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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 Pearson’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)
Pearson’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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https://doi.org/10.1016/j.ctarc.2021.100321

Available online 23 January 2021
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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/tcgaData Type.jsp) and the European Genome-Phenome Archive for METABRIC data (https://www.ebi.ac.uk/ega/studies) accession no: EGA5000000000833 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 Pearson’s correlation co-efficient (pc). All statistical analysis was carried out using the software XLSTAT 2015 (Windows).

Additional validation of the analyses was done using the The Bio 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          |  |
| 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) |
| Menopausal status | Pre 55 (23) |
| Post           | 181 (77) |

Clinico-pathological characteristics of HER2-enriched patients from METABRIC dataset used for disease-free survival analysis.
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 \( n = 67; \) \( \text{pcc} = 0.33; (p = 0.006) \) and METABRIC cohorts \( n = 236; \) \( \text{pcc} = 0.46; (p < 0.0001) \) (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 \( n = 236; \) \( \text{pcc} = 0.18; (p = 0.004) \), Vimentin \( n = 236; \) \( \text{pcc} = 0.154; (p = 0.01) \) and stemness marker ALDH1A1 \( n = 236; \) \( \text{pcc} = 0.216; (p = 0.001) \) 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 on all samples and HER2-enriched samples from METABRIC dataset.

|                   | All; \( N = 1898 \) |                     | HER2-enriched; \( N = 236 \) |                     |
|-------------------|---------------------|---------------------|-----------------------------|---------------------|
|                   | Univariate          | Multivariate        | Univariate                  | Multivariate        |
|                   | HR (95% CI)         | p-value             | HR (95% CI)                 | p-value             |
| Age               | 1.0 (0.99–1.0)      | 0.53                | 0.98 (0.97–1.0)             | 0.05                |
| T-size            | \(<3\text{cm}\) \(2.2\ (1.8–2.6)\) \(<0.00\) | \(1.8\ (1.5–2.1)\) \(<0.00\) | \(1.7\ (1.1–2.5)\) \(0.00\) | \(1.4\ (0.9–2.0)\) \(0.09\) |
| Lymph Node status| Reference            | \(2.2\ (1.9–2.6)\) \(<0.00\) | \(1.8\ (1.5–2.2)\) \(<0.00\) | \(2.8\ (1.9–4.2)\) \(<0.00\) |
| ER status         | Reference            | \(1.9\ (1.2–2.9)\) \(0.00\) | \(1.8\ (1.1–2.7)\) \(0.00\) | \(1.14\ (0.27–4.8)\) \(0.85\) |
| Menopausal status| Reference            | \(3.1\ (2.1–4.7)\) \(<0.00\) | \(2.3\ (1.5–3.6)\) \(<0.00\) | \(1.14\ (0.28–4.6)\) \(0.84\) |
|                   |                     |                     |                             |                     |
|                   |                     |                     |                             |                     |
|                   | 1.0 (0.9–1.3)       | 0.3                 | 1.45 (0.96–2.1)             | 0.07                |
| ER status         | 1.7 (1.4–2.0)       | \(<0.00\)           | 0.6 (0.0–0.89)              | 0.01                |
| ACE2 RNU          | 1.4 (1.2–1.7)       | 0.00                | 1.54 (1.0–2.24)             | 0.02                |

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.
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 [ρ = 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 mouse models 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 VEGFα 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 HER2 enriched subtypes are known to have high grade, mitotically active tumors, with high KI-67 labeling index. How both Basal-like and HER-2 enriched subtypes are known to have high levels of EGFR, and an inhibitor of EMT. ACE2 a carboxypeptidase, 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 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 VEGFα 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 HER2 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 BC12, CCNE1, CCND1 and BIRC5. Neither were high ACE2 levels correlated with tumor size (data not shown). These data argue against any synergetic 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 α1 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 carboxypeptidase, 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 authors have no conflict of interest.

Acknowledgement and funding

The authors are grateful to Nadadur Estates and the Bagaria Education Trust for their support of all the breast cancer research activities at SRJIR since 2008. We thank the Department of Health Research, Ministry of Health & Family Welfare, India, for the Young Scientist fellowship to M.G.N.

Supplementary materials

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

References

[1] I. Hamming, M.E. Cooper, B.L. Haugmann, N.M. Hooper, R. Korstanje, A. D. Oosterhuis, W. Timens, A.J. Turner, G. Nannis, 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 System. (2015) 185–189, https://doi.org/10.1002/978-0-12-801364-9.00025-0.

[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.M508941200.

[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.M505112200.

[5] H.A. Shaltout, B.M. Westwood, D.B. Aveill, C.M. Ferrari, J.P. Figueroa, D.I. Diaz, 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) F78–F91, https://doi.org/10.1152/ajprenal.00139.2006.

[6] M. Donoghue, F. Hsieh, E. Baronas, K. Godbold, M. Gosselin, N. Staglano, 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. Nannis, 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) 651–667, 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 glyemia, 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 (2020) 185–192, https://doi.org/10.1002/664-620-0794.0.

[11] Y. J.-Y. 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 (2020) 8(2020) 481–627, https://doi.org/10.1002/path.1570.

[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. Sorlie, M.B. Eisen, M. van de Rijn, S.S. Jeffrey, C.A. Rees, J.R. Pollack, D.T. Ross, H. Johnsen, L.A. Akdsen, O. Fluge, A. Parmigenschikov, C. Williams, S.X. Zhu, P.E. Løie, C.M. Perou, R. Tibshirani, T. Aas, S. Geisler, H. Johnsen, T. Hastie, M. B. Eisen, M. van de Rijn, S.S. Jeffrey, T. Thorzen, H. Quist, J.C. Matse, P.O. Brown, Molecular portraits of human breast tumours, Nature 406 (2000) 747–752, https://doi.org/10.1038/35021903.

[14] T. Sorlie, C.M. Perou, R. Tibshirani, T. Aas, S. Geisler, H. Johnsen, T. Hastie, M. B. Eisen, M. van de Rijn, S.S. Jeffrey, T. Thorzen, H. Quist, J.C. Matse, P.O. Brown,
D. Botstein, P.E. Lonning, A.-L. Botresen-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.191567998.

[15] J.S. Parker, M. Mullins, M.C.U. Cheang, S. Leung, D. Voduc, T. Vickery, S. Davies, C. Baudier, X. He, Z. Hu, J.F. Quackenbush, I.J. Stijlemans, 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. Lovrot, S. Egghazi Brage, M. Frostvik Stolt, L. Carlsson, Z. Einbeigi, B. Linderholm, N. Loman, M. Malmberg, T. Walz, M. Ferno, C. M. Perou, J. Bergh, T. Hatschek, L.S. Lindstrom, 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.