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High expression of *ACE2* in HER2 subtype of breast cancer is a marker of poor prognosis

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ABSTRACT

Background: ACE2 a key molecule of the Renin-Angiotensin system has been identified as the receptor for SARS-CoV-2 entry into human cells. In the context of human cancers, there is evidence that ACE2 might function as a tumor suppressor. The expression levels of ACE2 among the different subtypes of breast cancer has not been investigated.

Methods: We have examined the differential expression of *ACE2* and its correlation with prognosis in breast cancer subtypes using the METABRIC ($n = 1898$) and TCGA ($n = 832$) cohorts. Correlations were evaluated by Pearsons's correlation co-efficient and Kaplan-Meier analysis was used to estimate differences in disease-free survival between the *ACE2* high and *ACE2* low groups.

Results: There is minimal expression of *ACE2* in the luminal classes, but significantly higher levels in the Basal-like and HER2-enriched subclasses. Metastatic biopsies of these tumor types also show enhanced expression of *ACE2*. High levels of *ACE2* correlated with decreased disease-free survival in the HER2-enriched subtype, and it was positively correlated with *EGFR* expression.

Conclusion: These observations suggest *ACE2* might function as a context dependent factor driving tumor progression in breast cancer and permit new opportunities for targeted therapy.

Abbreviations
Angiotensin I (Ang I)
Angiotensin-converting enzyme 2 (ACE2)
EGFR (Epidermal Growth Factor Receptor)
Hazard Ratio (HR)
HER2-enriched (Human Epidermal Growth Factor Receptor 2-enriched)
Non-small cell lung cancer (NSCLC)
Pearsons's correlation co-efficient (pcc)
Receptor tyrosine kinase (RTK)
Renin-Angiotensin-Aldosterone system (RAAS)
Risk of relapse (ROR)
Tyrosine kinase inhibitors (TKIs)

Introduction

The COVID pandemic has catapulted angiotensin-converting enzyme 2 (ACE2) into the spotlight since it has been identified as the most likely receptor for SARS-CoV-2 entry into host cells (1). ACE2 is a key player in the Renin-Angiotensin-Aldosterone system (RAAS) that regulates vasoconstriction and dilation. The RAAS axis starts with Renin, secreted by the renal juxtaglomerular cells, that acts on angiotensinogen to release angiotensin I (Ang I). ACE is a carboxypeptidase that acts on Ang I and converts it to Ang II which is the main effector in the RAAS system with vasoconstrictive, pro-inflammatory and pro-fibrotic properties. A homologue of ACE, the ACE2 enzyme converts Ang I to Ang (1–9) and Ang II to Ang (1–7) that mediate vasodilation, apoptosis and anti-proliferation opposing the effects of Ang II [1,2].

ACE is equally expressed in apical and basolateral surfaces whereas, ACE2 is expressed on the apical surface of polarized cells [3]. Under normal conditions, the ACE2 does not internalize into the cell. However, binding of the spike protein of SARS-CoV and Cov2 to ACE2 triggers internalization reducing its activity on the cell surface. The surface activity is also reduced by shedding caused by the sheddase ADAM17 (TACE) [4] which results in the increased presence of the enzyme in plasma and urine [5]. ACE2 is expressed in many tissues primarily vascular endothelial cells and epithelial cells and exists in both secreted

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and membranous forms. The expression of ACE2 has been reported in the nasal and oral mucosa, colon, skin, nasopharynx, lymph nodes, spleen, human kidney, heart, testis, lung, liver, small intestine and brain [6,7]. It is also known to be expressed in arterial and venous endothelium and arterial smooth muscles [8]. It is also known to be expressed in glucose accumulating tissues like liver, β- cells and adipose tissue [9]. Through scRNA-seq data analysis, it was reported that ACE2 expression is highest in pulmonary AT2 cells, myocardium, esophageal epithelial cells, proximal tubule cells of kidney and bladder urothelial cells [10]. In a study on pan cancer tissues it was shown that six types of cancers including breast invasive carcinoma, prostate adenocarcinoma, thyroid carcinoma, liver hepatocellular carcinoma, kidney chromophobe and stomach adenocarcinoma had differential expression of ACE2 when compared to the corresponding normal tissues [11]. The role of ACE2 has been reported to be tumor suppressive in multiple cancer subtypes. A report on breast cancer suggests that levels of ACE2 protein is negatively correlated with the migratory ability of cells and downregulation of ACE2 contributes to invasion and metastasis of breast cancer cells [12].

Breast cancer is a heterogenous disease at the molecular level comprising multiple subtypes, and based on the PAM-50 classification it includes Luminal A, Luminal B, Basal-like, HER2-enriched (Human Epidermal Growth Factor Receptor 2-enriched) and the Normal-like subtypes [13]. PAM50 is a 50-gene q-PCR assay derived by the unsupervised clustering analysis of global gene expression of human breast cancer specimens, developed to identify sub classes that are segregated by biology and this was in turn used to develop a clinically validated assay that provides a risk of relapse (ROR) score [14,15]. Since the distribution of ACE2 expression amongst the breast cancer subtypes has not been investigated we have examined its expression and explored its associations with other genes that play an important role in the biology of breast cancer.

Material and methods

We have examined the expression of *ACE2* in breast cancer samples from the TCGA ($n = 832$) and the METABRIC cohort ($n = 1898$). The data was downloaded from the data portal on TCGA website (https://tcga-data.nci.nih.gov/tcga/) and were TCGA level 3 data, the most highly processed data (https://tcga-data.nci.nih.gov/tcga/tcgaDataType.jsp) and the European Genome-Phenome Archive for METABRIC data [(https://www.ebi.ac.uk/ega/studies) accession no: EGAS00000000083] respectively. We have also analyzed the data from the Swedish multicenter and randomized trial-TEX;

(http://www.clinicaltrials.gov/ct2/show/NCT01433614) which enrolled 287 breast cancer patients between December 2002 and June 2007 of whom 111 patients with a confirmed loco-regional or distant metastasis yielded at least one biopsy with adequate RNA for microarray analysis (Affymetrix array 2.0 GPL10379). All the patients had complete clinical details and follow-up information [16]. The gene expression microarray data was obtained from Gene Expression Omnibus under the accession number, GSE56493. Inter-group variability of gene expression data was analysed using the Kruskal-Wallis test. Correlations were evaluated by Pearsons's correlation co-efficient (pcc). All statistical analysis was carried out using the software XLSTAT 2015 (Windows). Additional validation of the analyses was done using the The cBio Cancer Genomics Portal. Kaplan-Meier analysis was used to estimate differences in disease-free survival between the *ACE2* high and *ACE2* low groups in the METABRIC cohort. To maximize the specificity, we chose to take a cut-off for *ACE2* transcript at the 3rd quartile (3rd quartile at 5.59) and divided the samples into *ACE2* high and low. Disease-free survival was calculated as the time from the date of first diagnosis to the time when either a local recurrence or a distance metastasis occurred. Patients without an event or those that had succumbed to non-breast cancer related causes were right-censored. Log rank test (Mantel-Cox) was used to compare the disease-free survival between groups. Both univariate and multivariate Cox-proportional hazard analysis were done to validate the prognostic importance of *ACE2* in comparison to other clinico-pathological characteristics.

Results

High levels of ACE2 is prognostic in HER2-enriched samples and is positively associated with EGFR expression

We first examined the relative abundance of *ACE2* across the PAM50 subtypes. The general level of expression of *ACE2* is rather low in breast cancer subtypes except for the HER2-enriched and Basal-like subtypes. *ACE2* expression was highest in HER2-enriched samples ($n = 236$; Table 1) when compared to the other subtypes in the METABRIC cohort ($p<0.0001$) (Fig. 1.a). In the TCGA dataset also we observed the same association and an average third quartile fold difference of 30 (ΔΔCt of 4.9) between the HER2 ($n = 67$) and the luminal subtypes (Lum A $n = 416$; Lum B $n = 185$; $p<0.0001$) (Fig. 1.b). Given this very significant over expression in the Basal-like and HER2 subtypes which are clinically aggressive sub classes of the disease compared to the luminal subtypes, we examined the effect of *ACE2* expression on disease-free survival by performing Kaplan Meier survival analysis on the set of patients in the METABRIC cohort ($n = 1898$). We chose to take a cut-off for *ACE2* transcript at the 3rd quartile (3rd quartile at 5.59) and based on this divided the samples into *ACE2* high and low groups. Stratification of all tumor samples by *ACE2* mRNA levels produced significant separation of the groups based on disease-free survival [(Hazard Ratio (HR) of 1.44 (1.20–1.73); log rank $p<0.0001$)]. However, given that the enhanced expression was restricted to the HER2-enriched and Basal-like sub classes we performed the Kaplan-Meyer survival analysis on these subgroups of patients. High levels of *ACE2* was not found to be prognostic within the basal-like class (Fig. 1.c) but only in the HER2-enriched class, where there was a significant higher probability of disease-free survival in the *ACE2* low group ($n = 123$) compared to the *ACE2* high group ($n = 113$) (Fig. 1.d). The mean disease-free survival time was 212 months [SD of 14.6 at 95% CI (183.98–241.27)] in the low *ACE2* group compared to 144 months [SD of 11.2 at 95% CI (122.06–166.17)] in the *ACE2* high group. The prognostic value was also validated using both univariate

Table 1
Clinico-pathological characteristics.

	METABRIC Her2-enriched N (%) ($n = 236$ patients)
Age (y)	
Mean	58
Median	58
Tumor Size (cm)	
Mean	2.9
Median	2.5
Stage	
0	2 (1)
I	38 (16)
II	94 (40)
III	25 (11)
IV	0 (0)
Nx	77 (33)
Grade	
I	4 (2)
II	55 (23)
III	166 (70)
Nx	11 (5)
Lymph Node status	
Positive	136 (58)
Negative	100 (42)
Nx	
Menopausal status	
Pre	55 (23)
Post	181 (77)

Clinico-pathological characteristics of HER2-enriched patients from METABRIC dataset used for disease-free survival analysis.

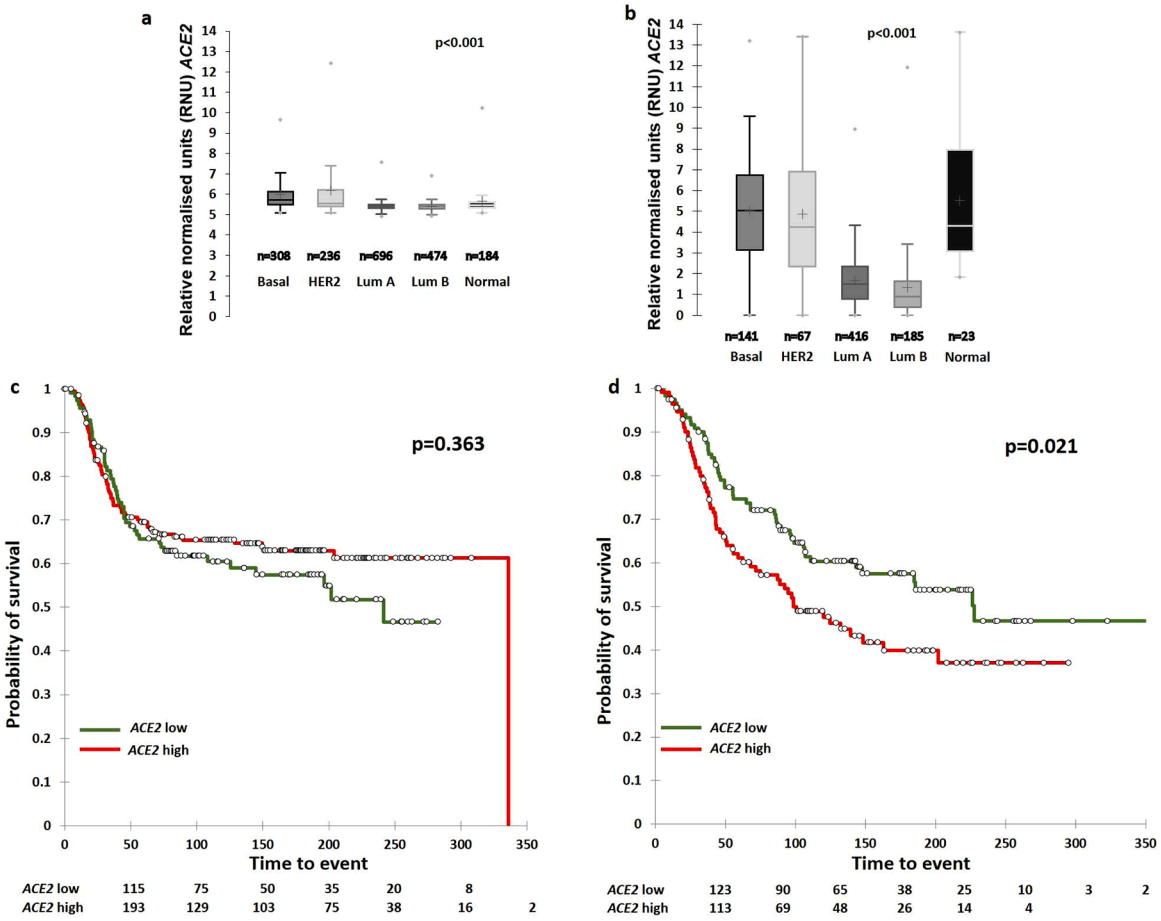

Fig. 1. Association of *ACE2* with PAM50 subtypes and prognosis- Tumors were divided into groups based on PAM50 classification in **a.** METABRIC cohort ($n = 1898$) **b.** TCGA ($n = 832$) and distribution of *ACE2* mRNA analysed. **c.** Kaplan Mayer survival analysis of *ACE2* in the basal-like ($n = 308$) and **d.** the HER2-enriched samples ($n = 236$) in the METABRIC cohort.

and multivariate Cox-proportional hazard analysis (Table 2).

Interestingly, in the HER2-enriched subtype, we also observed that *ACE2* mRNA levels had a strong positive correlation with *EGFR* (Epidermal Growth Factor Receptor) mRNA in the TCGA [$n = 67$; pcc= 0.33; ($p = 0.006$)] and METABRIC cohorts [$n = 236$; pcc= 0.46; ($p<0.0001$)] (Supplementary figure S1) but *EGFR* was not able to independently predict disease-free survival in this group ($p = 0.135$). We also observed a positive correlation with invasive markers like *ITGB1* [$n = 236$; pcc= 0.18; ($p = 0.004$)], *Vimentin* [$n = 236$; pcc= 0.154; ($p = 0.01$)] and stemness marker *ALDH1A1* [$n = 236$; pcc= 0.216; ($p = 0.001$)] in the METABRIC cohort.

Further analysis of gene expression data from metastatic breast cancer samples from the translational component of the Swedish Multicenter TEX trial, showed that the basal-like ($n = 30$) and HER2-

Table. 2
Univariate and Multivariate cox proportional hazard analysis. Univariate and Multivariate cox proportional hazard analysis on all samples and HER2-enriched samples from METABRIC dataset.

	All; $N = 1898$				HER2-enriched; $N = 236$			
	Univariate HR (95% CI)	p-value	Multivariate HR (95% CI)	p-value	Univariate HR (95% CI)	p-value	Multivariate HR (95% CI)	p-value
Age	1.0 (0.99–1.0)	0.53			0.98 (0.97–1.0)	0.05	0.9 (0.9–1.0)	0.8
T-size								
<=3cm	Reference				Reference			
>3cm	2.2 (1.8–2.6)	< 0.00	1.8 (1.5–2.1)	< 0.00	1.7 (1.1–2.5)	0.00	1.4 (0.9–2.0)	0.09
Lymph Node status								
Negative	Reference				Reference			
Positive	2.2 (1.9–2.6)	< 0.00	1.8 (1.5–2.2)	< 0.00	2.8 (1.9–4.2)	< 0.00	2.5 (1.6–3.9)	< 0.00
Grade								
I	Reference				Reference			
II	1.9 (1.2–2.9)	0.00	1.8 (1.1–2.7)	0.00	1.14 (0.27–4.8)	0.85		
III	3.1 (2.1–4.7)	< 0.00	2.3 (1.5–3.6)	< 0.00	1.14 (0.28–4.6)	0.84		
Menopausal status								
Post	Reference				Reference			
Pre	1.0 (0.9–1.3)	0.3			1.45 (0.96–2.1)	0.07		
ER status	1.7 (1.4–2.0)	< 0.00	1.2 (1.0–1.5)	0.03	0.6 (0.0–0.89)	0.01	0.7 (0.5–1.2)	0.2
ACE2 RNU	1.4 (1.2–1.7)	0.00	1.0 (0.8–1.3)	0.48	1.54 (1.0–2.24)	0.02	1.2 (0.8–1.9)	0.2

enriched ($n = 38$) metastatic lesions continued to show the highest mean expression of *ACE2* compared to the other PAM50 subtypes ($p<0.0001$) and there was a positive correlation between *EGFR* and *ACE2* expression in the HER2-enriched metastatic lesions [pcc= 0.36; ($p = 0.024$)].

Discussion

The RAAS system is known to play an important role in regulation of blood pressure and fluid balance in the human physiology. However, clearly ACE2 has independent and distinct cellular roles. Recent reports suggest a role for RAAS in tumor progression. Ang (1–7) has been reported to reduce migration and invasion in lung adenocarcinoma cell line and xenograft mice model systems through inhibition of pathways like the MAPK and the PI3K/Akt [17,18]. It is also known to reduce metastasis and angiogenesis in prostate cancer xenografts [19]. ACE2 has also been shown to be a tumor suppressor in various cancer types and an inhibitor of EMT. ACE2 downregulates VEGFa in breast cancer cells bringing down the process of neoangiogenesis and increased ACE2/ Ang (1–7)/ Mas axis decreases the ability of breast cancer cells to invade and metastasize in vivo and in vitro [12,20,21]. We are reporting for the first time that ACE2 might have a context dependent role in driving tumor progression in breast cancer.

Most gene-expression signatures, with small gene-sets, that have demonstrated utility in clinical prognostication, either include genes directly regulating proliferation or are strongly correlated to such genes. Both Basal-like and HER-2 enriched subtypes are known to have high grade, mitotically active tumors, with high KI-67 labeling index. However, in the tumors with high *ACE2* expression we did not find any positive correlation with proliferation associated genes like *BCl2, CCNE1, CCND1* and *BIRC5*. Neither were high *ACE2* levels correlated with tumor size (data not shown). These data argue against any synergistic role for ACE2 in further enhancing rates of proliferation in these tumor types. But high *ACE2* expression was positively associated with EMT associated genes like Integrin β1 and Vimentin. This observation coupled with the fact that metastatic lesions of both HER2-enriched and Basal-like breast cancer examined in the Swedish TEX trial continue to have high levels of *ACE2* suggests, that in conjunction with over expressed receptor tyrosine kinase (RTK) growth factors, ACE2 a carboxy peptidase, capable of promoting internalization upon binding as demonstrated by SARS-Cov-2, might help promote signaling mechanisms that enhance metastatic behavior. The fact that *ACE2* had a positive association with *EGFR* in all the datasets further supports the conjecture that its metastasis promoting role may only be in the context of growth factor RTKs. These observations need further validation using in-vitro model systems.

The results mirror the observation in a recent report on non-small cell lung cancer (NSCLC) cells where high ACE2 levels correlated with high levels of ERBB1, ERBB2 and ERBB3 and increased sensitivity to EGFR tyrosine kinase inhibitors (TKIs) [22]. High ACE2 levels were also found to be more in an EGFR mutant lung adenocarcinoma cell line model system [23]. EGFR inhibitors for treatment of breast cancer have been evaluated in several studies but results from clinical trials have been disappointing so far unlike in colorectal and lung cancer [24]. Hence identification of signaling mechanisms and biomarkers that work hand in hand with the EGFR pathway is very crucial. Another potential therapeutic application could be to use ACE2 inhibitors as a mode of targeted therapy in this class of breast tumors that over-express ACE2. Since there are monoclonal antibodies against ACE2 like H8R64 that are known get internalized by cells [23], it may be used as a conjugate with a targeting drug against HER2 or EGFR. Moreover, since centrally active ACE2 inhibitors like captopril and fosinopril can cross the blood brain barrier [25], they may be used in conjugation with HER2 targeting drugs for the treatment of brain metastasis; the most common site of metastasis for HER2 subtype of breast cancer. However, these conjectures need further in-vitro validations.

Authors' contributions

All authors have read and approved the final manuscript.

Declaration of Competing Interest

The authors have no conflict of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ctarc.2021.100321.

References

[1] I. Hamming, M.E. Cooper, B.L. Haagmans, N.M. Hooper, R. Korstanje, A. D. Osterhaus, W. Timens, A.J. Turner, G. Navis, H. van Goor, The emerging role of ACE2 in physiology and disease, J Pathol 212 (2007) 1–11, https://doi.org/10.1002/path.2162.

[2] A.J. Turner, ACE2 Cell Biology, Regulation, and Physiological Functions, Protective Arm Renin Angiotensin Syst. (2015) 185–189, https://doi.org/10.1016/B978-0-12-801364-9.00025-0.

[3] F.J. Warner, R.A. Lew, A.I. Smith, D.W. Lambert, N.M. Hooper, A.J. Turner, Angiotensin-converting Enzyme 2 (ACE2), but not ACE, is preferentially localized to the apical surface of polarized kidney cells, J. Biol. Chem. 280 (2005) 39353–39362, https://doi.org/10.1074/jbc.M508914200.

[4] D.W. Lambert, M. Yarski, F.J. Warner, P. Thornhill, E.T. Parkin, A.I. Smith, N. M. Hooper, A.J. Turner, Tumor necrosis factor-alpha convertase (ADAM17) mediates regulated ectodomain shedding of the severe-acute respiratory syndrome-coronavirus (SARS-CoV) receptor, angiotensin-converting enzyme-2 (ACE2), J Biol Chem 280 (2005) 30113–30119, https://doi.org/10.1074/jbc.M505111200.

[5] H.A. Shaltout, B.M. Westwood, D.B. Averill, C.M. Ferrario, J.P. Figueroa, D.I. Diz, J.C. Rose, M.C. Chappell, Angiotensin metabolism in renal proximal tubules, urine, and serum of sheep: evidence for ACE2-dependent processing of angiotensin II, Am J Physiol Renal Physiol 292 (2007) F82–F91, https://doi.org/10.1152/ajprenal.00139.2006.

[6] M. Donoghue, F. Hsieh, E. Baronas, K. Godbout, M. Gosselin, N. Stagliano, M. Donovan, B. Woolf, K. Robison, R. Jeyaseelan, R.E. Breitbart, S. Acton, A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1-9, Circ Res 87 (2000) E1–E9, https://doi.org/10.1161/01.res.87.5.e1.

[7] S.R. Tipnis, N.M. Hooper, R. Hyde, E. Karran, G. Christie, A.J. Turner, A Human Homolog of Angiotensin-converting Enzyme: CLONING AND FUNCTIONAL EXPRESSION AS A CAPTOPRIL-INSENSITIVE CARBOXYPEPTIDASE, J, Biol. Chem 275 (2000) 33238–33243, https://doi.org/10.1074/jbc.M002615200.

[8] I. Hamming, W. Timens, M.L.C. Bulthuis, A.T. Lely, G.J. Navis, H. van Goor, Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis, J Pathol 203 (2004) 631–637, https://doi.org/10.1002/path.1570.

[9] K.H. Chhabra, H. Chodavarapu, E. Lazartigues, Angiotensin converting enzyme 2: a new important player in the regulation of glycemia, IUBMB Life 65 (2013) 731–738, https://doi.org/10.1002/iub.1190.

[10] X. Zou, K. Chen, J. Zou, P. Han, J. Hao, Z. Han, Single-cell RNA-seq data analysis on the receptor ACE2 expression reveals the potential risk of different human organs vulnerable to 2019-nCoV infection, Front Med 14 (2020) 185–192, https://doi.org/10.1007/s11684-020-0754-0.

[11] Y.-.J. Dai, F. Hu, H. Li, H.-.Y. Huang, D.-.W. Wang, Y. Liang, A profiling analysis on the receptor ACE2 expression reveals the potential risk of different type of cancers vulnerable to SARS-CoV-2 infection, Ann Transl Med 8 (2020) 481, https://doi.org/10.21037/atm.2020.03.61.

[12] C. Yu, W. Tang, Y. Wang, Q. Shen, B. Wang, C. Cai, X. Meng, F. Zou, Downregulation of ACE2/Ang-(1-7)/Mas axis promotes breast cancer metastasis by enhancing store-operated calcium entry, Cancer Lett 376 (2016) 268–277, https://doi.org/10.1016/j.canlet.2016.04.006.

[13] C.M. Perou, T. Sørlie, M.B. Eisen, M. van de Rijn, S.S. Jeffrey, C.A. Rees, J. R. Pollack, D.T. Ross, H. Johnsen, L.A. Akslen, O. Fluge, A. Pergamenschikov, C. Williams, S.X. Zhu, P.E. Lønning, A.L. Børresen-Dale, P.O. Brown, D. Botstein, Molecular portraits of human breast tumours, Nature 406 (2000) 747–752, https://doi.org/10.1038/35021093.

[14] T. Sørlie, C.M. Perou, R. Tibshirani, T. Aas, S. Geisler, H. Johnsen, T. Hastie, M. B. Eisen, M. van de Rijn, S.S. Jeffrey, T. Thorsen, H. Quist, J.C. Matese, P.O. Brown,

D. Botstein, P.E. Lønning, A.-L. Børresen-Dale, Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications, Proc Natl Acad Sci 98 (2001) 10869–10874, https://doi.org/10.1073/pnas.191367098.

[15] J.S. Parker, M. Mullins, M.C.U. Cheang, S. Leung, D. Voduc, T. Vickery, S. Davies, C. Fauron, X. He, Z. Hu, J.F. Quackenbush, I.J. Stijleman, J. Palazzo, J.S. Marron, A.B. Nobel, E. Mardis, T.O. Nielsen, M.J. Ellis, C.M. Perou, P.S. Bernard, Supervised risk predictor of breast cancer based on intrinsic subtypes, J Clin Oncol 27 (2009) 1160–1167, https://doi.org/10.1200/JCO.2008.18.1370.

[16] N.P. Tobin, J.C. Harrell, J. Lövrot, S. Egyhazi Brage, M. Frostvik Stolt, L. Carlsson, Z. Einbeigi, B. Linderholm, N. Loman, M. Malmberg, T. Walz, M. Fernö, C. M. Perou, J. Bergh, T. Hatschek, L.S. Lindström, Molecular subtype and tumor characteristics of breast cancer metastases as assessed by gene expression significantly influence patient post-relapse survival, Ann Oncol 26 (2015) 81–88, https://doi.org/10.1093/annonc/mdu498.

[17] L. Ni, Y. Feng, H. Wan, Q. Ma, L. Fan, Y. Qian, Q. Li, Y. Xiang, B. Gao, Angiotensin-(1-7) inhibits the migration and invasion of A549 human lung adenocarcinoma cells through inactivation of the PI3K/Akt and MAPK signaling pathways, Oncol Rep 27 (2012) 783–790, https://doi.org/10.3892/or.2011.1554.

[18] J. Menon, D.R. Soto-Pantoja, M.F. Callahan, J.M. Cline, C.M. Ferrario, E.A. Tallant, P.E. Gallagher, Angiotensin-(1-7) inhibits growth of human lung adenocarcinoma xenografts in nude mice through a reduction in cyclooxygenase-2, Cancer Res 67 (2007) 2809–2815, https://doi.org/10.1158/0008-5472.Can-06-3614.

[19] B. Krishnan, F.M. Torti, P.E. Gallagher, E.A. Tallant, Angiotensin-(1-7) reduces proliferation and angiogenesis of human prostate cancer xenografts with a decrease in angiogenic factors and an increase in sFlt-1, Prostate 73 (2013) 60–70, https://doi.org/10.1002/pros.22540.

[20] Y.R. Qian, Y. Guo, H.Y. Wan, L. Fan, Y. Feng, L. Ni, Y. Xiang, Q.Y. Li, Angiotensin-converting enzyme 2 attenuates the metastasis of non-small cell lung cancer through inhibition of epithelial-mesenchymal transition, Oncol Rep 29 (2013) 2408–2414, https://doi.org/10.3892/or.2013.2370.

[21] Q. Zhang, S. Lu, T. Li, L. Yu, Y. Zhang, H. Zeng, X. Qian, J. Bi, Y. Lin, ACE2 inhibits breast cancer angiogenesis via suppressing the VEGFa/VEGFR2/ERK pathway, J Exp Clin Cancer Res 38 (2019) 173, https://doi.org/10.1186/s13046-019-1156-5.

[22] A. Stewart, C. Gay, K. Ramkumar, K. Cargill, R. Cardnell, M. Nilsson, S. Heeke, E. Park, S. Kundu, L. Diao, Q. Wang, L. Shen, Y. Xi, C.M.D. Corte, Y. Fan, K. Kundu, C. Pickering, F. Johnson, J. Zhang, H. Kadara, J. Minna, D. Gibbons, J. Wang, J. Heymach, L.A. Byers, SARS-CoV-2 infection induces EMT-like molecular changes, including ZEB1-mediated repression of the viral receptor ACE2, in lung cancer models, bioRxiv (2020), 2020;2020.05.28.122291.

[23] M. Yamaguchi, S. Hirai, T. Sumi, Y. Tanaka, M. Tada, Y. Nishii, T. Hasegawa, H. Uchida, G. Yamada, A. Watanabe, H. Takahashi, Y. Sakuma, Angiotensin-converting enzyme 2 is a potential therapeutic target for EGFR-mutant lung adenocarcinoma, Biochem. Biophys. Res. Commun. 487 (2017) 613–618, https://doi.org/10.1016/j.bbrc.2017.04.102.

[24] K. Nakai, M.-C. Hung, H. Yamaguchi, A perspective on anti-EGFR therapies targeting triple-negative breast cancer, Am J Cancer Res 6 (2016) 1609–1623.

[25] K.M. Sink, X. Leng, J. Williamson, S.B. Kritchevsky, K. Yaffe, L. Kuller, S. Yasar, H. Atkinson, M. Robbins, B. Psaty, D.C. Goff, Angiotensin-converting enzyme inhibitors and cognitive decline in older adults with hypertension: results from the Cardiovascular Health Study, Arch Intern Med 169 (2009) 1195–1202, https://doi.org/10.1001/archinternmed.2009.175.