Angiotensin-converting enzyme and the tumor microenvironment: mechanisms beyond angiogenesis

Okwan-Duodu D, Landry J, Shen XZ, Diaz R. Angiotensin-converting enzyme and the tumor microenvironment: mechanisms beyond angiogenesis. Am J Physiol Regul Integr Comp Physiol 305: R205–R215, 2013. First published June 5, 2013; doi:10.1152/ajpregu.00544.2012.—The renin angiotensin system (RAS) is a network of enzymes and peptides that coalesce primarily on the angiotensin II type 1 receptor (AT1R) to induce cell proliferation, angiogenesis, fibrosis, and blood pressure control. Angiotensin-converting enzyme (ACE), the key peptidase of the RAS, is promiscuous in that it cleaves other substrates such as substance P and bradykinin. Accumulating evidence implicates ACE in the pathophysiology of carcinogenesis. While the role of ACE and its peptide network in modulating angiogenesis via the AT1R is well documented, its involvement in shaping other aspects of the tumor microenvironment remains largely unknown. Here, we review the role of ACE in modulating the immune compartment of the tumor microenvironment, which encompasses the immunosuppressive, cancer-promoting myeloid-derived suppressor cells, alternatively activated tumor-associated macrophages, and T regulatory cells. We also discuss the potential roles of peptides that accumulate in the setting of chronic ACE inhibitor use, such as bradykinin, substance P, and N-acetyl-seryl-aspartyl-lysyl-proline (Ac-SDKP), and how they may undercut the gains of anti-angiogenesis from ACE inhibition. These emerging mechanisms may harmonize the often-conflicting results on the role of ACE inhibitors and ACE polymorphisms in various cancers and call for further investigations into the potential benefit of ACE inhibitors in some neoplasms.

angiotensin-converting enzyme; myeloid-derived suppressor cells; macrophage polarization; T regulatory cells; angiogenesis; cancer; tumor immunology
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Fig. 1. The peptide network of the renin-angiotensin system (RAS). Angiotensinogen is converted by renin to angiotensin I, which is further converted into angiotensin II by angiotensin converting enzyme (ACE). Angiotensin 1–7 and 1–9 (ANG 1–7, ANG 1–9) are byproducts of the catalytic activity of angiotensin converting enzyme-2 (ACE2). Other peptidases, such as aminopeptidase N, M, and B (APN, APM, APB) and neutral endopeptidase (NEP), may cleave peptides of the RAS. These substrates may act on angiotensin receptors (AT1R or AT2R) or the Mas oncogen receptor (MAS). Other non-RAS peptides such as bradykinin are degraded by ACE.

neutral or even negative impact of ACE inhibitor or RAS blocker use on specific cancers in clinical models (6, 132, 153). These conflicting data on the effect of ACE inhibition are further complicated by studies of insertion/deletion polymorphisms in the ACE gene, where different genotypes that influence varying levels of ACE expression in the circulation or in tissues (119) may be associated with increased, reduced, or no cancer risk (32, 56, 63, 152, 156). That there are such incongruities regarding the role of ACE inhibitors in cancer suggests a complicated role of the RAS far beyond AT1R-mediated angiogenesis effects. It also suggests potential multiple effects of the peptide products and substrates of ACE that may impact the immunological and nonimmunological microenvironment of the tumor. In this review, we present an overview of the complex immune network within the tumor microenvironment, and then highlight aspects of tumor immunology affected by ACE inhibition. We shall place special emphasis on the mechanisms of immunosuppression that allow for tumor escape from efficient anti-tumor immunity and how substrates of ACE may modulate these different facets, with the goal to harmonize the seemingly conflicting role of ACE in human cancer.

The Tumor Microenvironment

Tumor cells do not grow in isolation (31, 73). A complex host-tumor interplay is required to facilitate tumor proliferation, escape from appropriate host-mediated immune clearance, matrix remodeling, as well as metastasis to distant sites (31). A substantial component of the tumor mass comprises myelomonocytic lineage cells [myeloid-derived suppressor cells (MDSC), tumor-associated macrophages] as well as regulatory T cells, fibroblasts, stromal, and endothelial cells (61). Indeed, the number of macrophage infiltration within the tumor bed is a positive predictor of metastatic potential, poor prognosis, and shortened survival for several cancers (19, 135). Macrophages may differentiate along a spectrum of phenotypes, the alternatively activated M2 and classically activated M1 macrophages representing the extremes of the spectrum (90). While M1 macrophages are broadly considered anti-tumor, M2 macrophages (which are predominantly expressed within the tumor microenvironment) are considered pro-tumor (90). In contrast to mature macrophages, MDSCs are a heterogeneous group of immature myeloid lineage cells (53). They comprise poorly differentiated neutrophils, dendritic cells, and monocytes. In mice, MDSCs have the phenotype of Ly6C(+/−)CD11b(+)Gr-1(+) (69, 111). Along with M2 macrophages, MDSCs orchestrate a complex immunosuppressive milieu by inhibiting tumor-specific CD8 T cells, inducing regulatory T cells, promoting angiogenesis, extracellular matrix remodeling, and enhancing cancer cell metastasis (90, 114).

Regulatory T cells are another subset of immunosuppressive cells that are found in the tumor microenvironment as well as in the systemic circulation of cancer patients (22, 44). These activated CD4(+)CD25(+)Foxp3(+) T cells are induced in part by MDSCs (68). They have been described in nearly all types of cancers and function primarily to promote tumor escape by interfering with appropriate anti-tumor CD8 T cell responses (110). Through the production of cytokines and other factors, regulatory T cells may also facilitate angiogenesis (42).

The major culprit of angiogenesis within the tumor microenvironment is endothelial cell activation following the engagement of VEGF and other factors (such as fibroblast growth factors) with their respective receptors and downstream effect within these cells (16). As we shall discuss further below, it is important to note that ACE and other components of the RAS are expressed in all the cell types found within the tumor microenvironment, including endothelium, monocytes, macrophages, dendritic cells, fibroblasts, and T cells (11, 38, 83, 125). It is therefore imperative to delineate the possible role and function of ACE in these different compartments to evaluate and maximize pharmacological targeting of the RAS in cancer.

Renin Angiotensin System and Angiogenesis

Angiogenesis, the formation of a neovascular blood supply derived from preexisting blood vessels, is central to sustaining proliferative cell growth (23). Blockade of angiogenesis has long been proposed to be an effective mechanism to control tumor growth (48, 74, 89). The role of the RAS in angiogenesis and cellular growth has been demonstrated in several models. In vitro, angiotensin II mediates VEGF induction in a number of cell types, including mesenchymal stem cells (128), as well as primary pancreatic (4), breast (71), and endometrial cancer cells (112). Angiotensinogen and its cleaved derivatives, similar to other serpins (serine protease inhibitors) such as anti-thrombin III, possess antiangiogenic properties (27, 35). It appears that AT1R, AT2R, and other receptors may be involved in a tissue-specific manner (137, 138). The role of the AT1R as a potent G protein-coupled receptor (GPCR) in modulating facets of angiogenesis through vascular smooth muscle cells (VSMCs) has been extensively reviewed (35, 118). Accordingly, suppression of the RAS inhibits angiogenesis in a number of in vivo cancer models, such as esophageal carcinoma (147), endometrial (129), and pancreatic cancer (102).
In addition to generating angiotensin peptides, ACE may also function independent of the RAS-AT\textsubscript{1}R cascade as a member of transmembrane proteases involved in the degradation of extracellular matrix, an important initial step of angiogenesis (15). Direct ACE signaling has also been reported (76, 77), although its involvement in angiogenesis has not been described. Currently, ACE inhibitors are receiving attention as potential additional therapy to standard chemotherapeutic regimens with the proposed mechanism being angiogenesis inhibition (102, 148). However, the effect of ACE via AT\textsubscript{1}R-mediated angiogenesis is insufficient to account for the reported discrepancies on the role of ACE inhibitors in cancer. The inference is that ACE likely mediates other aspects of the tumor microenvironment.

We shall next focus on myelomonocytic and T cell functions.

**ACE and Myeloid Lineage Cells**

Hematopoietic stem cells (HSCs) give rise to a clonogenic common myeloid progenitor cells, from which arises all myeloid lineages, including granulocytes, monocytes, and myeloid-derived dendritic cells (1). HSCs themselves arise from bipotential hemangioblasts, which differentiate into hematopoietic and endothelial cells (14). Human and mouse cell culture assays have shown that ACE is one of the earliest true markers of hematopoietic potential (72, 141, 154). Indeed, ACE and components of the RAS were found in the earliest stage of embryogenesis in chicken yolk. Culture of these embryos showed that ACE(+) cells were endowed with hematopoietic potential, whereas ACE(−) cells were not (122).

While ACE (also referred to as CD143) expression is dramatically increased during differentiation and maturation of monocytes into macrophages or dendritic cells (38, 49, 125), the role of ACE in myeloid differentiation and myeloid-mediated immune responses to tumors remain largely unknown. Important advances were made toward that end by the advent of the ACE 10/10 mouse model (126, 127). In brief, the ACE 10/10 mice were generated by a promoter-swapping technique, where ACE expression was directed by the myelomonocytic lineage-specific c-fms promoter (65). Accordingly, these animals express nearly 20-fold increased ACE levels in macrophage and myeloid lineage cells (125, 126). When these mice were challenged with several cancer cell lines including the B16-F10 melanoma and EL-4 lymphoma, they significantly resisted tumor growth compared with the wild-type (WT) counterparts (126). Blockade by ACE inhibitors reversed the improved response to tumors. Interestingly, the ACE 10/10 mice maintained enhanced response to tumors when treated with AT\textsubscript{1}R blockers, suggesting that the effect of ACE was to some degree not modulated through AT\textsubscript{1}R (Fig. 2A) (126).

The promoter-swapping approach employed in the ACE 10/10 model leads to an absence of ACE in endothelial tissue (126). To address any impact of this modification on the inflammatory response, the tumor response was evaluated in a separate mouse model in which ACE expression was limited in the liver by using an ovalbumin promoter, thereby abrogating endothelial ACE as well. These animals, called the ACE 3 (29, 30), were not equivalent to the ACE 10/10 in response to tumor challenge. Rather, they showed similar response to tumor challenge as the WT mice (Fig. 2A). Thus the enhanced response to tumor in the ACE 10/10 is not due to absence of endothelial ACE but rather from ACE overexpression specifically in myelomonocytic cells. Indeed, bone marrow transplant of ACE 10/10 cells into WT mice endowed the recipient animals with enhanced immunity to tumors (126). This was unassailable proof of principle that

![Fig. 2. B16-F10 tumor growth in wild-type (WT), ACE 3, and the ACE 10/10 mice. A: mice were challenged with 1 × 10^6 tumor cells. Fourteen days later, tumor volume was measured (n ≥ 6 mice per group). Some mice were left untreated, while others were treated with losartan, an ACE inhibitor (ACEi), losartan (Los), an AT\textsubscript{1}R blocker, or hydralazine (Hyd), an agent that lowers blood pressure independent of the RAS. Figure is adapted from Shen et al. (126). ACE 3 mice overexpress ACE in the liver. ACE 10/10 mice, but not ACE 3, demonstrated statistically significant reduction in tumor volume compared with WT. Lisinopril, but not losartan, reversed the improved tumor response in the ACE 10/10. There was no significant (ns) difference between tumor volumes in untreated mice and those treated with Hyd. B: measurement of blood pressure after treatment with Hyd, Los, and ACEi. Blood pressures of mice treated above were measured by standard tail-cuff methodology to verify efficacy of pharmacological inhibition in A. Blood pressures of mice treated above were measured by standard tail-cuff manometry to verify efficacy of pharmacological inhibition in A.](http://ajpregu.physiology.org/)
myelomonocytic cells transformed with increased ACE expression demonstrated robust anti-tumor responses. Thus, while endothelium-mediated angiogenesis is crucial for tumor growth, it is likely an insignificant player in the observations of the ACE 10/10 tumor response. Furthermore, the effects in the ACE 10/10 mice were independent of blood pressure abnormalities (Fig. 2B). The enhanced immune response in the ACE 10/10 mice have been duplicated in a methicillin resistant Staphylococcus aureus (MRSA) and Listeria monocytogenes model, both of which require a robust M1 inflammatory response (105).

ACE and MDSC. A hallmark of cancer progression is the induction of dysfunctional myelopoiesis leading to accumulation of MDSC and the polarization of macrophages toward the M2 phenotype (53). The study of the ACE-deficient [ACE knockout (KO)] mouse provides useful lessons regarding the potential effect of ACE inhibition on the accumulation and function of these cells types. Recently, it has become apparent that ACE and its peptide network may play an important role in ensuring proper myelopoiesis (124). It has been demonstrated that ACE KO mice undergo extensive extramedullary myelopoiesis to compensate for bone marrow incompetence. ACE KO mouse expressed increased levels of CD11b+Gr1+ cells (83), which is a characteristic feature of the MDSCs (53). However, the function of these cells and their ability to suppress CD8+ T cell function in the ACE KO has not been tested. By contrast, although the tumor microenvironment was infiltrated by myelomonocytic lineage cells in the ACE 10/10 animals during tumor challenge, there was enhanced CD8+ T cell responses that contributed to tumor resistance in this animal model (126). This suggests that the infiltrating myeloid cells in the ACE 10/10 mice are functional antigen-presenting cells and likely not of the CD8+ T cell-inhibiting MDSC phenotype. Thus, ACE, required for normal myelopoiesis (83), may also favor differentiation of immature myeloid cells away from the MDSC subset during tumorigenesis. Further work is required to delineate the role of MDSC in the ACE 10/10 model in vivo, as well as the role of ACE in influencing development of myelomonocytic cells in vitro and in vivo.

ACE and M1/M2 macrophage polarization. Thioglycollate broth is commonly employed in the induction and recruitment of macrophages into the peritoneum of mice, which may then be harvested ex vivo and purified for various functional assays (39). Stimulation of these macrophages with different agents such as lipopolysaccharide (LPS) and chitin (a polymer of N-acetylgalcosamine) may induce the pro-inflammatory M1 and alternatively activated M2 macrophage, respectively (121). Work on the ACE KO mice showed that macrophages from these mice produce reduced pro-inflammatory cytokines such as IL-12, nitrite, and TNF-α in response to LPS stimulation (Fig. 3A). By contrast, equivalent stimulation of the ACE 10/10 induced relatively increased pro-inflammatory cytokines (Fig. 3A). We and others have also shown that pharmacological inhibition of ACE reduces these pro-inflammatory cytokine in primary cultured macrophages or transformed macrophage cell lines (52, 59, 83). Clinically, ACE inhibitors reduced the pro-inflammatory cytokine dysregulation in patients with cardiovascular disease (57). Thus ACE blockade does reduce M1 inflammatory response, whereas ACE overexpression in macrophages may augment the M1 response as demonstrated by the increased pro-inflammatory cytokine production in the ACE 10/10 model. Indeed, granulocyte-macrophage colony stimulating factor (GM-CSF), a potent inducer of M1 macrophages (47, 121), has been shown to upregulate ACE expression in human monocytes (79). Of note, a subset of inflammatory ACE-expressing monocytes have been found in patients with heart failure and chronic renal disease, with deleterious implications owing to their association with overt production of inflammatory cytokines (13, 144). Thus the role of ACE in the M1 inflammatory response is robust. By contrast, the role of ACE in M2 polarization is rather unclear. We found no difference in the induction and function of M2 macrophages in the ACE 10/10, ACE KO, and the WT animals, as demonstrated by expression of surface markers such as Siglec-F(+)CD11b(+) and mannose receptor (MR) following stimulation with chitin (Fig. 3B). Other groups, however, found increased or decreased M2 response by RAS blockade (2, 78, 93, 151). Further work is required to clarify the role of ACE in M2 macrophages in vivo and in vitro, as this may influence response to tumor challenge.

Although the ACE 10/10 and ACE KO animal models are important advances toward our understanding of the role of ACE in myelomonocytic cell function, it is necessary to note some of the limitations from this work. Human monocytes express minimal ACE, which is further upregulated as these cells differentiate into dendritic cells (38). We used Ly6C expression to sort monocytes in mouse and also observed a surge in ACE expression during differentiation into macrophages and dendritic cells (125). However, Balyasnikova et al. (12) have previously demonstrated reduced ACE expression during differentiation of mouse monocytes into macrophages, based on CD11b, F4/80 and Gr-1 expression. Furthermore, we have not tested if forced expression of ACE in human myeloid progenitor cells would facilitate differentiation into mature macrophages and dendritic cells, thereby eliminating the pool of immature, pro-tumor myelomonocytic cells as we observed in the ACE 10/10 model. Thus the differential expression of ACE in mouse and human monocytes, and its role in differentiation and function, requires further elucidation. The hope of translational implications of the ACE 10/10 model must be duly balanced with these caveats.

ACE in T Cells

Accumulating evidence over the past few years suggests that all components of the RAS are also expressed in T lymphocytes (66, 116). Indeed, ACE expression levels, absent in B cells, are genetically determined in T cells by polymorphism of the ACE gene (37). Although T cell RAS have been recently implicated in different models of hypertension (58, 62), no direct evidence has been demonstrated for modification of T cell function by ACE in tumor immunology. Maeda et al. (88) have recently showed that CD8+ T cells express AT1R, the levels of which are enhanced by T cell receptor ligation with anti-CD3 antibody. Their work, corroborated by others, show that angiotensin II increases cytokine production by CD8+ cells, a phenomenon that is reversed by both losartan (an AT1R) or on ACE inhibitor. In a similar fashion, CD4 T cells proliferation and function may also be impacted by the RAS (103, 130). The work of Nataraj et al. (103) suggests...
that in the absence of AT1R expression on T cells, proliferation of T cells is impaired, dampening the inflammatory response and permitting cardiac transplant without overt host reaction. Akin to their influence on monocyte recruitment, ACE inhibitors could also affect recruitment of T cells (130).

Strong evidence supporting the role of the RAS in T cell function is provided from autoimmunity models, where ACE inhibition potently induced regulatory T cells and significantly ameliorated the course of experimental autoimmune encephalomyelitis (EAE) in mouse (113, 134). These two separate groups both demonstrated that RAS inhibition improved multiple sclerosis by favoring activation of regulatory CD4\(^{+}\)CD25\(^{+}\)Foxp3\(^{+}\) T cells, while dampening Th1/Th17 response. It is therefore plausible that the use of ACE inhibitors could similarly stimulate this subset of T cells that hamper tumor antigen-specific immune responses, while dampening anti-tumor CD8 T cell responses. The mechanisms by which the RAS influences T cell development are currently of prime interest. A newly developed genetic model that ablates AT1R expression specifically on T cells (155) should provide powerful insights into this question. This animal model will also prove valuable when evaluating whether all the effects of ACE inhibitors on T cells are mediated through the AT1R.

**Role of ACE Outside the RAS**

ACE is widely known for its key role in conversion of angiotensin I to angiotensin II. However, our in vivo and in vitro data in the ACE 10/10 mouse as well as the promiscuity of ACE as a metallopeptidase suggest that other peptide substrates or product of ACE may mediate aspects of tumor biology. It is important to address the effects of the ACE substrates in tumor microenvironment so as to fully understand the complicated role of ACE inhibitors in carcinogenesis. Here, we shall focus on some substrates of ACE, including bradykinin, substance P (SP), and Ac-SDKP.

**Bradykinin.** Bradykinin is a nonapeptide substrate of ACE that mediates vasodilation (123). There are two major receptors of bradykinin: kinin B(2) receptors [B(2)R] are constitutively expressed on neurons and smooth muscle cells, while kinin B(1) receptors [B(1)R] are upregulated in the context of tissue injury and inflammation (20). Because kinin metabolites such as des-Arg\(^{9}\)-bradykinin and des-Arg\(^{10}\)-bradykinin are substrates of ACE, ACE inhibition leads to accumulation of kinins...
and subsequent activation of B(1)R (70, 117). There is evidence to suggest a role for bradykinin in carcinogenesis. Bradykinin is implicated in the increased secretion of endothelin-1 in melanoma cells, which stimulates their growth and survival (5). Bradykinin also enhances migration of human cholangiocarcinoma cells through B(1)R (92). Furthermore, bradykinin has been implicated in altering blood-tumor barrier permeability via cytoskeletal rearrangements (87). In fact, so profound is this effect that use of low-frequency ultrasound irradiation with low-dose bradykinin recapitulates this response (149, 157). By influencing barrier permeability and potentially stimulating the release of pro-inflammatory cytokines (21), kinins may play important roles in tumorigenesis. Accordingly, bradykinin antagonists may inhibit tumor growth in rodents (45). These data suggest that although ACE inhibition has a profound effect on angiogenesis versus AT1R, this benefit may be blunted and even possibly reversed by the secondary effects of accumulated bradykinin and activation of the B(1)R.

Bradykinin accumulation in the setting of ACE inhibitor use may also influence the cellular components of the tumor microenvironment. For example, bradykinin activation of B(2)R on dendritic cells promotes IL-12 induction, a response that is augmented by ACE inhibitor use (3). Interestingly, B(1)R antagonism, contrasting with agonism by ACE inhibitor, also led to suppression of EAE in part by inhibiting Th1 and Th17 responses in T cells (40, 55). Furthermore, bone marrow cells from B(1)R-deficient mouse demonstrated reduced numbers of primitive myeloid precursor cells and expression of myeloid-lineage markers (106). Indeed these animals mount impaired response to LPS. These conflicting data echo an incomplete understanding of the confluence between ACE inhibitors and bradykinin activation of B(1)R or B(2)R, or more broadly, the RAS and the kinin system. How these systems influence T lymphocytes, dendritic cells, and macrophages during tumor challenge are not known. Additional work is also required to evaluate the clinical implications of the activation of B(1)R in patients undergoing ACE inhibitor therapy.

Substance P. Another substrate of ACE is the neuropeptide SP (24, 67). It is an 11-amino-acid tachykinin released from the ends of specific sensory neurons to mediate pain and inflammation (46). Although SP primarily produced by neurons, immune cells may express appropriate receptors and be activated by neurokinin-1 (NK-1) (115). Not only does SP collaborate in the peptide network regulating hematopoiesis (124), but the SP-NK-1 activation may be involved in stimulating pro-inflammatory cytokine production via NF-kB (143, 150). The role of SP in cancer is most thoroughly studied in gastrointestinal malignancies. SP activates the NK-1 receptor, which is expressed on primary colon and gastric adenocarcinoma cells (120). Binding and activation of the NK-1 receptor facilitates cell proliferation, angiogenesis, cell survival, and migration of the tumor cells for invasion and metastasis (91, 109). In fact, the NK-1 receptor is also expressed on a number of other cancer cells, including melanoma, glioblastoma, laryngeal, and breast cancers (25, 95, 97, 98). SP may facilitate migration of certain cancer cells such as gastric cancer cells (43). The SP-NK-1 interaction has currently received increasing attention and is an attractive target for cancers (94, 96, 109). The clinical implication of potentially elevated SP in the setting of ACE inhibitor use is unclear, but this could represent an interesting confounder in the already murky role of ACE inhibition in cancer.

AcetylSDKP. Another substrate of ACE worthy of mention is the N-acetyl-seryl-aspartyl-lysyl-proline (Ac-SDKP). It is a naturally occurring tetrapeptide released from thymosin β4 by prolyl oligopeptidase (26). Ac-SDKP is hydrolyzed almost exclusively by the NH₂-terminus of ACE and thus accumulates in the setting of ACE inhibitor use or NH₂-terminus ACE inactivity (9, 10, 50). Intriguingly, some studies have reported increased accumulation of Ac-SDKP in cancer patients (84–86). Although the role of Ac-SDKP in carcinogenesis is not completely understood and likely multifaceted, robust evidence has been presented for its role in angiogenesis (86, 99). Thus ACE inhibitor use blocks angiotensin II-mediated angiogenesis but also may increase the accumulation of the proangiogenic Ac-SDKP.

Several models of inflammation and fibrosis have provided insight into the potential role of Ac-SDKP in modulating the inflammatory cells that participate in carcinogenesis. A study of angiotensin II-induced hypertension in the NH₂-terminus inactivated mice (called N-KO) showed augmented hypertension compared with WT (107). Interestingly, ex vivo studies showed that macrophages from the N-KO had augmented pro-inflammatory cytokine profile, suggesting that accumulated Ac-SDKP may impact macrophage polarization toward the M1 phenotype (107). By contrast, several other models of inflammation, including experimental autoimmune myocarditis, found reduced pro-inflammatory cytokine markers in the presence of Ac-SDKP (100). Thus the role of Ac-SDKP in shaping macrophage function is not resolved. Neither has the role of Ac-SDKP in T cell maturation and function, if any, been elucidated. Nonetheless, it is fairly well established that Ac-SDKP is a negative regulator of hematopoiesis, driving progenitor cells into nonproliferative stages of the cell cycle (80). Whether this tetrapeptide confounds the generally beneficial impact of ACE on tumor immunology remains an interesting area of investigation. The availability of the N-KO mouse model (50, 107) should not only provide answers to these important questions but also enable evaluation of the role Ac-SDKP on immune cells without the potential complicated effects of other ACE substrates and peptides that accumulate in the setting of routine ACE inhibitors, which block both termini of the ACE protein.

Summary

The wide substrate specificity and diverse function of ACE in different cell types within the tumor microenvironment presents a perplexing and potentially antithetic role of ACE in tumorigenesis (Fig. 4). Thus, not surprisingly, there has been conflicting reports on the role of ACE inhibition and ACE polymorphism in cancer progression and survival (81, 132). As we have shown, in addition to blocking angiogenesis, ACE inhibitors could potentially impair myeloid differentiation, induce regulatory T cells, or lead to accumulation of certain substrates that may influence various aspects of tumor biology. Indeed, use of ACE inhibitors (or RAS blockers) as combination therapy with other agents continues to show promise in various studies (75, 101). One intriguing but poorly explored
area is combination ACE inhibitor use with antagonists of some of the pro-tumor peptides such as bradykinin that accumulates in the setting of ACE inhibitor use. For example, it is likely that combination ACE inhibition and bradykinin antagonists may augment anti-tumor responses (136). A better understanding of the role of the peptide network of ACE in the tumor microenvironment provides further rationale for which combination therapies would be suitable, as well as which cancers would benefit from such therapies. One area worthy of further investigation is the characterization of the differential expression ACE and RAS components in specific tumor types as a potential predictor of response to ACE inhibitor use in combination therapy with appropriate, standard chemotherapy regimen (71, 112, 146).

Understanding the role of the RAS in myelomonocytic lineage cells is particularly pertinent not only for cancer but also in cardiovascular diseases. For example, it has recently been shown that the recruitment of reservoir of monocytes from the spleen after tissue injury (such as myocardial ischemia) is dependent on AT_1R signaling (140). Interestingly, the spleen may also be a source of MDSCs (33). Because, MDSCs regulate diverse inflammatory diseases, appreciating the role of ACE in myeloid cell recruitment, differentiation and function could provide mechanistic understanding not only for anti-tumor immunity but also in inflammation and end-organ damage pertaining to cardiac, renal, and other organ systems where RAS regulation is crucial. That the components of the RAS are expressed in almost all immune cells is evidence of the central role of this system in inflammation and disease.

**Perspectives and Significance**

Despite robust preclinical evidence that ACE inhibitors reduce tumor growth likely via AT_1R-mediated angiogenesis inhibition (41), controversy still remains regarding the effect, if any, of this agent on outcomes in cancer patients (60, 81, 131, 132). One possible reason is the inherent limitations associated with statistical approaches employed in these broad populational-based analyses (133). Another potential factor is that patients on ACE inhibitors have underlying cardiovascular and/or renal indications that by themselves possess inflammatory components (82) that may confound the potential effect of ACE inhibitor. Unfortunately, very few prospective double-blinded studies have been described to evaluate the benefit of this drug without the confounding cardiovascular factors. However, another implication is that the promiscuous activity of ACE as a peptidase may result in the clearance or accumulation of certain peptides that may exert multiple, perhaps antithetic, roles not only on angiogenesis, but also on the inflammatory milieu of the tumor microenvironment. By exploring some of the mechanistic underpinnings of ACE on the immune cellular tumor microenvironment beyond angiogenesis, this review sheds important light on this controversy. Because millions of patients take ACE inhibitors, we believe this work is critical toward our understanding of this puzzling, evolutionarily preserved system that appears pivotal in multiple human physiology and pathophysiology, while highlighting the importance of integrative biology.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**

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