Carcinogenesis vol.29 no.9 pp.1675–1684, 2008
doi:10.1093/carcin/bgn171
Advance Access publication July 16, 2008

REVIEW

The renin–angiotensin system and malignancy

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The renin–angiotensin system (RAS) is usually associated with its systemic action on cardiovascular homoeostasis. However, recent studies suggest that at a local tissue level, the RAS influences tumour growth. The potential of the RAS as a target for cancer treatment and the suggested underlying mechanisms of its paracrine effects are reviewed here. These include modulation of angiogenesis, cellular proliferation, immune responses and extracellular matrix formation. Knowledge of the RAS has increased dramatically in recent years with the discovery of new enzymes, peptides and feedback mechanisms. The local RAS appears to influence tumour growth and metastases and there is evidence of tissue- and tumour-specific differences. Recent experimental studies provide strong evidence that drugs that inhibit the RAS have the potential to reduce cancer risk or retard tumour growth and metastases. Manipulation of the RAS may, therefore, provide a safe and inexpensive anticancer strategy.

Introduction

Cancer is the leading cause of death worldwide (World Health Organization) (1). Therapeutic strategies usually involve a combination of surgical ablation, radiotherapy and chemotherapy. Apart from conventional chemotherapy, targeting of the tumour vasculature by vascular disrupting agents or inhibitors of angiogenesis has also been used.

Recent evidence suggests an alternative pathway for targeted therapy in cancer. Several paracrine mechanisms existing at local tissue sites have been implicated in tumourigenesis. One such system is the renin–angiotensin system (RAS) that exists in several organs at a local tissue level. Epidemiological and experimental studies now suggest that the RAS may contribute to the paracrine regulation of tumourigenesis. Blockade of the RAS may, therefore, provide an alternative, adjunctive therapy for the treatment of solid tumours.

The RAS

Commonly, the RAS has been associated with the systemic regulation of cardiovascular homoeostasis. However, there is now increasing evidence that local RASs may influence tissue angiogenesis, cellular proliferation, apoptosis and inflammation (2). Components of the RAS are expressed in several adult organs including the liver, kidney, pancreas, brain and reproductive organs (3). It is the paracrine mechanisms of locally expressed RASs, not its circulating counterpart, that appear important for tumourigenesis.

A variety of physiological responses can be induced through activation of the RAS, some of which can have antagonistic consequences for tumour growth. The physiological malleability of the RAS is achieved by alternative peptides and receptors. Angiotensin (ANG) II is the main effector of the RAS (Figure 1). ANG II is an octapeptide cleaved from ANG I by the angiotensin I-converting enzyme (ACE). The majority of ANG II effects are mediated by the AT1 receptor (AT1R). The *AT1R* is expressed in many adult tissues, including blood vessels, adrenal cortex, liver, kidney, and brain (4). A second receptor encoded by a different gene, the *Angiotensin II type 2 receptor AT2R*, is predominantly expressed during foetal life, but is present at a low level in a few adult tissues such as the adrenal medulla, uterus and ovarian follicles (5–7). Whereas the AT1R induces angiogenesis, cellular proliferation and inflammatory responses, as well as being anti-apoptotic (8,9), the AT2R appears to functionally antagonize many of these actions (4,6,10). There is some evidence, however, that signalling via the AT2R can also be pro-angiogenic (11) and pro-inflammatory (12).

The Mas1 oncogene (MasR) represents a fifth RAS receptor and binds the ANG-(1–7) peptide (13). ANG-(1–7) may be generated directly from ANG II by the enzymatic activity of ACE2 or from ANG I, via ANG-(1–9), a pathway that utilizes both ACE2 and ACE (14,15). ACE2 is present in many tissues with high concentrations in the heart, kidney and gastrointestinal track (14). *ACE2* expression is increased in animal models of liver injury and in human cirrhosis and is associated with increasing plasma and tissue levels of ANG-(1–7) (16).

ANG-(1–7) appears to have an inhibitory influence on many of the events induced by ANG II (15). ANG-(1–7) has depressor, vasodilator, apoptotic and anti-proliferative actions (17). ANG-(1–7) is suggested to inhibit angiogenesis (18,19), although further investigations are needed to confirm these effects in a wider range of pathological/physiological conditions. In contrast, ANG-(1–7) may also mimic some actions of ANG II. For example, ANG-(1–7) induces the release of prostanoids (17) and may increase proliferation of some cells, such as epidermal stem cells after injury (20) and haematopoietic progenitors in the bone marrow of myelosuppressed mice (21).

The variety of physiological responses to the RAS reflects the alternative peptides and receptors and the different signalling pathways they induce. The balance between these signalling events will influence the proliferative and angiogenic phenotype of cells that are either directly or indirectly responsive to the RAS. Therefore, it can be hypothesized that the balance between components of the RAS will contribute to tumour growth, angiogenesis and metastatic potential (Figure 2).

Evidence for a RAS contribution to cancer development

Given the expression of local RASs in many tissues, it is perhaps not surprising that many components of the RAS are also expressed in malignant tissue. However, the RAS, in particular the AT1R, is often up-regulated during the progression from normal to malignant phenotypes, indicating at the very least a correlation between the RAS and tumour progression.

Components of the RAS are frequently differentially expressed in various cancers including brain, lung, pancreatic, breast, prostate, colon, skin and cervical carcinomas in comparison with their corresponding non-malignant tissue (2). In particular, over-expression of the *AT1R* is common. Changes in the expression of RAS components appear to correlate with tumour grade (22,23). These changes, however, are not consistent and vary for individual tumour types. For example, high levels of *AT1R* are found in breast hyperplasia but decrease when breast cancer becomes invasive (24), while in ovarian carcinoma up-regulation of *AT1R* correlates with tumour invasiveness (25). These examples of *AT1R* expression in breast cancer suggest that, while up-regulation of AT1R is common to abnormal breast tissue, whether this increase is associated with higher or lower grades of tumour may depend on the expression of other components of the RAS.

Abbreviations: ACE, ANG I converting enzyme; ANG, Angiotensin; AT1R, Angiotensin II type 1 receptor; AT2R, Angiotensin II type 2 receptor; EMT, epithelial to mesenchymal transition; ET-1, endothelin-1; HSCs, hepatic stellate cells; MasR, mitochondrial assembly receptor; MCP, macrophage/monocyte chemoattractant protein; PDGF, platelet-derived growth factor; RAS, renin-angiotensin system; VEGF, vascular endothelial growth factor.

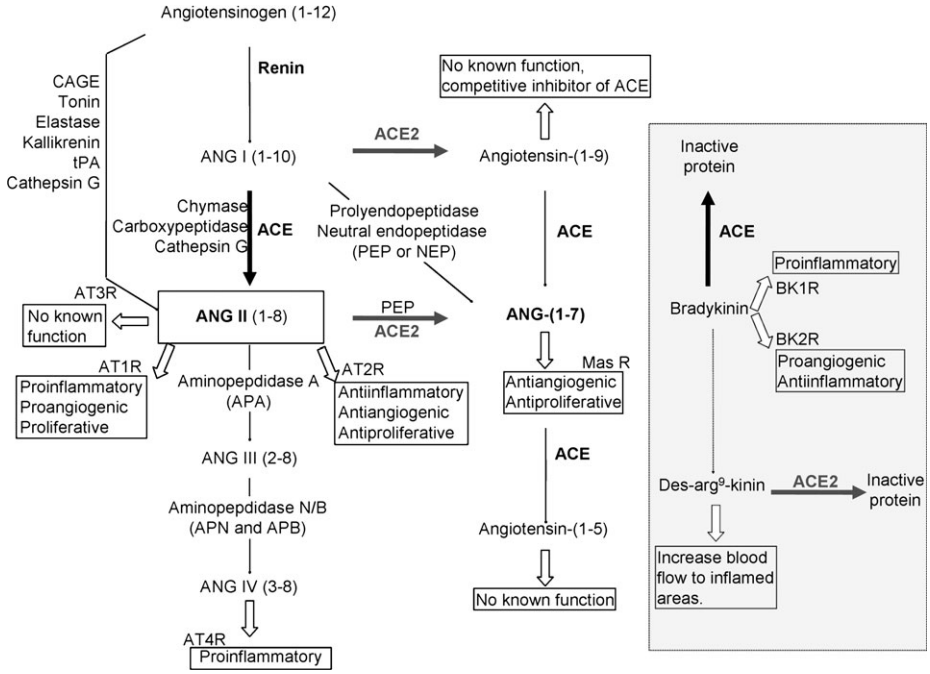

Fig. 1. The RAS. Several enzymes catalyze the generation of angiotensinogen-derived peptides (thin black arrows). The most prominent of these, however, are ACE (thick black) and ACE2 (thick grey). Similarly, there are several receptors, but most of the RAS effects are mediated by AT1R, AT2R and Mas receptor. Mas R, Mas receptor; BK1R and BK2R, bradykinin receptors; tPA, tissue plasminogen activator; CAGE, chymostatin-sensitive ANG II-generating enzyme. Thick white arrows indicate receptor–ligand interactions and black/grey arrows indicate enzymatic conversion of RAS components. See References in text.

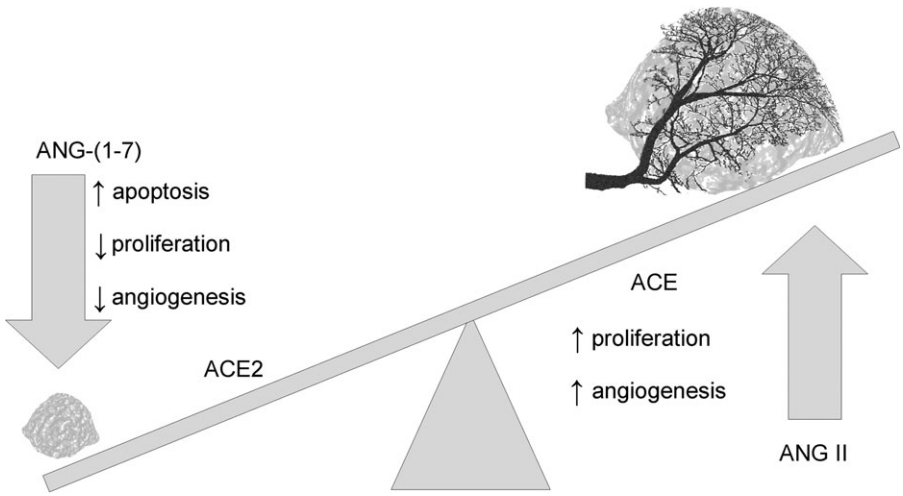

Fig. 2. The balancing effects of the RAS on tumourigenesis. The RAS can promote or inhibit angiogenesis and cellular proliferation, thus supporting or blocking tumour neovascularization, growth and metastasis.

Epidemiological studies provide further evidence that the RAS may influence tumour progression. Drugs that target the RAS, in particular ACE inhibitors and AT1R antagonists, are commonly used in the treatment of hypertension. A retrospective cohort study based on 5207 patients found that the incidence of fatal cancers was reduced in patients treated with ACE inhibitors for >3 years (26). A second cohort study with nested case–control analysis found that captopril, an ACE inhibitor, but not other classes of anti-hypertensive drugs, was associated with a lower risk of developing prostate cancer (27). A reduced risk of developing oesophageal (55%), pancreatic (48%) and colon cancer (47%) was observed in an assessment of 483 733 veterans, 38% of which were taking ACE inhibitors (28). Other epidemiological investigations, however, have failed to find a protective effect of ACE inhibitors on the rates or development of some types of cancers (29,30). The variable conclusions from these studies may be due to differences in population profiles, the types of cancer examined, the agents used and the dose and length of administration of those agents. In an attempt to avoid these variables, other epidemiological studies have utilized the ACE insertion (I)–deletion (D) polymorphism.

In humans, there are two ACE alleles; the I allele is associated with lower circulating and tissue levels of ACE compared with the D allele (31). Lower risks of breast cancer and a 50% reduced risk of advanced versus localized prostate cancer have been linked to the II genotype (32). Conversely, the DD genotype is associated with tumour progression and lymph node metastases of gastric cancer (33). Most

recently, the Rotterdam Study, a population-based prospective cohort study with 6670 useful participants, found that the DD genotype had an increased risk of breast cancer compared with the low-activity II/ID genotypes, but no association was demonstrated for colorectal, lung or prostate cancer (34). Further complicating our understanding of this system, patients with the II/ID genotype on short term, high doses of RAS blockers had an increased risk of progression of colorectal cancer.

The failure of epidemiological studies to conclusively show an effect of either pharmacological RAS blockade or endogenous differences in ACE level and activity with various cancers should not be taken a conclusive evidence that this system is not important for tumour growth, but rather that this is a complex system and that if it is to be used in the treatment of cancer, we need a better understanding of how it can regulate tumour growth and, importantly, how best to manipulate the system to reduce tumour growth. This may involve a combination of RAS targeting agents, something that as yet to be tested in *in vivo* experimental models, let alone in a clinical or epidemiological setting.

A range of RAS blockers (Table I) have, however, been used singly to assess various cancer cell lines in rodent models of common human primary and metastatic carcinomas (Table II). These experiments provide strong evidence for the possibility of RAS agents as anticancer treatments. In a murine model of hepatocellular carcinoma, the ACE inhibitors captopril and perindopril suppressed tumour growth (39). Perindopril has also been shown to decrease tumour growth and reduce angiogenesis in head and neck squamous cell carcinoma (45). In contrast, in renal cancer-bearing immunocompetent, but not immunocompromised mice, captopril decreased survival and promoted immunogenic MethA sarcoma tumours (46). Similarly, patients on ACE inhibitors were found to have higher rates of kidney cancer (29). However, these were suggested to result from a correlation between hypertension and kidney cancer. Although, these results illustrate potentially important differences in the pathological/physiological role of the local RAS in different host and neoplastic tissues, the vast majority of experimental models have found a reduction in tumour growth following RAS blockade. Further, these models suggest possible mechanisms by which these effects are achieved.

Candesartan, an AT1R antagonist, reduced tumour-related angiogenesis and the number of lung metastases in a murine Lewis lung cancer model (47) and, when explanted into nude mice, inhibited tumour-associated angiogenesis (48). In a mouse model of colorectal cancer liver metastases, both captopril (an ACE inhibitor) and irbesartan (an AT1R antagonist) decreased tumour growth, the percentage of liver metastases and tumour-associated angiogenesis (43). A significant reduction in tumour growth and vascularization has also been observed in response to candesartan in mouse melanoma syngeneic tumours (49) and in xenograft models of human prostate (50) and ovarian cancer cells (25). These studies indicate that the RAS may influence tumour neovascularization.

The RAS influences tumour angiogenesis

A major mechanism by which the RAS exerts its pro-tumour effect may be through modulation of tumour angiogenesis, which is critical for tumour growth (51). ANG II stimulates the expression of several pro-angiogenic agents and growth factors including *vascular endothelial growth factor* (*VEGF*) (9,44), angiopoietin 2 (45), *basic fibroblast growth factor* (*b-FGF*) (46), and *platelet-derived growth factor* (*PDGF*) (47). RAS blockade is frequently associated with reduced expression of the potent angiogenic factor *VEGF* (36,48–50). For example, a mouse xenograft model of human gastric cancer reduced tumour volume and a reduction in tumour-associated expression of *VEGF* in candesartan-treated animals (51).

The pro-angiogenic effects of ANG II appear to be mediated by the AT1R. In models of ischemia-induced angiogenesis, ANG II promotes revascularization of damaged vessels by increasing VEGF and endothelial nitric oxide synthase levels via activation of the AT1R (57). In contrast, the AT2R appears to antagonise these actions. In a study using AT2R deficient mice, Silvestre *et al.* (2002)(53) confirmed that the ANG II-induced increases in VEGF and eNOS are regulated by the AT1R, since both responses were observed in *AT2R* gene-deleted mice. This study also illustrated that the AT2R can negatively modulate ischaemia-induced angiogenesis by increasing apoptotic processes. The AT2R has also been shown to inhibit signals from VEGFR2/Flk-1 and is suggested to reduce endothelial cell migration and tube formation (59). However, high AT2R expression was found in intratumoural blood vessel of human pituitary adenomas (60) and blockade of the AT2R has been associated with inhibition of angiogenesis (61), suggesting that the AT2R can also be pro-angiogenic. In contrast to ANG II, the ANG-(1–7) peptide appears to inhibit angiogenesis. ANG-(1–7) inhibited both angiogenesis and the proliferation of fibrovascular tissue in a murine sponge model of

Table I. Agents utilized to manipulate the RAS

Target inhibited or blocked	Name	Mechanism of action
ACE	Enalapril (enalaprilate)[a] Captopril[a] Lisinopril[a] Ramipril[a] Perindopril[a] Benazepril[a] Fosinopril[a] Quinapril[a]	These drugs are competitive inhibitors of ACE. Some are active drugs, whereas others are administered as prodrugs that are converted *in vivo* into their active metabolites. ACE inhibitors directly block the formation of ANG II (and also increase the level of bradykinin). Captopril has a sulfhydryl group that can also inhibit matrix metalloproteinases.
AT1R and AT2R	Saralasin	A peptide analogue capable of binding, and thereby blocking, both AT1R and AT2R.
AT1R	Candesartan (TCV 116, CV 11974)[a] Losartan (DuP753)[a] EXP3174 (active metabolite of losartan) Telmisartan[a] Irbesartan[a] L-158 809	These drugs belong to a class of biphenylimidazoles. They can be competitive inhibitors (peptide analogues) or insurmountable receptor antagonists (non-peptide). AT1R antagonists inhibit ANG II receptor binding and, therefore, prevent signal transduction.
AT2R	PD 123319 PD 123177 CGP 42114	These drugs are tetrahydroimidazolepyridines. AT2R antagonists inhibit ANG II receptor binding and, therefore, prevent signal transduction.
AT4R	Divalinal-ANG IV	Peptide analogue
MasR	A-779 D-Pro7-ANG-(1–7)	Peptide analogues

[a]There are several ACE inhibitors and AT1R antagonists that are currently in clinical use as anti-hypertensive agents.

Table II. RAS blockade *in vivo*

Cell line/model	Agent	Tumour volume	Metastases	Reference
Lewis lung carcinoma 3LL	Captopril	Decreased	Decreased	Kowalski *et al.* (35)
Rat fibrosarcoma	Captropril	Decreased	N/A	Volpert *et al.* (36)
Renal carcinoma SN12K-1	Captopril	Decreased	N/A	Hii *et al.* (37)
Lewis lung carcinoma 3LL	Captoril (alone and combined with batimastat)	Decreased	Decreased	Prontera *et al.* (38)
Murine hepatocellular carcinoma	Captopril, perindopril and temocapril	Decreased	N/A	Yoshiji *et al.* (39,40)
Lung metastases of renal carcinoma	Candesartan	Decreased	Decreased	Miyajima *et al.* (41)
Ovarian carcinoma SKOV-3	Candesartan	Decreased	N/A	Suganuma *et al.* (25)
Bladder cancer KU-19-19	Candesartan	Decreased	N/A	Kosugi *et al.* (42)
Mouse colorectal cancer liver metastases	Captopril	Decreased	Decreased	Neo *et al.* (43)
MKN-28 human gastric cancer mouse xenograft	Candesartan	Decreased	N/A	Huang *et al.* (44)

N/A, not assessed. Several cancer cell lines have been used in xenograft or allograft animal models of metastases. These experiments have shown decreased tumour volume and when assessed decreased metastases.

angiogenesis (18,19). Therefore, the balance between ANG-(1–7) and ANG II as well as the AT1R and AT2R may be important in determining if tumours gain an angiogenic phenotype.

These angiogenic effects of the RAS are also evident in several models of malignancy. Ovarian cancer cells positive for AT1R secrete VEGF in response to ANG II stimulation (25) and AT1R antagonists inhibit VEGF-induced affects on bovine retinal endothelial cells (62). Indicative of the angiogenic potential of ANG II, a reduction in tumour microvascular density is a common effect of ACE inhibitors (43). However, ACE inhibition has also been associated with pro-angiogenic outcomes in several models of vascular injury. In a clinical study of congestive heart failure, ACE inhibition increased hepatocyte growth factor (63), a potent growth and angiogenic factor (64), and in a mouse model of ischaemic injury, vessel density and capillary number increased when treated with an ACE inhibitor (65).

While it is clear that the RAS can mediate angiogenic processes, the pro-angiogenic responses to ACE inhibitors appear to be, at least in part, associated with the inhibition of ACE-mediated bradykinin degradation and the ensuing increased bradykinin levels (65–68). Indeed, many of the cardiovascular benefits resulting from treatment with ACE inhibitors are now suggested to arise from the actions of these inhibitors on blocking the production of ANG II in conjunction with the increased activity of bradykinin (66). Given the potential pro-angiogenic responses of ACE inhibitors, AT1R blockade may provide a more suitable option for the treatment of cancers. However, pro-angiogeneic responses to ACE inhibition have not been reported in experimental models of cancer or in epidemiological investigations of the association between RAS blockade and tumour development. Moreover, it is unclear whether normalization of tumour vessels is in part responsible for the antitumour effects of ACE inhibitors, AT1R blockers and other classes of anti-angiogenic agents (69).

The vasoactive properties of ANG II and other vasoactive peptide hormones such as endothelin (ET)-1 could also potentially be used to increase blood flow to tumours (70–72). Increasing tumour blood flow would presumably provide a mechanism to increase the efficacy of radiotherapy as well as improving chemotherapeutic drug delivery. Lower expression of AT1Rs in many tumours compared with non-neoplastic tissue is suggested to result in a comparative hyporesponsiveness of tumours to ANG II and may provide an explanation for the observed specific increase in tumour blood flow following ANG II infusion (73,74). The systemic delivery of ET-1 has also been shown to selectively increase tumour vasodilation in a rat model of breast cancer (75). However, others have failed to find a significant effect on tumour blood flow after treatment with ET-1 (71). Also, whereas ANG II infusions have been shown to increase drug delivery to small tumours (76–79), in larger tumours ANG II infusion did not alter tumour blood flow (80). Alterations in tumour responsiveness to ANG II may reflect changes in the expression of AT1R and AT2R as tumours grow and/or gain a more aggressive phenotype. Moreover, it is unclear what overall effect ANG II infusion may have on tumour growth as the mitogenic and pro-angiogenic effects of increased ANG II may counteract its potential benefit in increasing drug delivery. Bouzin *et al.* (73) and Sonveaux (81) present excellent reviews on the potential of pro-vascular approaches in the treatment of cancer.

ANG II stimulates the synthesis of several vascular permeability factors including prostaglandins, nitric oxide, nuclear factor-κß (NF-κß), VEGF, and endothelin. Interestingly, there are many similarities between the RAS and the ET-1 system in addition to their vasoactive properties. Given the similarities between the RAS and the ET-1 system, it is perhaps not surprising that the ET-1 system is also now gaining interest as a potential target for anticancer treatments. Like ANG II, ET-1 effects are mediated via two subtypes of G-protein-coupled receptors (ET_A and ET_B) and ET-1 is generated by the action of ET-1-converting enzyme, a metalloprotease belonging to the same family as ACE. ET-1, similar to ANG II, can stimulate hypertrophy, proliferation and pro-inflammatory responses in several pathological/physiological conditions (82,83). Although the interactions between ANG II and ET-1 are incompletely understood, recent studies indicate that ANG II can stimulate ET-1 generation in endothelial cells and increase expression of ET_B receptor in hepatic stellate cells (HSCs) (84,85). ET-1, acting via its ET_B receptor, increases endothelial cell proliferation, migration and capillary-like tube formation *in vitro* and *in vivo* models of angiogenesis suggest that ET-1 augments the pro-angiogenic effects of VEGF (86). However, in a rat ischaemia-induced angiogenesis model, ET-1 infusion was not pro-angiogenic (87). In contrast, blockade of both ET_A and ET_B receptors by bosentan markedly increased vessel density, an effect apparently mediated by an increase in VEGF and endothelial nitric oxide synthase levels. These results suggest that, as with the RAS, there may be counter-regulatory mechanisms within the ET-1 system and that the balance of these determines the physiological outcomes.

Several studies have investigated the potential of inhibiting the ET-1 system to reduce tumour growth and metastasis. Both AT1R and ET_A receptor are expressed in many human cancer cell lines and tumours and autocrine activation of these receptors is associated with increased tumour growth (25,75,83,88). Moreover, inhibition of ET_A receptor was found to inhibit ovarian tumour growth *in vivo* and was associated with a suppression of epithelial-to-mesenchymal transition (EMT) (75). Parallels between the RAS and the ET-1 system raise the possibility of potentially synergistic effects resulting from the therapeutic targeting of both systems simultaneously.

Of the varied angiogenic factors stimulated by ANG II, VEGF/VEGF-A is particularly important because of its potency and selectivity for vascular endothelial cells (89). VEGF is also over-expressed in many malignant carcinomas (90–93). The initial response to VEGF appears similar in many tissues (94). Microvascular permeability increases, extravascular fibrin is deposited and the extracellular matrix degrades. Endothelial cells then migrate into surrounding tissue stroma, forming enlarged, thin-walled, pericyte-poor vessels, termed mother vessels. After these initial events, angiogenesis can proceed differently in different tissues with mother vessels differentiating into smaller daughter vessels, disorganized tangle of vessels (glomeruloid

bodies) and/or medium-sized muscular arteries and veins (94). Structures resembling mother vessels and mother vessel derivatives are observed in benign and malignant tumours (94), further supporting a VEGF-induced angiogenesis pathway (Figure 3).

Because VEGF is over-expressed in many malignancies, it has been the subject of intense clinical interest (89,91,92) with ~170 clinical trials targeting VEGF or VEGF signalling currently listed on the USA National Institutes of Health clinical trials database

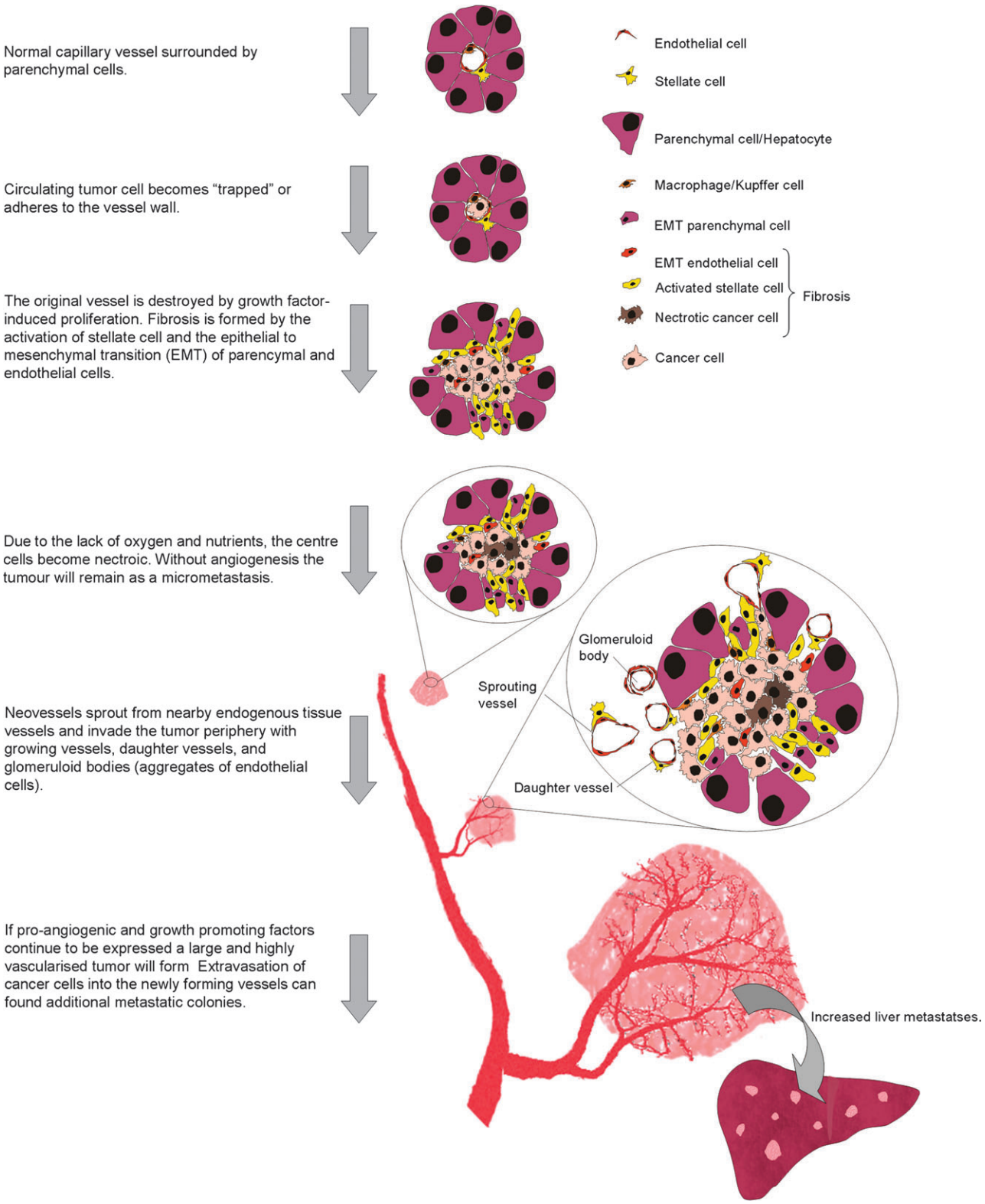

Fig. 3. Tumour angiogenesis, growth and metastasis. Angiogenesis is critical for growth of solid tumours and also aids in metastasis. The tumour can induce angiogenesis either itself or via the stimulation of host cells. The tumour can also induce changes in the surrounding parenchymal tissue leading to the infiltration of immune cells and fibrosis, both of which can further promote tumour progression.

(ClinicalTrials.gov; http://clinicaltrials.gov/). Several anti-VEGF therapies currently in clinical trials are listed in Table III. However, these anti-angiogenic agents are not without side effects. Bevacizumab has been associated with proteinuria, bleeding and wound-healing complications, gastrointestinal perforation, thromboembolic events and, most commonly, hypertension (97). Indeed, for all anti-VEGF clinical trials listed on National Institutes of Health ClinicalTrials.gov, where data were available, hypertension was a listed side effect. Treatments with anti-hypertensive agents, including ACE inhibitors, are frequently described in clinical trials of anti-VEGF therapies, but with no reference to the potential of these treatments to also influence tumourigenesis (98,99). Also, for tumours that express additional angiogenic or proliferative factors, anti-VEGF therapies alone may not provide an optimal strategy. Targeting multiple aspects of angiogenesis, including the VEGF pathway, may provide a more effective treatment. The RAS also contributes to cellular proliferation and tumour-associated fibrosis and blockade of these systems may provide additional benefits beyond those predicted for anti-VEGF strategies.

Proposed effects of the RAS on cellular proliferation

The RAS can also effect the cell survival and or proliferation and may, therefore, have a direct effect on the number of live cancer cells within tumours. ANG II can stimulate or inhibit proliferation depending on whether the AT1R or AT2R is activated. It is also now becoming evident that ANG-(1–7) also has a role in defining the proliferative potential of some cells. ANG II is a mitogen for smooth muscle cells, fibroblasts and endothelial cells (6) and increases the expression of growth-related oncogenes (100–102) and growth factors (2) in several cell types. However, ANG II stimulation of the AT1R may increase senescence of bone marrow-derived endothelial progenitor cells (103), which are important for tumour angiogenesis (104). These results suggest that the effect of ANG II on proliferation may differ for different cell types, possibly due to the alternative physiological pathways that can be initiated by the RAS. Although AT2R is commonly thought to mediate the anti-proliferative effects of ANG II (105), this may not always be the case. For example, in a normotensive rat model, infusion of ANG II in conjunction with AT1R blockade induced aortic hypertrophy (106). In contrast, the number and size of aortic smooth muscle cells remained normal in rats infused with ANG II in the presence of PD123319 (an AT2R-specific antagonist), suggesting that at least part of the vasotropic effects of ANG II were mediated by the AT2R.

ANG-(1–7) is generally thought to inhibit cellular proliferation (107). However, ANG-(1–7) also appears to increase proliferation of some cell types including fibroblasts, epidermal stem cells, keratinocytes and haematopoietic progenitor cells (20,21). ANG-(1–7) clearly has a complex role in regulating cellular proliferation. Therefore, whether ANG-(1–7) is pro- or anti-proliferative for a particular tumour/host cell may be an important consideration for the applicability of RAS blockade as a cancer treatment. However, at least for human lung cancer cells, it has been shown that ANG-(1–7) upregulation can inhibit proliferation (108).

Immunomodulatory effects of the RAS

Tumour-infiltrating immune cells indicate an immune response by the host to the developing tumour. ANG II can stimulate the release of macrophage/monocyte chemoattractant protein (MCP)-1, MCP-2 and gonocyte colony-stimulating factor, thus increasing macrophage infiltration (42,49,109). In low-density lipoprotein receptor-deficient mice, ANG II infusion was found to promote macrophage infiltration, whereas treatment with valsartan, an AT1R inhibitor, reduced macrophage accumulation and atherosclerosis (110). Similarly, in a rabbit aortic balloon injury model, AT1 blockade attenuated atherosclerosis in association with a reduction in plaque distribution and macrophage accumulation (111). Surprisingly, however, in a rat model of nephritis, ANG II infusion induced a significant reduction in glomerular monocyte infiltration (112). Therefore, the overall effect of the RAS on macrophage infiltration may be different in different tissues depending on other immunomodulatory factors and the relative expression of various RAS components.

AT1R is highly expressed in tumour-associated macrophages and mice mutant for AT1R have fewer infiltrating macrophages following

Table III. Inhibition of angiogenesis by blockade of the VEGF pathway

Drug	Cancer	Treatment	Outcome
Bevacizumab (Avastin)—a monoclonal antibody that binds and inhibits VEGF	Metastatic renal cell cancer	Monotherapy	Benefit in progression-free survival, but not in overall survival
	Colorectal cancer	In combination with chemotherapy (FOLFOX4) as a second-line therapy	Benefit in progression-free survival and overall survival
	Non-small cell lung cancer	In combination with chemotherapy (paclitaxel + carboplatin) as a first-line treatment	Benefit in progression-free and overall survival
	Metastatic breast cancer	In combination with paclitaxel in a first-line treatment	Benefit in progression-free survival
Vatalanib—inhibits VEGF and platelet-derived growth factor receptors	Metastatic colorectal cancer	In combination with chemotherapy (FOLFOX4) as a second-line therapy	Benefit in progression-free survival, but not overall survival
VEGF-Trap (aflibercept)—a soluble VEGF receptor that binds and sequesters circulating VEGF	Non-small cell lung cancer	Second-line monotherapy or in combination	In phase I trials, there was a radiographic improvement
	Acute myeloid leukaemia	Monotherapy	Phase II trials are underway
Sunitinib (Sutent)—binds to PTK receptors, blocking signal transduction. Includes VEGF and PCGF receptors and c-KIT and Flt-3 receptors	Cytokine-refractory metastatic renal cell cancer	Monotherapy	High rate of objective tumour responses
Sorafenib (Nexavar)—inhibits RAF kinase (part of the RAS oncogene pathway) and also inhibits platelet-derived growth factor and VEGF receptors	Metastatic renal cell cancer	Second-line monotherapy	Improvement in progression-free survival, but low partial tumour responses

PTK, protein tyrosine kinase receptors; RAF, product of v-raf-1 murine leukemia viral oncogene homolog 1; PCGF, Platelet derived growth factor.
There are many phase II or phase III clinical trials currently underway. Examples of representative drugs, their mechanism of action and the cancers targeted are provided [data cited in Kerbel (95), Cao (96), Los *et al.* (97) and table modified from Kerbel (95)].

tumour induction (49). Blockade of AT1R inhibits ANG II-stimulated increases in MCP-2. In contrast, AT2R antagonists enhanced ANG II-induced up-regulation of MCP-2 (109). Therefore, whereas AT1R stimulation increases MCP-2 expression and presumably macrophage infiltration, AT2R stimulation opposes this effect.

Classical macrophage activation is thought to eliminate microorganisms and kill tumour cells (113–116). However, the role of macrophages in late-stage tumour development is less clearly defined. There is some evidence that tumour-infiltrating macrophages can promote growth and metastasis (117,118). The alternatively activated or M2 macrophage pathway, which normally participates in debris salvaging and wound healing, is suggested to be associated with these pro-tumour roles. During the later stages of tumour metastases, host defences may no longer be capable of eliminating metastatic tumour cells due to rapid proliferation of cancer cells, angiogenesis and/or weakened host defences. For example, binding of circulating tumour cells by Kupffer cells, resident macrophages in the liver, is associated with promoting immune escape and facilitating the formation of metastatic colonies once the initial induction phase has past (113). Tumour-associated macrophages also release many cytokines that can induce angiogenesis and these may facilitate tumour growth and metastases (49,118–120). In a B16-F1 melanoma model, sites of AT1R expression co-localized with VEGF protein and it is possible that ANG II may promote stimulation of macrophage infiltration and macrophage-mediated angiogenesis (49).

Macrophages themselves also release ACE and can participate in ANG II synthesis. When macrophages were engineered to produce high levels of ACE, by driving ACE expression from the macrophage-specific c-fms promoter, mice became resistant to melanoma growth (116). ACE inhibitors were capable of reversing this phenotype, but inhibition of ANG II or AT1R did not, suggesting that these effects were not dependent on ACE–ANG II interactions. Instead, it was found that mice over-expressing ACE in their macrophages responded to tumour challenge with an enhanced tumour-specific CD8+ T-cell response (121). Further, it was suggested that the increased peptidase activity of ACE by these macrophages may enhance the presentation of major histocompatibility class I-associated peptides to T cells.

Effects of the RAS on tumour-induced fibrosis

Fibro-proliferative diseases such as pulmonary fibrosis, liver cirrhosis, cardiovascular disease, progressive kidney disease and macular degeneration are typified by increased extracellular matrix formation and the differentiation of myofibroblasts. However, the process of fibrosis is also closely associated with carcinogenesis (122,123).

The RAS, in particular activation of the AT1R by excess ANG II, is associated with a number of renal and cardiac fibro-proliferative diseases (124–128). It is perhaps not surprising, therefore, that numerous experimental and clinical investigations have demonstrated a benefit of ACE inhibitors and AT1R blockers in renal and cardiac disease. While some of the positive effects of ACE inhibitors and AT1R blockers can be explained by the reduction in systemic blood pressure, there is also substantial evidence for a direct anti-fibrotic effect.

A non-hypertensive mouse model of renal fibrosis, which allows the effects of RAS blockade on fibrosis to be assessed irrespective of blood pressure involvement by the RAS, found that both ramipril (an ACE inhibitor) and candesartan (an AT1R blocker) delayed the onset and reduced the extent of proteinuria, postponed the onset of uraemia and prolonged life (129). However, ramipril treatment was associated with a greater increase in life span and resulted in a more pronounced anti-fibrotic effect. A mouse model of cyclosporine A-induced kidney damage implicated the inhibition of bradykinin degradation by ACE inhibitors in its anti-fibrotic effects. Benazepril appeared to facilitate matrix degradation via activation of the bradykinin B2 receptor on tubular epithelial cells (130). Activation of the B2 receptor was associated with decreased plasminogen activator inhibitor-1 expression, which was suggested to increase production of plasmin and activation of matrix metalloproteinases. Indeed, B2 receptor activation has been demonstrated to reduce renal fibrosis in unilateral ureteral obstruction

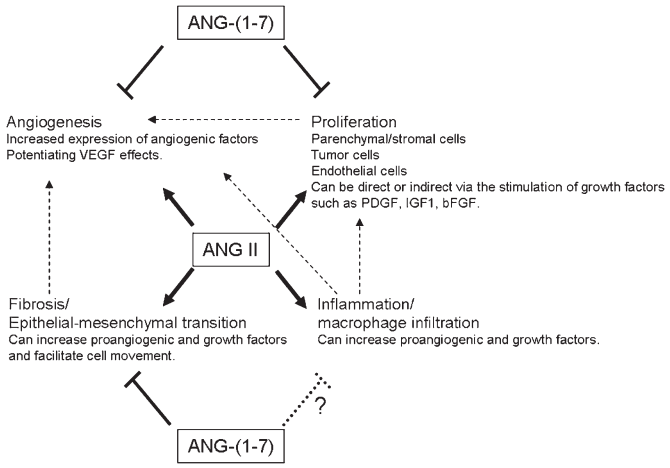

Fig. 4. The role of ANG II and ANG-(1–7) in tumour-associated processes. The RAS has developed in its complexity with increased knowledge but we are now beginning to elucidate the feedback and feedforward mechanisms that regulate this system. With these and other studies, there is also now a greater understanding of the crosstalk that occurs between the physiological and phenotypic processes induced by the RAS in a cancerous environment.

models by increasing extracellular matrix degradation through the activation of plasminogen activator (124).

In the liver, HSCs are recognized as essential for fibrogenesis and the RAS appears, at least in part, to mediate their fibrogenic role. Myofibroblasts can arise from activated HSCs, hepatocytes, bone marrow-derived cells and possibly endothelial cells. Activated stellate cells secrete ANG II that can promote fibrosis via reduced nicotinamide adenine dinucleotide phosphate oxidase, myofibroblast proliferation and differentiation and increased collagen synthesis (132). Also, the number of α-smooth muscle actin-positive cells (a marker of activated HSCs) was noticeably suppressed by candesartan and perindopril. Interestingly, ANG II activates NF-κB through both AT1 and AT2Rs, in turn leading to increased expression of pro-inflammatory cytokines such as IL-6, TNFα, TGF-ß1 and intercellular adhesion molecule (12,126,127).

Fibrotic processes such as increased intratumoural collagen I or EMT of host and/or tumour cells are associated with increased tumour invasiveness (135,136). EMT refers to a series of cellular and structural changes, which allow cells to separate, lose apico-basal polarity and gain changeable cell adhesions, all of which potentially facilitate cell motility. In addition, the extracellular matrix can be induced to undergo changes that permit cell movement and the expression of several growth and angiogenic factors, such as VEGF, can be induced in response to EMT (137). A key inducer of EMT is TGF-ß1 which has tumour suppressor and oncogenic activities. In colorectal cancer, TGF-β1 changes from an inhibitor of proliferation to a stimulator of growth and invasion at late stages of tumour progression (137). ANG II can increase TGF-β1 expression amongst other cytokines and this is associated with an accumulation of fibrotic matrix proteins (119,138–141). ANG II also increases α-smooth muscle actin and decreases in E-cadherin, both of which regulate EMT (142,143).

Both ANG II and ANG-(1–7) influence the formation of fibrosis following hepatic bile duct ligation (BDL) and other forms of tissue injury (15,16,144). However, whereas plasma ANG II levels increase in the first week after BDL and then return to normal, ANG-(1–7) levels increase 3 weeks after BDL, but are normal prior to this point (15). ACE and AT1R are also up-regulated following BDL and localize to areas of active fibrogenesis (144). Changes in the RAS may reflect a balance whereby up-regulation of hepatic ACE2 and ANG-(1–7) may provide a counter-regulatory response to ANG-II-mediated acute responses. Therefore, it is possible that by manipulating the RAS tumour-induced fibrosis and EMT may also be reduced. Because

tumour-induced fibrosis can produce growth and angiogenic factors, its inhibition may initiate positive feedback mechanisms that further impede tumour growth and metastases. However, in some instances, reduced fibrotic responses have been linked to more aggressive cancer development (145).

Conclusion

There is accumulating evidence that ACE inhibitors and AT1R antagonists reduce tumour growth and metastatic potential. Our understanding of the RAS in normal and pathological conditions, including cancer, has increased markedly over recent years, but has also demonstrated a complexity not initially evident. Newly discovered components of the RAS, such as the enzyme ACE2 and the ANG-(1–7) peptide, may provide novel targets for the treatment of cancer.

Not only does the RAS influence several important physiological processes such as angiogenesis, cellular proliferation, inflammation and fibrosis but also there is crosstalk between these processes that will further determine tumour potential (Figure 4). This complexity highlights the need for greater knowledge of the role of the RAS in specific tissues and tumour types. Nevertheless, anti-hypertensive agents based on ACE inhibition and AT1R antagonism are already in clinical use without serious side effects and if these drugs can inhibit tumour progression at comparable doses, then they may provide a useful adjunctive therapeutic strategy in the treatment of cancer.

Funding

This work was funded by a Cancer Council of Victoria Grant.

Acknowledgements

Conflict of Interest Statement: None declared.

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Received April 23, 2008; revised July 10, 2008; accepted July 10, 2008