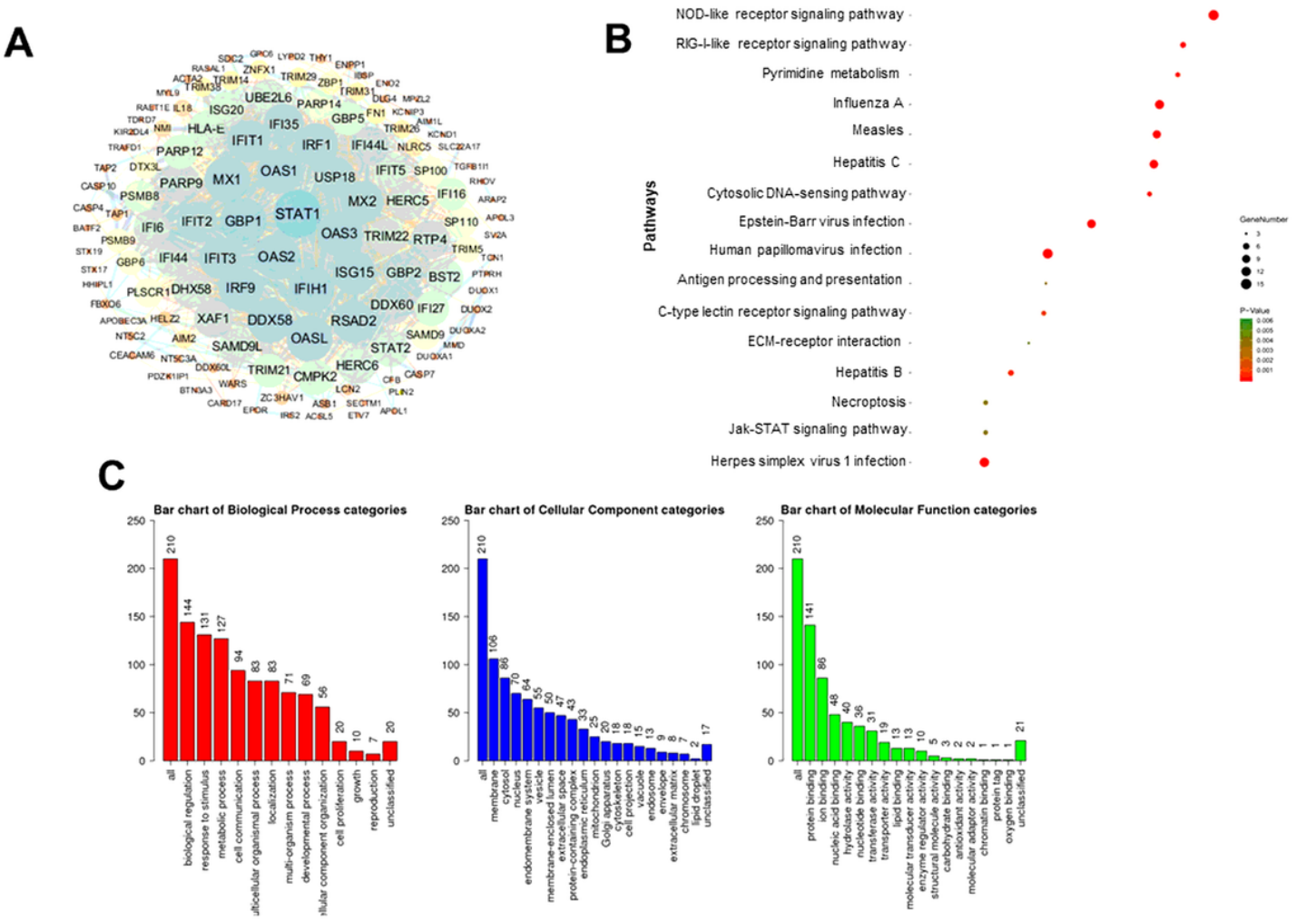

Figure 4

Analysis of ACE2 co-expression network A: Using cBioPortal database, string public information website website and Cytoscape software, the network of co-expression gene with ACE2 was constructed. B: The KEGG enrichment pathway of co-expressed genes was analyzed by KOBAS biological website, and bubble diagram was drawn by R language package. C: The analysis of GO biological processes, molecular functions and cell components is from WebGestalt website.

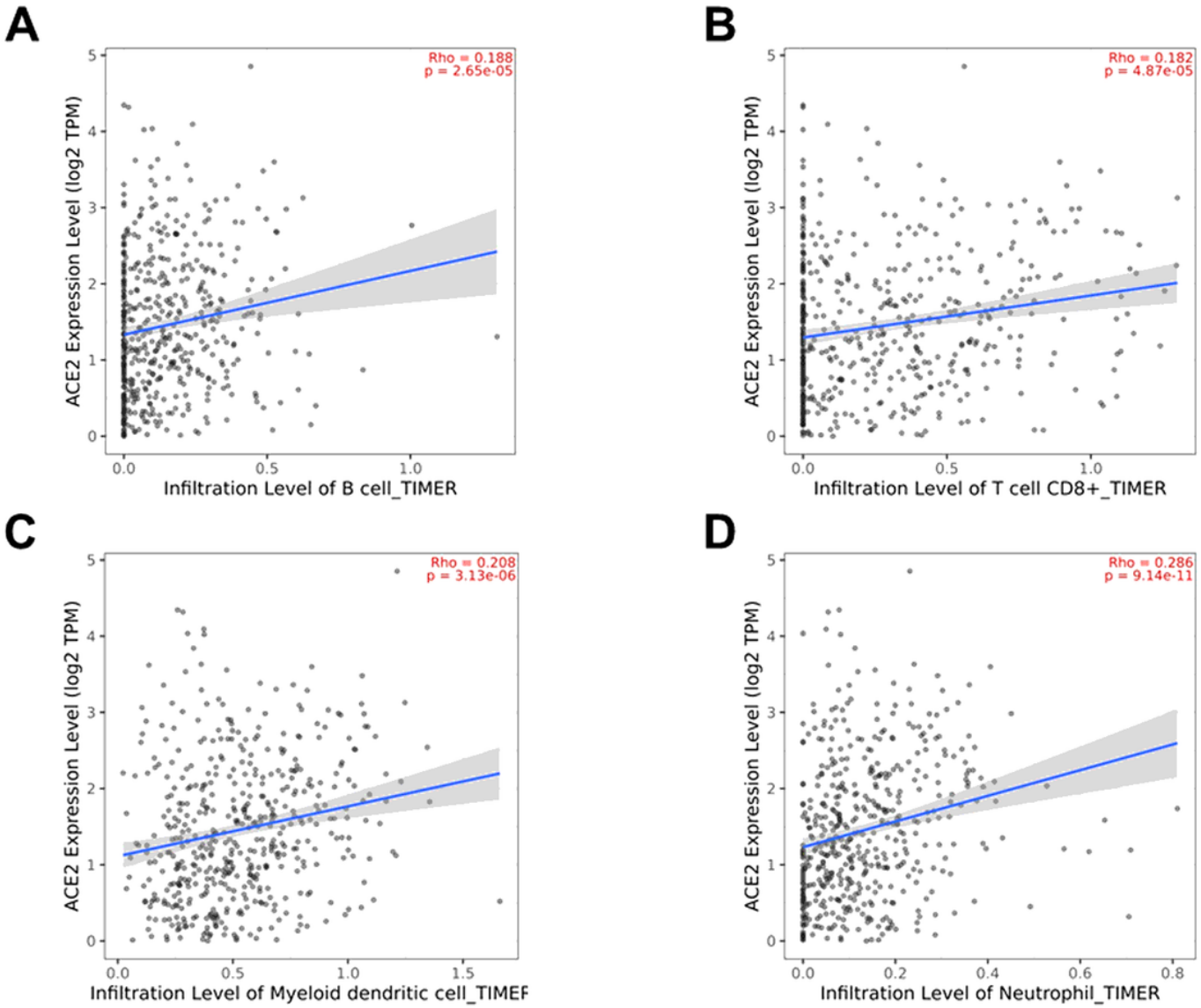

Figure 5

Correlation between the expression of ACE2 and the level of immune infiltration in HNSCC was analyzed by TIMER A: The correlation between the expression of ACE2 and the immune infiltration of B cells in HNSCC. B: The correlation between the expression of ACE2 and the immune infiltration of CD8+ T cells in HNSCC. C: The correlation between the expression of ACE2 and the immune infiltration of myeloid dendritic cells in HNSCC. D: The correlation between the expression of ACE2 and the immune infiltration of neutrophils in HNSCC.

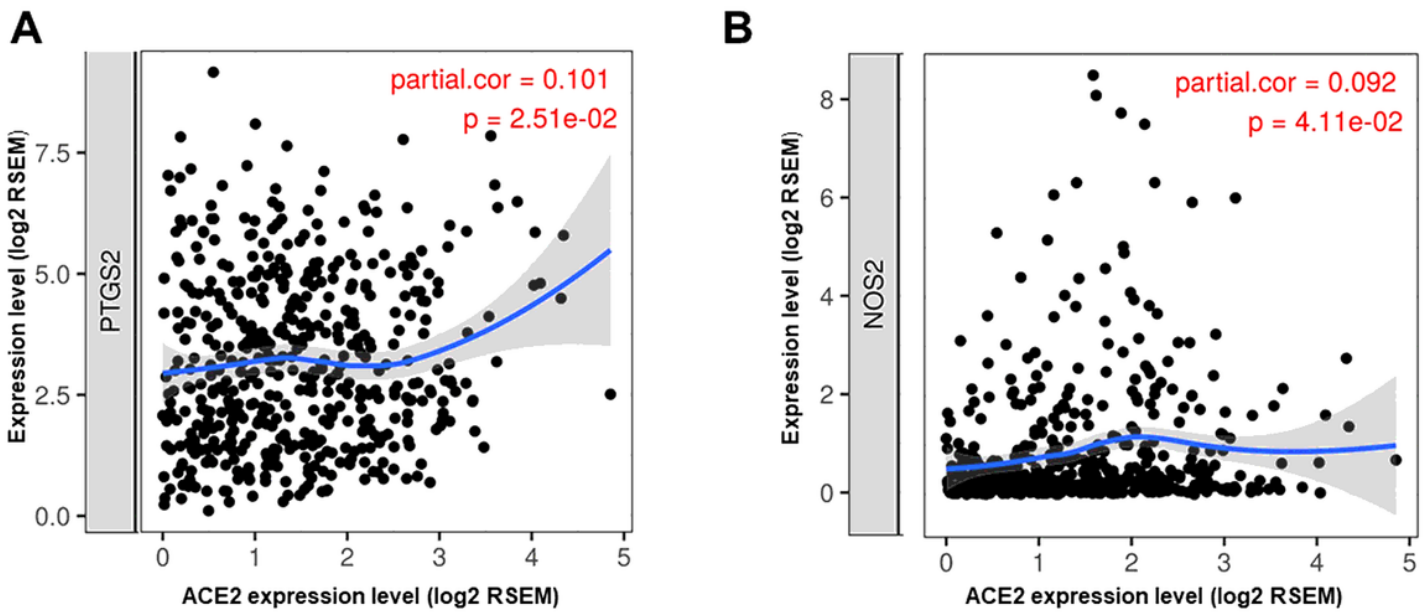

Figure 6

Correlation between ACE2 expression and tumor markers in HNSCC was characterized by TIMER A: Correlation between ACE2 expression and tumor associated gene markers PTGS2 in M1 macrophages cells. B: Correlation between ACE2 expression and tumor associated gene markers NOS2 in M1 macrophages cells.

Supplementary Files

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

- Additionalfile1TableS1.docx
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