Renin Angiotensin System and Hepatic Malignancy

Abdulmalik Alkatheri, Abdulkareem Albeairy and Mahmoud Mansour
College of Pharmacy, King Saud bin Abdulaziz University for Health Sciences, P.O. Box 22490, Riyadh 11426, Kingdom of Saudi Arabia

ABSTRACT
The renin-angiotensin system (RAS) is usually associated with its systemic action on cardiovascular homeostasis. Cancer is the leading cause of death worldwide. Previous studies suggested that at a local tissue RAS influences tumor growth. It has been reported that local RAS appears to influence tumor growth and metastases and there is evidence of tissue- and tumor-specific differences. These include modulation of angiogenesis, cellular proliferation, immune responses and extracellular matrix formation. Therefore, apart from conventional chemotherapy, targeting of the tumor vasculature by vascular disrupting agents or inhibitors of angiogenesis has also been used. Knowledge of the RAS has increased dramatically in recent years. Manipulation of the RAS may, therefore, provide a safe and inexpensive anticancer strategy. Therefore, Blockade of the RAS may, therefore, provide an alternative, adjunctive therapy for the treatment of solid tumors.

Key words: Hepatocellular carcinoma, renin-angiotensin system, growth factors

INTRODUCTION
Hepatocellular carcinoma: Hepatocellular carcinoma (HCC) is the fifth most common cancer and the third leading cause of cancer related mortality worldwide. Nearly 500,000 cases of HCC, representing more than 5% of all cancers are diagnosed each year. HCC has a striking geographic variability in its incidence, causes and presentation. In the Far East and sub-Saharan Africa, the incidence is 15 cases per 100,000 despite under-diagnosis of HCC patients. In Europe, Australia and the United States the incidence is approximately 3 cases per 100,000. There has been an increase in the incidence of HCC in developed countries as a consequence of chronic hepatitis C virus (HCV) infection and the improved survival of patients with cirrhosis. The incidence in the United States increased from 1.4 cases per 100,000 during 1976–1980 to 2.4 cases per 100,000 during 1990–1995. The associated mortality rates and hospitalizations also increased by 41 and 48%, respectively. Nearly 15,000 new cases of HCC are diagnosed each year in the United States. The rising incidence of HCC suggests that the management of HCC patients will continue to pose a significant challenge.

Molecular targeted therapies for hepatocellular carcinoma: During the past years, there has been a remarkable improvement of the knowledge of the molecular basis underlying liver carcinogenesis, which has allowed the identification of novel molecular targets and the design of therapeutic approaches aimed at overcoming this devastating disease. The vast majority of these drugs interfere with signaling pathways involved in cell proliferation and survival. On the contrary, some therapeutics are addressed to block those signals related to neo-angiogenesis, a key feature of the malignant phenotype in HCC.

In 2007, a major breakthrough has significantly changed the clinical management of HCC. The positive results in terms of survival obtained in the phase III clinical trial that evaluated sorafenib vs. placebo in patients with advanced HCC have spurred extensive research on the molecular pathogenesis of HCC. Sorafenib, a multikinase inhibitor of VEGFR2, PDGFR, Raf-1, B-Raf and c-KIT has previously demonstrated efficacy in renal cancer. In addition, preclinical studies demonstrated its activity in experimental models of HCC by inhibiting cell viability and inducing significant apoptosis. Several molecular therapies have been tested in phase II trials, including monoclonal antibodies (mAbs) and tyrosine kinase inhibitors blocking different molecular targets such as EGFR, IGF-1R, c-MET, VEGFR, PDGFR, among others. The renin-angiotensin system is frequently activated in patients with chronic
liver diseases. Perindopril, an angiotensin converting enzyme inhibitor, inhibits angiogenesis by reducing vascular endothelial growth factor (VEGF) production.

**Renin-angiotensin system (RAS):** The RAS was, for many years, thought of as an endocrine system with enzymes and peptides released into the systemic circulation to act on target organs. More recently, it has been recognized that most organs including the brain, kidney, heart, liver, pancreas, reproductive organs, skin and the gastrointestinal tract constitutively express all the components required to allow autonomous function of a local intra-organ RAS, where it performs both paracrine and autocrine function. Commonly, the RAS has been associated with the systemic regulation of cardiovascular homeostasis. However, there is increasing evidence that local RAS may influence tissue angiogenesis, cellular proliferation, apoptosis and inflammation. Components of the RAS are expressed in several adult organs including the liver, kidney, pancreas, brain and reproductive organs. It is the paracrine mechanisms of locally expressed RAS, not its circulating counterpart, that appear important for tumorigenesis. A variety of physiological responses can be induced through activation of the RAS, some of which can have antagonistic consequences for tumor growth. The physiological malleability of the RAS is achieved by alternative peptides and receptors. Angiotensin (ANG) II is the main effector of the RAS (Fig. 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 angiotensin II type 1 receptor (AT1R). The AT1R is expressed in many adult tissues, including blood vessels, adrenal cortex, liver, kidney and brain. A second receptor encoded by a different gene, the angiotensin II type 2 receptor (AT2R) is predominantly expressed during fetal life, but is present at a low level in a few adult tissues such as the adrenal medulla, uterus and ovarian follicles. Whereas the AT1R induces angiogenesis, cellular proliferation and inflammatory responses, as well as being antiapoptotic, the AT2R appears to functionally antagonize many of these actions. There is some evidence, however, that signaling via the AT2R can also be pro-angiogenic and pro-inflammatory.

![Diagram](https://example.com/ras-diagram.png)

**Fig. 1:** The RAS several enzymes catalyze the generation of angiotensinogen-derived peptides; thin black arrows, thick grey: ACE, White arrows, AT1R: Angiotsenin type 1 receptor, AT2R: Angiotsenin type 2 receptor, Mas R: Mas receptor, BK1R: Bradykinin type 1 receptor, BK2R: Bradykinin type 2 receptor, tPA: tissue plasminogen activator, CAGE: chymostatin-sensitive ANG II-generating enzyme.
The Mas1 oncogene (MasR) represents a fifth RAS receptor and binds the ANG-(1-7) peptide. 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. ACE2 is present in many tissues with high concentrations in the heart, kidney and gastrointestinal tract. 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). ANG-(1-7) appears to have an inhibitory influence on many of the events induced by ANG II. ANG-(1-7) has depressor, vasodilator, apoptotic and anti-proliferative actions, 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 prostanooids and may increase proliferation of some cells, such as epithelial stem cells after injury and hematopoietic progenitors in the bone marrow of myelo-suppressed mice.

The variety of physiological responses to the RAS reflects the alternative peptides and receptors and the different signaling pathways they induce. The balance between these signaling 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 tumor growth, angiogenesis and metastatic potential.

CONCEPT OF LOCAL RAS

There is considerable evidence that most or all of the components of the RAS are present in a variety of organs, supporting the theory that local expression and modulation of the RAS play important roles in tissue homeostasis. These roles may be summarized as (1) Fluid and electrolyte transport, (2) regional blood flow regulation and (3) promoting the wound healing response, including cell proliferation, inflammation and fibrosis. The liver expresses renin, angiotensinogen, Ang II, ACE, AT1R, Ang (1-7), ACE2 and mas receptors, all of which are upregulated in the diseased liver.

Intra-hepatic renin-angiotensin system: Apart from the circulating RAS, the existence of local or intra-organ RASs has been described in a number of organs, including the heart, kidney, lung, pancreas and liver. These local systems have been shown to be responsive to various stimuli of physiological and patho-physiological importance. Moreover, the locally generated angiotensin peptides fragments have a plethora of actions and have been implicated in cell growth, anti-proliferation, apoptosis, reactive oxygen species (ROS) generation, hormonal secretion, pro-inflammatory and pro-fibrogenic actions.

The roles of the hepatic RAS in the function of normal liver and in liver fibrosis are less well-described than that of the heart and kidney. However, it is clear that most of the key components of the enzymatic cascade which lead to the formation of angiotensin II in other organs are the same and are present in the liver. One common theme throughout the literature is that with liver injury an up-regulation and/or redistribution of RAS components, including angiotensinogen, renin, ACE, angiotensin II and AT1R, is observed. The main source of the RAS precursor angiotensinogen is the hepatocyte, but low levels of protein have also been detected in Kupffer cells and in bile duct epithelium. Studies in humans and rodents show plasma renin concentration and activity and its substrate angiotensinogen, are increased in cirrhotic livers compared with that of controls. The product of angiotensinogen cleavage by renin, angiotensin I, has not been demonstrated in the liver tissue; however, there is evidence to suggest de novo generation of angiotensin I may be produced locally in hepato-mesenteric vascular beds as well as in circulating plasma. In contrast, angiotensin II is present in both plasma and liver tissue from normal animals and is increased significantly in rat models of liver disease and in cirrhotic patients. Other RAS components expressed in the normal liver tissue include ACE and AT1R proteins which are both predominantly localized to vascular endothelium, but are also observed in hepatocytes and bile duct epithelial cells. In the fibrotic liver, ACE and AT1 receptor protein expression is also localized to fibrous septa, mesenchymal cells (HSCs and myo-fibroblasts) and Kupffer cells. Although the AT1R is abundant in the liver, the AT2R gene is very low or not detectable in normal or diseased liver. The only report so far to attribute AT2R gene expression to a particular liver cell type is that of Bataller et al., who detected the receptor message in isolated human hepatocytes and stellate cells (quiescent, culture-activated and in vivo activated). Despite the existence of AT2R in the liver and a recent study showing that ablation of AT2 receptors augments liver injury and fibrosis, the vast majority of reports support the concept that AT1R mediate the inflammatory, proliferative and vascular effects of angiotensin II in the liver. Moreover, the gene expression of AT1R on septal myo-fibroblasts appears to correlate with the extent of fibrosis and the degree of portal hypertension.

Role of RAS in the hepatic cancer development: Evidence for the inhibitory effect of the RAS blockade in cancer and stimulation in liver regeneration suggests that
this system offers an anticancer target in the context of liver regeneration and further studies specifically addressing this are warranted. Patients with impaired liver regeneration either as a result of chemotherapy, obesity may also benefit from targeting of the RAS. Blockade of ACE with captopril or AT1R blockade has been shown to retard tumor growth and decrease the extent of liver metastases in a mouse model of CRC liver metastases. This effect was in part because of a significant reduction in tumor vascularization, although the liver surrounding tumors appeared unaffected by either treatment.

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 tumor progression. Components of the RAS are frequently differentially expressed in various cancers including liver, lung, pancreatic, breast, prostate, colon, skin and cervical carcinomas in comparison with their corresponding non-malignant tissue. In particular, over-expression of the AT1R is common. Changes in the expression of RAS components appear to correlate with tumor grade. These changes, however, are not consistent and vary for individual tumor types. For example, high levels of AT1R are found in breast hyperplasia but decrease when breast cancer becomes invasive, while in ovarian carcinoma up-regulation of AT1R correlates with tumor invasiveness. 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 grade of tumor may depend on the expression of other components of the RAS.

The low prevalence of cancer in hypertensive patients receiving angiotensin converting enzyme inhibitors has been reported, however, the molecular mechanisms have not been elucidated. It is known that angiotensin-II (Ang-II) plays a fundamental role not only as a vasoconstrictor in controlling blood pressure and electrolyte and fluid homeostasis, but also as a mitogenic factor through the AT1R in cardiovascular cells. Interestingly, there is increasing evidence that the RAS is implicated in the development of various cancers. As previously reported, AT1R Blockers (ARBs), a class of antihypertensive agent, have the potential to inhibit the growth of different cancer cells and tumors through the AT1R.

There is emerging evidence that the incidence of cancer is reduced in patients undergoing long-term treatment with drugs that inhibit the RAS. For instance, a retrospective cohort study on 5207 patients receiving ACE inhibitors or other antihypertensive drugs with a 10 years follow up showed that ACE inhibitors decreased the incident of cancer and fetal cancer (Glasgow study). The other antihypertensive drugs example, calcium channels blockers, diuretics and beta blockers had no apparent effects on the risk of cancer development and the Rotterdam study concluded that drugs that inhibit the RAS protected against cancer in individuals with a genotype that is associated with high levels of ACE. ACE inhibitors have also been shown to inhibit tumor angiogenesis and tumor growth following the injection of sarcoma and fibro-sarcoma cell lines in mice and to reduce lung metastasis following intravenous injection of Lewis lung carcinoma cells. Angiotensin (1-7) also reduces DNA synthesis in human adeno-carcinoma SK-LU-1 and A549 cells and in non-small lung cancer SK-MES-1 cells. In murine hepatocellular carcinoma cells, ACE inhibition suppressed VEGF-mediated tumor development and neo-vascularisation and a deletion polymorphism of the ACE gene increases risk of breast cancer risk in postmenopausal women. Other evidence suggested that Ang II directly stimulates cell growth via the AT1R and that its blockade inhibits tumor growth. For instance, AT1R antagonists suppress growth of human pancreatic cancer cells and, like ACE inhibitors, inhibit tumor angiogenesis, growth and lung metastasis resulting from injection of lung cancer cell lines. AT1-blockade also reduced tumor volume, vascular density, mitotic index and cell proliferation following injection of C6 glioma cells and in mice injected subcutaneously with B16-F1 melanoma, angiogenesis was prominent in WT but not in AT1-R deficient mice. Moreover, Ang II, acting via the AT1-R, enhanced the invasive potential and VEGF secretion in SKOV-3 ovarian cancer cells and AT1-blockade resulted in a reduction in peritoneal dissemination, VEGF expression and tumor angiogenesis. In addition, AT1-R expression was shown to correlate with VEGF expression and poor patient outcome in ovarian cancer biopsies from 67 patients. In human gastric cancer cells, Ang II increased the expression of matrix metalloproteases and AT1-blockade decreased microvessel density and VEGF expression. Finally, in prostate cancer cell lines AT1-blockade inhibits Ang II-mediated prostate cancer cell growth and was shown to improve performance status and reduce Prostate Specific Antigen (PSA) levels in some patients with advanced hormone-refractory prostate cancer.

Evidence for a RAS contribution in hepatic cancer angiogenesis: A major mechanism by which the RAS exerts its pro-tumor effect may be through modulation of tumor angiogenesis, which is critical for tumor growth. ANG II stimulates the expression of several
pro-angiogenic agents and growth factors including vascular endothelial growth factor (VEGF)\(^2\), angiopoietin 2, basic fibroblast growth factor (b-FGF) and platelet-derived growth factor (PDGF)\(^3\). RAS blockade is frequently associated with reduced expression of the potent angiogenic factor VEGF\(^6\). For example, a mouse xenograft model of human gastric cancer reduced tumor volume and a reduction in tumor-associated expression of VEGF in candesartan-treated animals\(^5\). 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, endothelial nitric oxide synthase levels via activation of the AT1R\(^5\). In contrast, the AT2R appears to antagonise these actions.

In a study using AT2R deficient mice, it has been reported 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\(^6\). This study also illustrated that the AT2R can negatively modulate ischemia-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. However, high AT2R expression was found in intra-tumoral blood vessel of human pituitary adenomas and blockade of the AT2R has been associated with inhibition of angiogenesis, 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 fibro-vascular tissue in a murine sponge model of angiogenesis\(^9\). Therefore, the balance between ANG-(1-7) and ANG II as well as the AT1R and AT2R may be important in determining if tumors 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\(^8\) and AT1R antagonists inhibit VEGF-induced effects on bovine retinal endothelial cells. Indicative of the angiogenic potential of ANG II, a reduction in tumor micro-vascular density is a common effect of ACE inhibitors\(^8\). 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, a potent growth and angiogenic factor and in a mouse model of ischaemic injury and capillary number increased when treated with an ACE inhibitor\(^9\).

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\(^7\). 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\(^8\). Given the potential pro-angiogenic responses of ACE inhibitors, AT1R blockade may provide a more suitable option for the treatment of cancers. However, pro-angiogenic responses to ACE inhibition have not been reported in experimental models of cancer or in epidemiological investigations of the association between RAS blockade and tumor development. Moreover, it is unclear whether normalization of tumor vessels is in part responsible for the antitumor effects of ACE inhibitors, AT1R blockers and other classes of anti-angiogenic agents.

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 tumors\(^5\). Increasing tumor 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 tumors compared with non-neoplastic tissue is suggested to result in a more responsive tumors to ANG II and may provide an explanation for the observed specific increase in tumor blood flow following ANG II infusion\(^8\). The systemic delivery of ET-1 has also been shown to selectively increase vaso-dilatation in a rat model of breast cancer. However, others have failed to find a significant effect on tumor blood flow after treatment with ET-1. Also, whereas ANG II infusions have been shown to increase drug delivery to small tumors\(^6\), in larger tumors ANG II infusion did not alter tumor blood flow. Alterations in tumor responsiveness to ANG II may reflect changes in the expression of AT1R and AT2R as tumors grow and/or gain a more aggressive phenotype. Moreover, it is unclear what overall effect ANG II infusion may have on tumor growth as the mitogenic and pro-angiogenic effects of increased ANG II may counteract its potential benefit in increasing drug delivery.

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 tumorogenesis\(^9\). Also, for tumors 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 tumor-associated fibrosis and blockade of these systems.
RESEARCH ARTICLE

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 tumors. 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 and increases the expression of growth-related oncogenes and growth factors in several cell types. However, ANG II stimulation of the AT1R may raise senescence of bone marrow-derived endothelial progenitor cells, which are important for tumor angiogenesis. 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, 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. 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 vasoactive effects of ANG II were mediated by the AT2R. ANG-(1-7) is generally thought to inhibit cellular proliferation. However, ANG-(1-7) also appears to increase proliferation of some cell types including fibroblasts, epidermal stem cells, keratinocytes and haematopoietic progenitor cells. 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 tumor/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) up-regulation can inhibit proliferation. Our previous study showed that ACE inhibitors, (captopril and perindopril) inhibit hepatocarcinogenesis with equal potency with AT1R blocker losartan. Recently, it has been reported that Ang-(1-7) is considered a potential anti-cancer treatment since it is able to inhibit cell proliferation and angiogenesis. Thus the ACE2/Ang-(1-7)/Mas pathway seems to be involved in many physiological and pathophysiological processes in several systems and organs especially by opposing the detrimental effects of inappropriate over-activation of the ACE/AngII/AT1R axis. In addition it has been shown that captopril may provide a beneficial treatment option for the management of patients with selected patients with colorectal cancer liver metastases.

CONCLUSION

These findings suggested that Ang II and AT1R interaction may have a pivotal role in hepatocellular carcinoma and therefore, AT1R blocker and ACE inhibitors may have therapeutic potential in the treatment of hepatic cancer. The use in this context of molecules which have shown an unexpected efficiency in cancer cells may be a way to improve cancer treatment and overcome resistance mechanisms. The therapeutic agents developed in the context of cardiovascular disorders and which target the renin-angiotensin system (RAS) has shown such potential and deserves evaluation in the field of cancer. This approach offers the advantages of getting immediate access to drugs already tested in humans, without long chemical and biological development.

REFERENCES


