

Renin angiotensin system deregulation as renal cancer risk factor (Review)

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Abstract. For numerous years, the non-cardiovascular role of the renin-angiotensin system (RAS) was underestimated, but recent studies have advanced the understanding of its function in various processes, including carcinogenesis. Numerous evidence comes from preclinical and clinical studies on the use of antihypertensive agents targeting the RAS, including angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers. It has been demonstrated that the use of ACEIs can alter the incidence of renal cell carcinoma (RCC) and may have a positive effect by prolonging patient survival. It has an effect on the complex action of ACEI, resulting in decreased angiotensin II (Ang-II) production and altered levels of bradykinin or Ang 1-7. The present review discusses the existing knowledge on the effects of ACE and its inhibitors on RCC cell lines, xenograft models, and patient survival in clinical studies. A brief introduction to molecular pathways aids in understanding the non-cardiovascular effects of RAS inhibitors and enables the conduction of studies on combined cancer treatment with the application of ACEIs. Recent evidence regarding the treatment of hypertension associated with tyrosine kinase inhibitors, one of the most pronounced and common side effects in modern RCC treatment, are also outlined. Captopril, an ACEI, may be used to lower blood pressure in patients, particularly due to its additional renoprotective actions.

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1. Introduction

Kidney cancer accounts for approximately for 3% of all malignant tumors in humans but its incidence worldwide is growing. Around 90% of all cases is classified as renal cell carcinoma (RCC). RCC is characterized as highly aggressive with 20-30% of the patients presenting metastasized disease at the moment of diagnosis, and another 30% of the patients treated for localized disease developing metastases during follow-up. RCC comprises a heterogeneous group, of which clear cell RCC (ccRCC) is the most common (70-80%) followed by papillary RCC (pRCC) (15%) (1,2). Molecular bases of RCC are complicated and not completely discovered. The most common alteration include inactivation of Von Hippel-Lindau gene (VHL), mutations in cMET and TP53 genes, upregulation of vascular endothelial growth factor A (VEGFA), and platelet-derived growth factor B (PDGFB) (3,4). In the case of localized disease, RCC is curable with surgery. However, the prognosis is poor for patients with distant metastases. RCC is not responsive to conventional radiotherapy and chemotherapy but in recent years significant improvement was achieved with use of small targeted molecules, like tyrosine kinase inhibitors (TKIs) and mammalian target of rapamycin (mTOR) inhibitors. New molecular targets are tested aiming to improve survival of patients with RCC. Some authors report the role of the renin-angiotensin system (RAS) in cancer development (5-7) but studies concerning RCC have not been comprehensively revised so far. Derosa *et al* (8) has published recently review on RAS inhibitors in RCC concentrating mostly on hypertension and impact on patients survival. In this paper we present review of current knowledge about role of RAS in RCC development and progression, including not only clinical aspects but also molecular mechanisms and possible future directions in clinical and basic research in this field.

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2. Renin-angiotensin system

The RAS is one of the most comprehensively described hormonal and polypeptide axes, involved in many physiological and pathological processes (Fig. 1). The single and obligate precursor of all angiotensin peptides is angiotensinogen (AGT), which is synthesized and released from the liver. In response to such factors as blood pressure (BP) or plasma sodium level, the kidneys release renin-proteinase, which cleaves AGT to produce angiotensin I (Ang-I). Next, Ang-I is converted by angiotensin-converting enzyme (ACE) to angiotensin II (Ang-II), a key particle of the RAS posing a variety of functions (5). Ang-II acts through two types of G protein-coupled receptors: Angiotensin type 1 receptor (AT1-R) and angiotensin type 2 receptor (AT2-R) (9). Ang-II can also undergo further modifications by aminopeptidases A and N to produce angiotensin III (Ang-III) and angiotensin IV (Ang-IV), respectively. Ang-III binds to AT1-R and AT2-R while Ang-IV has its own receptor, AT4-R (5).

Recently, a new converting enzyme called ACE2 has been discovered. It is capable of cleaving carboxy-terminal amino acids from Ang-II to generate angiotensin 1-7 (Ang 1-7), which signals through the G protein-coupled receptor Mas (MasR) to antagonize the cardiovascular function of Ang-II (10,11). Ang-II can be also generated from Ang-I through an alternative pathway by cathepsin G, CAGE, or chymase (5). Ang-II, through AT1-R, promotes vasoconstriction, increases plasma aldosterone, retains water and sodium, and enhances thirst and salt appetite. Altogether, this results in maintaining fluid and salt homeostasis and increasing BP. Dysregulation or over-activity of the system is associated with cardiovascular diseases, predominantly hypertension (5). Besides classical RAS, many organs, such as the brain, kidneys, heart, and blood vessels, can locally produce RAS components that work independently or synergistically with circulating RAS molecules. Locally generated, angiotensins are likely to contribute to tissue homeostasis and dysfunction (12).

3. Angiotensin receptors

Angiotensin receptors in the kidneys. Angiotensin receptors play an important role in kidney development. Both AT1-R and AT2-R are present in the early days of embryogenesis and persist through embryonic life. AT1-R reaches peaks of its expression at embryonic day 20 and preserve this level until adulthood, whereas AT2-R is observed until day 28 of the post-natal period (13). In adult kidneys, its expression is significantly lower (14). Both receptor types co-localize at differentiated nephrons and blood vessels, while AT2-R also concentrates in actively differentiating cells of the cortex (13). AT1-R is the most common angiotensin receptor in human kidneys with an 8-10-fold higher mRNA expression than AT2-R. In healthy adult kidneys, AT1-R is predominantly expressed in the kidney glomeruli, interlobular arteries, and tubule-interstitial fibrous regions surrounding the interlobular arteries, while AT2-R is expressed in large preglomerular vessels of the human cortex and by interlobular endothelial arterial cells (14-16). Moreover, Ang-II through AT1-R is responsible for the proliferation of proximal tubule cells (17), and through AT2-R, it triggers tubular cell proliferation, apoptosis and neo-angiogenesis (18).

Angiotensin receptors in RCC. Goldfarb *et al* (16) analyzed with autoradiography the expressions of angiotensin receptors in RCC specimens. The receptors were present in all cancer samples in proportions of 60 and 40% for AT1-R and AT2-R, respectively. Dolley-Hitze *et al* (19) broadly analyzed the expressions of AT1-R and AT2-R in clear cell RCC (ccRCC) and correlated it with tumor aggressiveness and clinical outcome. In immunohistochemical staining, 82 out of 84 tumor samples expressed AT1-R and 76 out of 84 AT2-R. When the number of positive tumor cells/total tumor cells was expressed as a percentage, the AT1-R and AT2-R median ratios were 12.5 and 10%, respectively. Both types were significantly overexpressed by Fuhrman's grade 4. The correlation between receptor expressions and tumor grade was also confirmed by western blotting. Interestingly, at the mRNA level, a significant decrease in AT1-R mRNA levels according to Fuhrman's grade was present. This is one of the arguments proving that the altered function of the RAS is mainly an effect of post-transcriptional or post-translational modifications (19). The expressions of Ang-II receptors can be also used as a predictor of patient survival. The PFS of ccRCC patients is longer when the AT1-R and AT2-R expressions are below 12.5% (median) and 10% (median), respectively. By univariate analyses, AT1-R was correlated with PFS, but it was not confirmed in a multivariate analysis. AT2-R's influence on PFS was confirmed in both univariate and multivariate analyses with HR 1.021 (P=0.006), when adjusted for stage, grade, nodal invasion, distant metastasis, tumor size, and ECOG status (19). Even though, correlation between AT2-R expression and PFS was statistically significant, HR is very close to 1 meaning no differences between subgroups. Data should be analyzed in the bigger cohort to prove statistical improvement in PFS. Additional, Authors did not present confidence intervals for HR.

4. Angiotensin-converting enzyme

General information. ACE is a bivalent dipeptidil carboxyl metalloproteinase present in body fluids in a soluble form and in a membrane-bound form, attached to endothelial, epithelial, and neuroepithelial cells. The main function of ACE is the cleavage of Ang-I into Ang-II. Beyond Ang-II production, ACE cleaves many other substrates, including bradykinin, substance P, tetrapeptide AcSDKP, and many others (20). Altogether, ACE plays an important role in maintaining balance between the vasodilatory and natriuretic actions of bradykinin and the salt-retaining properties of Ang-II. Additionally, experiments on ACE in knock-out mice showed that a lack of the enzyme results in male infertility and a variety of hematological abnormalities, such as anemia and immature myeloid cells production (21,22). ACE2 is a carboxypeptidase that cleaves single amino acids from Ang-I to produce angiotensin-(1-9) or to degrade Ang-II to Ang 1-7. It is not expressed as widely as ACE and is mainly localized in the kidneys, heart, and testis. However, it shares 42% of the genomic structure of ACE (12).

ACE in healthy kidney. The kidneys are among the organs with the highest expression of ACE, which is mainly bound to cell membranes of endothelial, mesangial, and epithelial cells of proximal and distal structures of nephron. The highest

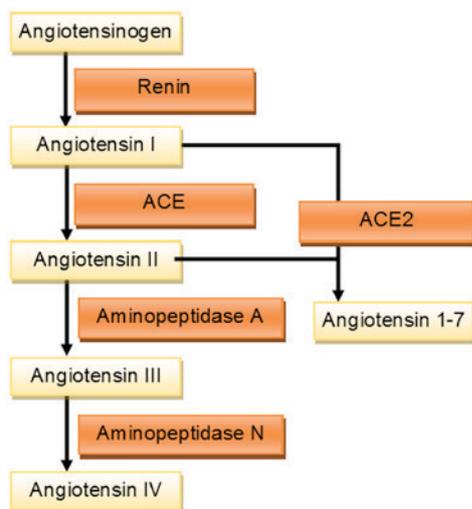


Figure 1. Scheme of renin-angiotensin system. Renin cleaves angiotensinogen to produce angiotensin I which is converted by angiotensin-converting enzyme (ACE) to angiotensin II. Angiotensin II can undergo further modification to angiotensin III and IV. Under action of ACE2 angiotensin 1-7 can be produced.

expression is present in the brush border of the proximal tubule. In kidney development, its activity is crucial to provide sufficient levels of Ang-II necessary for organogenesis (20). In physiological conditions in the kidneys, ACE is responsible for the production of Ang-II, a main effector of the RAS.

ACE2 is responsible for the production of Ang 1-7, acting on the kidneys through the MasR, predominantly expressed in the kidney's blood vessels and proximal tubules (12). Activation of the ACE2/Ang 1-7/Mas pathway causes a decrease in BP, vasodilatation of the renal vessels, and increases in renal blood flow, the glomerular filtration rate, and diuresis (23). Generally, Ang 1-7 counteracts the effects of Ang-II receptors.

ACE and ACE2 in RCC. Takada *et al* (24) had already reported on the presence of ACE in specimens of RCC, while it was not observed in extra-renal tumors. The enzyme activity of ACE is significantly decreased in homogenized tissue samples of chromophobe RCC (chRCC), ccRCC, and renal oncocytoma (RO) with respect to healthy tissue. In ccRCC and chRCC, the activity is four-fold lower and in RO, it is seven-fold lower. Similar results were also observed for ACE2 regarding activity, with a nearly two-fold decrease in ccRCC and four-fold decrease in chRCC (25). Larrinaga *et al* reported as well that ACE activity correlates positively with tumor grade, but not with stage. ACE activity was nearly twice as high in the high Fuhrman grade group (G3-G4) than in the low grade group (G1-G2). ACE activity levels could be used as predictors for poor prognosis in ccRCC. In the same study, the authors described the immunohistochemical pattern of the ACE and ACE2 expressions. ACE-specific staining was negative in cancer cells, whereas it was present at a high rate in tumor vessels. Contrarily, the ACE2 expression was observed within ccRCC cells, with differences between cancers originating from proximal and distal nephrons (25). Based on the presented data, distinct patterns of immunostaining for ACE and ACE2 may be helpful in clinics in differential diagnosis between distal and proximal nephron tumors and in the selection of

appropriate therapy. At the mRNA level, significantly, an almost 100-times decrease ACE2 mRNA in chRCC was noticed. In ccRCC, the mRNA expression was not different than in healthy tissue (25). Different trends in the expression of mRNA and enzymatic activity may rely on post-translation modifications, leading to decreased ACE activity. Hence, we cannot solely rely on mRNA levels to assess changes in protein levels in cancers.

ACE gene polymorphisms in RCC. It is hypothesized that ACEI influences cancer incidence, and prognosis may be associated with differences in ACE activity in plasma, which is strongly correlated with the insertion/deletion (I/D) polymorphisms of 287 base pairs in intron 16 of the ACE gene (rs4646994). Healthy homozygotes for allele D have been shown to increase ACE plasma levels and are correlated with high Ang-II levels, while homozygotes for the I allele have the lowest ACE activity (26,27). In the large cohort analyzed for a correlation between the rs4646994 polymorphism and cancer incidence among RAS inhibitors users, (28) Van der Knaap *et al* concluded that using RAS inhibitors is associated with a significant decrease in the risk of four of the most common non-skin cancers (colorectal, lung, breast and prostate) in individuals with the DD genotype, especially among long-term users. Among RCC patients, the DD genotype and D allele are more frequent than among controls (27). Earlier, Usmani *et al* found a greater incidence of the D allele in non-ccRCC patients vs. RCC, which almost reached statistical significance (26). Genotype distribution did not differ between controls and ccRCC or papillary RCC, while significant differences were present for chRCC. The receptor II genotype has not been observed in chRCC (27).

In a multivariate analysis, the ACE genotype was found to be an independent risk factor for RCC of any kind and for chRCC without a correlation with tumor grade, stage, or nodal and metastatic status (27). The DD genotype and D allele are associated with susceptibility for chRCC, and the II genotype might be protective. The rs4646994 polymorphism is linked with RCC development but not progression. The limitation of the study is a lack of data about plasma levels and ACE activity in relation to genotype and RCC type (27). Polymorphisms rs4295 and rs4343 are not associated with RCC risk, neither in hypertensive nor in normotensive patients (29). Andreotti *et al* (30) has examined another 11 polymorphisms in the ACE gene but did not discover any associations with RCC risk. Polymorphisms in two other genes encoding RAS proteins-AGT (preangiotensinogen) and AGTR1, were examined by Andreotti *et al*, and five AGT single nucleotide polymorphisms (SNPs) [rs1326889, rs2493137, rs7539020, rs3889728, rs3789662] are significantly associated with RCC when adjusted for age, gender, country, smoking status, BMI, hypertension, and lead exposure. Rs1326889 has the highest impact on kidney cancer with OR 1.26 (30).

5. RAS-targeting drugs

Due to the importance of the RAS in maintaining homeostasis and its involvement in the development of many cardiovascular diseases, considerable research efforts have focused on developing drugs that antagonize the RAS. Several

antagonists of enzymes and receptors are now available to inhibit the action of the system. The first class of such drugs includes angiotensin-converting enzyme inhibitors (ACEIs), which are commonly used in the treatment of hypertension, heart failure, myocardial infarction, proteinuria, and diabetic nephropathy. ACEIs inhibit ACE activity and thus decrease the conversion of Ang-I into Ang-II. Examples of this group of drugs are captopril, lisinopril, ramipril, and perindopril. The second-most common group of RAS inhibitors are angiotensin receptor blockers (ARBs), generally known as 'sartans.' Examples of this group are candesartan, irbesartan, losartan, telmisartan, and valsartan. They act as specific inhibitors of AT1 receptors and block the AT1-R-mediated effects of Ang-II. Specific inhibitors of AT2-R (PD123319, PD123117, CGP42114, saralasin), AT4-R (Divalnal-Ang-IV), and MasR (A-779, D-Pro7-Ang 1-7) receptors have been discovered, but so far they are used only in research and clinical studies (5).

6. Effect of ACEI use

Blocking ACE activity results in a decrease in Ang-II levels and an increase in the concentration of Ang-I. Reduced levels of Ang-II cause a decrease in the binding and activation of angiotensin receptors, mainly AT1-R, leading to an increase in renin release (31). It was proved in many studies that renin, independently on Ang-II, can promote fibrosis and cell growth (32,33). Increased levels of Ang-I, resulting from the blockade of ACE, lead to the activation of alternative pathways of its cleavage. ACE-2, which in physiological conditions converts mainly into Ang-II, can produce high levels of Ang 1-7 by converting Ang-I. Ang 1-7 binds to the MasR and promotes a variety of processes depending on the tissue type (34). ACE inhibition does not block the production of Ang-II completely, which can be present in the bloodstream as a result of the activation of alternative enzymes, such as cathepsin G, CAGE, and chymase, which in normal situations play a minor role in the conversion of Ang-I (5).

Additionally, ACEIs decrease the degradation of bradykinin, which stimulates the production of vasodilatory factors, such as NO, cGMP, prostaglandin E2, and prostacyclin (35). Increased levels of bradykinin may promote carcinogenesis by stimulating growth, survival, and cancer cells migration. Its B2 receptors are overexpressed in many tumors, including prostate, renal, and breast cancer tumors. Bradykinin can also increase the permeability of the blood-brain barrier and together with the stimulation of cell migration can result in a higher metastatic potential (36). ACEIs have a few lesser-known activities that may also play a role in their anti-cancer activities. Some ACEIs have intrinsic metal-chelating properties, which are thought to be responsible for the inhibition of matrix metalloproteinase (37). Plasminogen activator inhibitor (PAI) levels can be directly reduced by ACEIs. Drugs containing the free sulfhydryl group lead to the generation of angiostatin, a protein that inhibits angiogenesis (38). The same group also acts as a free radical scavenger (39). A reduction in reactive oxygen species prevents the subsequent activation of metalloproteinase and VEGF, thus reducing tumor invasion. The pleiotropic effects of ACEIs include anti-inflammatory, antioxidant, anti-thrombotic, and pro-fibrinolytic activities. It also improves arterial compliance

through the cytoprotection of the vascular endothelium (35). As presented above, ACEIs, through the blocking of ACE, cause a variety of actions with the activation or inhibition of numerous processes and pathways. Thus, it is difficult to access and evaluate the precise mechanism involved in the effect of this group of drugs on cancer cells.

Role of Ang 1-7 in RCC. The use of ACEI increases the plasma levels of Ang-I, Ang 1-7, as well as renin activity (31). Thus, it is crucial to underline the role of Ang 1-7. Zheng *et al* examined the influence of Ang 1-7 on cell migration and invasion in two RCC cell lines: Caki-1 and 786-O. The migration rates of both types of cells were strongly promoted by Ang 1-7. The invasion ability of the Caki-1 and 786-O cells was increased in the presence of Ang 1-7 by approximately 1.5-fold. Both activities are driven by the Mas receptor (40). Moreover, Ang 1-7 through the Mas receptor causes a pro-inflammatory effect in the kidney through the local activation of the NFκB pathway, as well as the upregulation of proinflammatory genes (41). This altogether suggests Ang 1-7 may have a significant impact on RCC development and progression. The role of Ang 1-7 in renal cancers is contrary to that of other tumors, where it was suggested that Ang 1-7 inhibits cell migration and invasiveness (42). Interestingly, the signaling of Ang 1-7 through MasR inhibits tumorigenesis, probably through its effect on angiogenesis. It has been proven that Ang 1-7 significantly reduces tumor growth and microvascular density and significantly reduces the VEGFA expression (43,44). The effect of this discrepancy in Ang 1-7 on tumors can be explained by different experimental conditions or cell-specific signaling. The presented data suggests that in RCC, Ang 1-7 tends to have a pro-cancerous rather than anti-cancerous effect, but this correlation should be examined in more detail at the molecular level and in xenograft models.

Role of Bradykinin in RCC. Physiologically, bradykinin works as a modulator of renal function, is responsible for electrolyte and water balance, and possesses vasodilatory properties. Via two G-protein-coupled receptors, B1 and B2, bradykinin can promote renal cell growth and proliferation (45-47). Both, the B1 and B2, receptors are expressed in the membranes of ccRCC cells (48). Kramarenko discovered that bradykinin promotes the proliferation of RCC cells (A498 cell line) through the B2 receptor. This activation is dependent on the PLC, PKC, and ERK pathways, as well as on Ca²⁺/Cam activity (49). Thus, the increased concentration of bradykinin observed in ACEI users may promote carcinogenesis and tumor cell proliferation.

Risk of RCC among ACEI inhibitor users. Lever, Hole and Gillis (50) reported a 28% reduction in relative risk of cancer incidence among ACEI users compared with general subjects (HR 0.72, 95% CI, 0.55-0.92), and more attention was paid to the role of angiotensin system inhibitors in carcinogenesis. Many analyses of the impact of ACEI on cancer risk were published, but only a few contained data about the specific risk of RCC. Yoon *et al* performed a meta-analysis of cancer risk among ACEI and ARB users. When stratified by cancer origin site, ACE use was associated with an increased risk of kidney cancer (RR 1.50, 95% CI, 1.01-2.23), as well as melanoma,

and a reduced risk of esophageal tumors. The correlation between RCC and ACEI usage was no longer observed when conventional case-control studies were excluded (51). Although, Fryzek *et al* reported a 1.6 times increased risk of RCC among users of any antihypertensive drug, the increased risk was not associated with any specific class of drugs. The number of prescriptions and length of follow-up did not influence RCC risk (52). In a large analysis of over 3,000 patients with glomerulonephritis, 1% developed any kind of cancer, but no differences in estimated cancer incidence were reported regarding the use of ACEIs and ARBs (53). The use of RAS inhibitors significantly decreased cancer mortality by 0.2% in ACEI/ARB users vs. 2.1% in non-users. ARB usage was an independent risk factor for cancer mortality, which was 0.124-fold compared to non-users of ARBs, while usage of ACEIs did not affect cancer mortality (53). ARBs and ACEIs are safe and do not increase cancer incidence, while ARBs are related with a decrease in all-cause and cancer-related mortality.

Influence of ACEIs on cancerogenesis in in vitro and xenograft models. There are contrary reports on the effect of ACEIs on renal cancer, suggesting they might have an inhibitory effect on RCC cells and xenograft tumors or could promote carcinogenesis. Wysocki *et al* showed that captopril promotes the growth of immunogenic tumors in immunocompetent mice through the regulation of the host's immune system. When mice with a RenCa cell-mediated tumor were treated with different concentrations of captopril, decreased survival was observed among the group treated with the highest concentration of the drug. Similar results were noticed among animals treated with captopril after nephrectomy in comparison to the control group (54). Alternatively, Hii *et al* first described the inhibitory effect of captopril on tumor growth in a xenograft model of RCC. Using SN12K-1 cells, they proved that captopril reduces the size of the tumor in a concentration-dependent manner with no side effects. A reduction in tumor growth is not a direct effect of ACEI action on RCC cells, but rather an indirect effect of decreasing Ang-II levels and inhibiting of angiogenesis (55).

Araujo *et al* performed experiments in a xenograft model using RenCa and a murine cell line of spontaneous origin. Mice treated with ACEI (captopril 10 mg/kg/day), ARB (losartan 1,000 mg/kg/day), or both together exhibited significantly reduced tumor growth, but ACEI and ARB alone had a better impact when compared with double blockage (ACEI + ARB). Treatment with ACEI reduced the frequency of lung metastases at 14.3% when used alone and at 28.6% when combined with ARB (56). ACEI and ARB use results in the decreased expressions of VEGF and CD34, a hemopoietic marker of stem cells, in xenograft models of renal cancer (56). Captopril at a clinically achievable concentration of 0.1-10 μ M has no effect on proliferation in SN12K-1 cells, but a higher concentration can reduce it by 14-31% (55).

Effects of ACEIs on RCC cell lines and xenograft tumors when combined with TKIs. To evaluate the impact of ACEIs on RCC, a few *in vitro* and *in silico* studies were conducted with a combination of ACEIs and TKIs, commonly used in the treatment of metastatic renal cancer. When the 769-P and

A-498 cell lines were incubated with sunitinib and captopril or lisinopril in increasing concentrations (from 0 to 1,000 μ mol/l), a decrease in cell viability was observed. None of the agents was able to cause a significant decrease alone. While under physiological conditions, sunitinib acts primarily on endothelial rather than cancer cell growth, in the same concentration, but combined with captopril and lisinopril, it can significantly reduce cell viability. A similar study was conducted with the use of temsirolimus, showing that the addition of captopril and lisinopril caused a decrease in cell viability, but only in high concentrations (1,000 μ mol/l) (57).

The combination of interferon alfa, cimetidine, COX-2 inhibitor, and ASI (I-CCA therapy) was evaluated in a Phase II trial by Tatokoro *et al* (2011). Patients were receiving combined therapy with ACEI perindopril, which during the trial, due to side effects, was replaced by the AT1-R antagonist candesartan. In the study, objective response rate (ORR) was 22%, complete response rate (CR) 8%, median progression free survival (PFS) 12 months and overall survival (OS) was 30 months, and all of those measurements were significantly better than were those presented in studies that used IFN- α in monotherapy. The efficacy of I-CCA therapy was comparable to that of sunitinib or IFN- α plus bevacizumab (58). The monthly cost of such treatment is approximately three times lower than sunitinib.

Impact of ACEIs on RCC survival

Impact of ACEIs on RCC survival in patients after nephrectomy. The use of drugs targeting the RAS (ACEIs or ARBs) at the moment of diagnosis or nephrectomy can improve prognosis and survival. Miyaima *et al* reported that five-year metastasis-free survival rates were at 93.7% for ARB/ACEIs users and 83.9% for their counterparts ($P=0.035$), while five-year disease-specific survival rates were 96.8 and 89.8%, respectively. Proportions are also significantly higher in ARB/ACEI users when compared to patients receiving other antihypertensive drugs (59). Neither ACEI nor ARB administration was an independent risk factor for a decrease in metastasis-free survival (HR 2.36) and disease-specific survival (HR 2.69) (59). The use of RAS inhibitors tends to be an independent predictive factor of metastasis-free survival and disease-specific survival.

Impact of ACEIs on RCC survival in patients with systemic treatment. Keizman *et al* first described the correlation between ASI use and the clinical outcome of sunitinib treatment. In the analysis of a small cohort of 127 patients, subjects receiving ACEIs or ARBs ($n=44$) showed higher ORRs at the first imaging follow-up after three months in this group vs. non-users (86% vs. 72%). In addition, a decrease in progression rates from 28 to 14% was observed. Recipients of ASIs had doubled the median PFS (13 vs. 6 months, HR 0.537, $P=0.0055$) and demonstrated an insignificant increase in the median OS (30 vs. 23 months, HR=0.688, $P=0.21$) (60). In a multivariate analysis, ASI use was independently associated with improved PFS (HR=0.54) (60). McKay performed an analysis of almost 5,000 RCC patients treated with targeted agents, almost 1,500 of whom used ACEIs, ARBs, or both before and during treatment. OS was significantly longer in ASI users than in non-users (adjusted HR 0.848, 95% CI 0.731-0.960) and individuals receiving no antihypertensive treatment (adjusted HR

0.81, 95% CI 0.707-0.929). Similarly, PFS was significantly longer in ASI users compared with users of other drugs (HR 0.786, 95% CI 0.707-0.876, median 8.4 vs. 6.70 months). In a multivariate analysis, a lack of ASI use was an independent predictor of a worsened OS. When stratified by therapy type, improvements in OS and PFS among ASI users were observed only in patients receiving VEGF-targeted therapy. ORRs in patients receiving ASIs were slightly higher, at 28.31% vs. 22.74%, but the correlation did not reach statistical significance and should be evaluated in clinical studies (57). Results similar to those of Keizman *et al* (60) and McKay *et al* (57) were presented by Izzedine *et al*, who in a multivariable Cox regression model also showed the significant association between ASI use and PFS and OS, with HR=0.4 (0.24-0.66; P<0.001) and HR=0.55 (0.35-0.86; P=0.009), respectively. The protector effect of an ASI user decreases over time (61). Another study of 1,120 patients (361 RCC patients) treated with VEGF signaling pathway inhibitors (sunitinib, sorafenib, pazopanib and others) showed no significant improvements in survival rates between ASI and other drug users (62).

Recently, a secondary pooled analysis of two Phase III randomized controlled trials of patients with mRCC (NCT00334282 and NCT00720941) was published. After adjustment for baseline systolic blood pressure (SBP) and the use of non-ASI antihypertensive drugs and standard prognostic factors in a multivariable model, no significant association was observed between ASI use and OS. When individual VEGF-targeted therapies were analyzed separately, there was a marginally significant association between ASI use and improved OS in patients receiving sunitinib (HR 0.73, P=0.03), but not for those receiving pazopanib or a placebo. A multivariable analysis did not produce a significant improvement in PFS among ASI users (63). In addition, Penttila *et al* (64) did not prove any association between ASI use and PFS or OS among patients treated with sunitinib or pazopanib. Because most studies combine in one study group patients receiving ACEIs and ARBs, it is hard to evaluate the influence of each drug class separately. An analysis by Sorich *et al* (63) did not show a significant association with OS, PFS, ACEIs, or ARBs using patients grouped separately. There is no clear evidence of the impact of other antihypertensive agents, such as beta blockers or calcium channel blockers. Some authors report no influence on ORRs, PFS, or OS among RCC patients (60), while others show a significant association between the baseline use of calcium channel blockers and improved OS (63). Different results concerning ASI correlation with patient survival do not help to specify the direct mechanisms of their activity in RCC patients. Many authors suggest that a blockage of the RAS leads to decreased cell proliferation and changes in the tumor micro-environment. Another theory underlines the role of sarcopenia, which predicts sunitinib-induced early dose-limiting toxicities in mRCC. The use of ACEIs helps to preserve muscle mass and therefore may improve the therapeutic index of VEGF-targeted therapies, subsequently resulting in a longer duration, higher dose-intensity, and improved outcomes (65).

7. Hypertension in TKI users

Incidence of TKI-induced hypertension. Currently, tyrosine kinase inhibitors (TKIs) targeting the VEGF pathway are

commonly used for the treatment of metastatic RCC. One of the on-side effects of this class of drugs is hypertension (HTN). Clinical trials with sunitinib malate have shown a 34% incidence of any-grade hypertension among RCC patients. Sunitinib-induced hypertension is defined as an SBP over 140 mmHg or DBP over 90 mmHg during treatment (66). In a study by Rini *et al* by the end of cycle two, up to 80% of patients treated with sunitinib had systolic-defined HTN and 68% had diastolic-defined HTN (67). The development of HTN is associated with better clinical outcomes. ORR was almost seven times better among patients who developed HTN (54.8 vs. 8.7%). The median PFS and OS are significantly longer among patients with HTN than among those without, with no difference in whether HTN was defined as SBP or DBP over the normal limit. The use of antihypertensive drugs does not reduce the antitumor efficacy of sunitinib and ORR, results that are similar in both groups (67).

The most likely mechanism of the rapid increase in SBP and DBP at the beginning of treatment is a decrease in nitric oxide synthase (NOS) activity caused by the inhibition of VEGF. This leads to reduced nitric oxide production, which in physiological conditions possess vasodilatory activity (68). In the long term, anti-angiogenic drugs can lead to the chronic remodeling of the capillaries, resulting in a reduction of their density, called *capillary rarefaction* (69). The RAS was considered one of the additional pathways involved in the development of VEGF inhibitor-mediated hypertension, but one clinical study showed a decrease in plasma renin concentration and plasma renin activity among patients treated with sunitinib (70) and no significant changes in renin and aldosterone levels in the plasma of patients receiving sorafenib (71). Clinical data were confirmed in a mouse model where plasma renin activity was decreased in rats exposed to TKI cediranib (72). Those observations provide evidence that the activation of the RAS is not involved in the development of hypertension during antiangiogenic treatment, which suppresses the system.

Treatment of TKI-induced HTN. According to TKIs' manufacturers recommendations, BP should be monitored during treatment, and when necessary, antihypertensive treatment should be started or intensified. No treatment algorithm suggesting the class of antihypertensive drugs has been proposed yet, but in most cases, hypertension can be controlled with standard medications. ACEI and ARBs are the most commonly prescribed drugs for patients who developed TKI-associated hypertension. However, some drugs can be more effective in treating anti-VEGF-associated hypertension and have less toxicity in combination with targeted agents. It is suggested that the ACEI enalapril and the ARB candesartan may inhibit angiogenesis and trigger the effects of anti-VEGF drugs. Those data were confirmed in the study on myocardial angiogenesis, but not tested in RCC models (73). In addition, the ASI has an additional benefit of improving endothelial function and microvascular density, key factors involved in anti-VEGF-induced hypertension (74).

Hamnvik *et al* presented data that 30.4% of patients receive ACEI/ARBs as a first class drug for TKI-induced HTN, while calcium channel blockers are started/intensified in 23.7% and beta blockers in 17.8% (62). Szmit *et al* (75) suggest that treatment with at least three antihypertensive drugs significantly

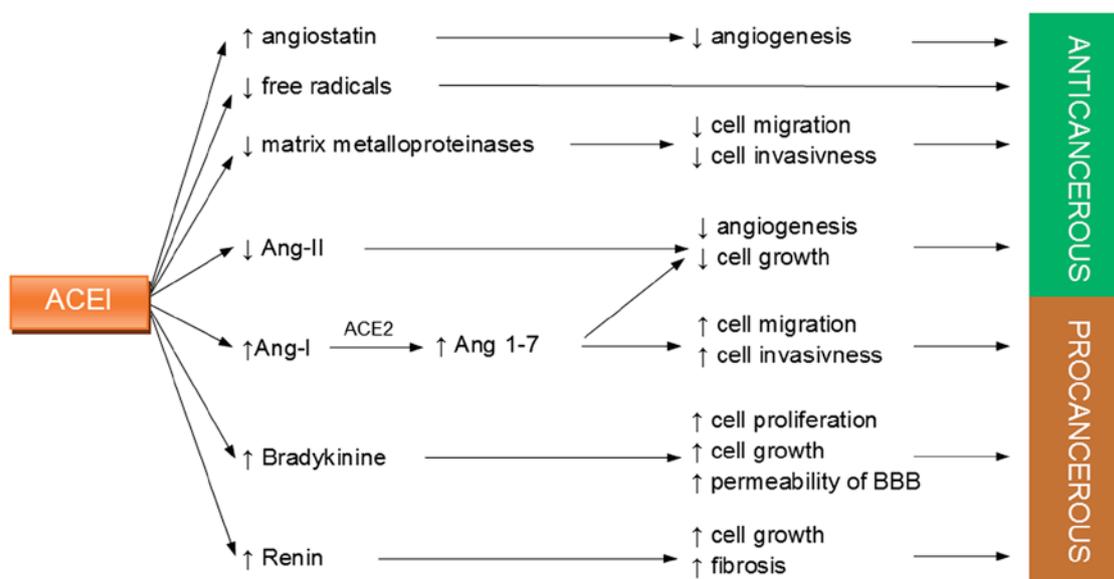


Figure 2. Pro- and anticancerous effects of ACEIs. ACEIs affects level of many different substances like Ang-I, Ang-II, Ang 1-7, renin, bradykinine and others that mediate both, pro- and anticancerous response. BBB, blood-brain barrier; ACEIs, angiotensin-converting enzyme inhibitors; Ang, angiotensin.

improves PFS on sunitinib and OS compared with patients who received one, two, or no medications.

Another important issue in the treatment of hypertension is drug-drug interactions. The metabolism of sunitinib, which undergoes oxidative metabolism mediated by cytochrome p450, mainly CYP3A4, may be potentially altered by anti-hypertensive drugs that inhibit CYP3A4. Such properties have non-dihydropyridine calcium channel blockers, such as verapamil (76). In rats with cediranib-induced hypertension, captopril showed activity of lowering a 10-mmHg increase in BP, but was ineffective with a 35-50-mmHg increase (72). The concomitant use of ACEI captopril with sorafenib can lead to a 30-mmHg decrease in a rise in BP in comparison with sorafenib alone (155 vs. 182 mmHg, $P < 0.05$) and can reduce albuminuria by 50%. Those findings demonstrate that ACEI due to its protective effect on the glomerular filtration barrier can reduce therapy-related glomerular and renal tubular injury, which often occurs during TKI treatment. Captopril improves the renal autoregulatory capacity (77). In another experimental model with rats exposed to sunitinib, captopril had no BP-lowering effect, an effect observed for amlodipine. On the contrary, captopril significantly attenuated a rise in proteinuria. The serum concentration of sunitinib was increased threefold while combined with captopril, confirming the renoprotective effect of ACEI is not associated with the reduced bioavailability of sunitinib. Sunitinib-induced hypertension was associated with a rise in endothelin-1 excretion, which was attenuated by captopril and sildenafil but not amlodipine (78). Recently, Penttila *et al* published a first analysis of the treatment of TKI-induced hypertension with ASIs in patients with metastatic RCC. The study showed that patients receiving ASI as a novel anti-HTN treatment had a significantly longer PFS and OS. It also demonstrated a better OS and PFS among patients with treatment-related HTN for ASI users vs. non-ASI users (64). The study did not assess the differences between the use of ACEI and ARB. High doses of captopril are effective in protecting against renal injury associated with TKIs, but its

effect on increased BP is visible only with a small increase in BP. Based on recent observations stating hypertension induced by TKIs is associated with changes in microvasculature and renin suppression, dihydropyridine calcium channel blockers rather than ASIs are more effective in lowering BP. With the beneficial effect on renal protection, ASIs could be combined with calcium channel blockers.

8. Summary

Molecular studies have revealed specific alterations in the RAS components in RCC. Much evidence concerns the over-expression of angiotensin receptors and the downregulation of ACE. These changes correlate with tumor aggressiveness and grading in Fuhrman's scale; thus, AT1-R, AT2-R, ACE, and ACE 2 can be used as additional biomarkers in histopathological examinations. A different staining pattern may help to determine the type and origin of RCC.

Data that are more inconsistent come from the evaluation of cancer incidence among ACEI users. Despite a general decrease in cancer incidence, one study showed an increase in RCC among ACEI recipients. Further studies did not correlate ACEI with any kind of RCC. Based on this knowledge, this group of drugs can be used without fear of developing kidney cancer.

Influence on survival was evaluated in both, a xenograft model and clinical practice. Some authors present data suggesting ACEIs decrease survival in a mouse model, while others prove decreases in tumor growth, cell viability, and proliferation when used alone or combined with sunitinib. Significant improvements in PFS, OS, and ORR are observed in clinical practice. Similar effects are described for ARBs, but not for any other class of antihypertensive drugs.

The molecular background of the described effects of ACEI on RCC is complex and not completely discovered. ACEI affects the levels of many RAS components: Ang-I, Ang-II, ACE, Ang 1-7, and ACE2, as well as bradykinin,

whose cleavage is mediated by ACE (Fig. 2). A decrease in Ang-II activity, NO, cGMP, prostaglandin E₂, and prostacyclin causes anti-cancerous effects intensified by an ACEI-mediated decrease in the PAI level and the inhibition of metalloproteinase activity. Less clear is the role of Ang 1-7, whose levels are elevated due to the inhibition of ACE and the over-reactivity of ACE2. In experiments on RCC cell lines, Ang 1-7 promoted the migration and invasion of cells; thus, it is suggested that Ang 1-7 promotes cancerogenesis. This activity is in opposition to other activities that increase tumor volume and inhibit angiogenesis and cell proliferation. Further studies in cell cultures and xenograft models should be carried out to define the role of Ang 1-7 in RCC.

Molecular and clinical data leave room for the possible use of ACEIs in RCC treatment combined with currently used drugs, such as TKIs. However, translational studies that are more detailed are necessary to describe molecular bases and prepare treatment algorithms to use in everyday practice with patients.

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