Advances in Biochemistry in Health and Disease

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The Renin Angiotensin System in Cancer, Lung, Liver and Infectious Diseases

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Preface

The renin-angiotensin system (RAS) has been an extensively studied system in human health and disease. The common precursor of all RAS hormones, angiotensinogen, which is primarily formed in the liver, is cleaved by enzyme renin (secreted from the kidneys) to produce angiotensin (Ang) I. This peptide is further cleaved to form Ang II by angiotensin-converting enzyme (ACE) in the lungs. Activation of different signal transduction pathways by Ang II is mediated by Ang-receptors (Ang II type 1 and Ang II type 2), resulting in proliferative or anti-proliferative effects. Increased Ang II levels caused by chronic activation of RAS influence several processes including proliferation, apoptosis, autophagy, migration, cellular metabolism, Ca^{2+}-handling, inflammation, oxidative stress and angiogenesis. Ang I is also converted to Ang (1-7) by ACE2, which activates mitochondrial assembly (MAS) receptors to produce anti-proliferative actions and other beneficial effects. Apart from the existence of RAS in the circulatory system, as well as locally in the heart, this system is also operational in the brain, pituitary gland, adrenal gland, prostate gland, endometrium, pancreas, mammary gland, ovaries, testis and epididymis. In addition to cardiovascular diseases (CVDs), the disruption in the expression or activity of RAS components also manifests in other multiple pathologies, including cancer, liver disease, lung abnormalities, infectious diseases and renal dysfunction. Furthermore, pharmacological or the therapeutic efficacy of the blockade of RAS by the Ang II receptor blockers and/or ACE inhibitors has been designed to normalize RAS over-activation for the treatment of these diseases.

The role of RAS in the pathogenesis of CVDs has been described in an earlier volume of this series. Since cancer and diseases of the liver, lung and kidney as well as infectious diseases are the leading cause of death worldwide after CVDs, exploration of underlying physiological, pathological, and pharmacological aspects of these diseases and the contribution of RAS to these processes have remained a global focus of investigation in health and disease. Indeed, there has been a remarkable expansion of studies on the implication of RAS over-activation in these diseases. Accordingly, this book is intended to summarize the role of RAS in the pathogenesis of these diseases as well as the potential of RAS blockade as a novel therapeutic

approach. In this regard, basic concepts of RAS and the role of RAS in the pathogenesis of infectious diseases such as COVID-19, viral hepatitis, pneumonia and influenza are also described. In addition, the role of RAS in the processes involved in carcinogenesis, such as proliferative signal transduction, microenvironment interactions, cellular and molecular dysregulation and metabolic disturbances, is discussed. Also included in this book are chapters that address pre-clinical and clinical studies which identify modulation of RAS as an effective treatment for cancer.

It is now well known that RAS elicits the pathophysiology of different liver diseases such as liver cirrhosis, hepatocellular carcinoma and liver failure, and thus, it represents a therapeutic target for the treatment of these conditions. This book therefore describes the role of RAS in the pathological and pharmacological characteristics of chronic liver diseases and includes advances in signaling pathways, biomarkers and associated events that are related to RAS, such as enhanced liver fibrosis, oxidative stress and inflammation. Alterations in RAS are also known to induce multiple lung diseases, including idiopathic pulmonary fibrosis, sarcoidosis, pulmonary hypertension, acute respiratory distress syndrome and lung cancer. It is thus important to describe RAS signaling pathways, molecular and cellular mechanisms in lung disease as well as role of this system in lung fibroblast and extracellular matrix production. In addition, the inhibition of RAS as an effective treatment of lung disorders is included in this book.

This book on the status of *The Renin Angiotensin System in Cancer, Lung, Liver and Infectious Diseases* has been assembled to consist of 24 chapters by highly productive investigators with expertise in the field of RAS in health and disease. It mainly deals with the pathophysiology and pharmacotherapy of various health hazards such as cancer, lung, liver, kidney and infectious diseases with respect to the involvement RAS. Particularly, the mechanisms such as inflammation, oxidative stress and signal transduction related to Ang-receptors have been discussed. All the information contained in this book has been arranged into three parts: Part I—contains six chapters on the general implications of RAS in health and disease; Part II—consists of eight chapters concerning the involvement of RAS in lung, liver and kidney ailments; and Part III—contains ten chapters, which are devoted to discussions regarding the role of RAS in the pathogenesis and therapy of different types of cancer. It is our contention that the information contained in this book will not only stimulate several biomedical and clinical investigators to carry out future research to understand the involvement of RAS in the genesis of diverse diseases, but will also help in identifying various targets for drug development. Furthermore, the state-of-the-art information regarding the molecular and cellular mechanisms in this book will be useful to graduate students, postdoctoral fellows, residents and health professionals for enhancing their careers in medical research and improving the scientific basis for the practice of medicine.

We are grateful to various members of the Advisory Board for this series on "Advances in Biochemistry in Health and Disease" for their valuable suggestions. The enthusiastic support of all contributors for preparing different chapters in this book is highly appreciated. We are indeed thankful to Dr. Gonzalo Cordova for his time and efforts in evaluating and approving this project. Our cordial thanks to the

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Part I
General Implications of RAS in Infectious and Other Diseases

Chapter 1
Implications of Renin-Angiotensin System in Health and Disease

Anureet K. Shah, Sushma Yadav, and Hoda Yeganehjoo

Abstract The renin-angiotensin system (RAS) is one of major neuro-endocrine entity which is intimately involved in regulating the cellular functions and metabolic activities in the body. The activation of RAS results in the formation and release of angiotensin II mainly as well as angiotensin 1–7 through the participation of angiotensin converting enzyme (ACE) and its homolog ACE2, respectively. Angiotensin II upon activating the angiotensin type 1 receptors (AT_1R) is known to produce a wide variety of effects namely vasoconstriction, fluid retention, fibrosis and thrombosis in addition to promoting oxidative stress, inflammation and hypertrophic process. On the other hand, angiotensin II by activating the angiotensin type 2 receptors (AT_2R) produces effects which are antagonists to those due to AT_1R activation. Furthermore, angiotensin 1–7 has been shown to activate Mas receptors and exert actions which are similar to those for AT_2R activation but antagonists to those for AT_1R activation. The effects of AT_1R activation during initial stages of pathological stimulus are considered to be of adaptive nature for maintaining homeostasis in all organs but over a prolonged period it is known to produce adverse effects which are associated with the development of diverse diseases. Although the activation of both AT_2R and Mas receptor is antagonistic to AT_1R activation, the exact role of these receptor systems at different stages of disease progression and organ dysfunction remains to be investigated.

Keywords Peripheral RAS · Local RAS · Angiotensin II · AT_1R activation · AT_2R activation · Mas receptors · Angiotensin converting enzymes

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Introduction

Among several neuro-endocrine systems, which regulate cellular and metabolic homeostasis of all organs in the body, the renin-angiotensin system (RAS) is known to play a pivotal role in health and disease by synthesizing and releasing different forms of angiotensin [1–12]. There are two types of RAS depending upon the location of angiotensin synthetic machinery. The term peripheral, conventional or circulating RAS is designated to the system in which angiotensin is synthesized due to the interaction of ingredients from kidney, liver and lung whereas the term local, tissue or endogenous RAS is commonly used for the system in which angiotensin is synthesized in different organs of the body. It should be mentioned that RAS is a multifunction system as it has been demonstrated to exert a wide number of effects in various organs in the body [1–6]. The present article is focused to provide information regarding different components of RAS which have been targeted for pharmacological modification of this system. It is also intended to describe the functional implications of different types of angiotensin receptors. In addition, some information regarding the involvement of RAS in the pathophysiology of a few diseases will be described to illustrate its role in health and disease.

Functional Components of RAS as Targets for Pharmacologic Manipulation

The peripheral RAS is a cascade of complex biochemical processes and enzymatic reactions that result in the formation of angiotensin [1, 3, 5, 13]. Briefly, the liver-derived angiotensinogen is degraded to form angiotensin I (Ang I) by the action of renin, which is secreted into the bloodstream from the renal juxtaglomerular apparatus. Ang I is subsequently converted to angiotensin II (Ang II) through the action of angiotensin converting enzyme (ACE), that is primarily located in the capillary blood vessels of the lungs. It may be mentioned that Ang II is also known as Ang (1–8) where the first and the eight amino acids are located at the peptide's N- and C-terminus, respectively. Furthermore, Ang II is the most predominant effector molecule of the peripheral RAS. Since 1980s extensive research has been carried out to identify several new components of RAS such as angiotensin converting enzyme 2 (ACE2), Ang (1–9), and Ang (1–7) [4, 5, 10–12, 14–16]. Although ACE2 is a homolog of ACE, it is resistant to ACE inhibitors. It generates Ang (1–7) either directly from Ang II or indirectly from Ang I. It is pointed out that Ang (1–7) is another effector molecule of RAS but is known to produce effects which are opposite to that of Ang II. In addition to generating the Ang (1–7), cleavage of Ang II by ACE has been shown to form other angiotensin peptides such as Ang (2–8) and Ang (3–8) which are also known as Ang III and Ang IV, respectively [17]. The physiological effects of Ang III are similar to Ang II whereas those of Ang IV are mainly localized in the central nervous system [17]. The biochemical pathway involving different

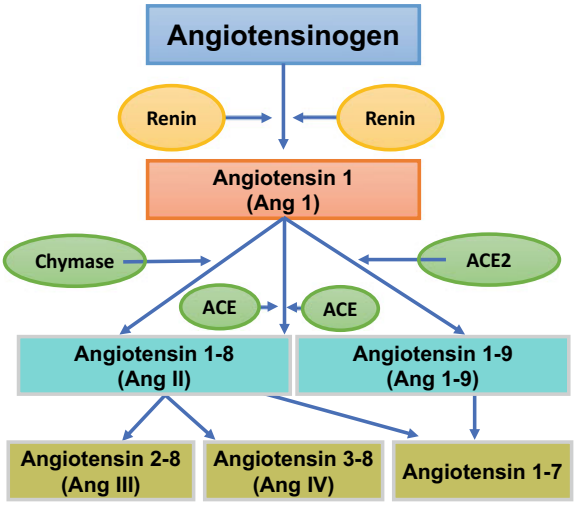

Fig. 1.1 Biochemical pathway of RAS showing the formation of angiotensin 1 from angiotensinogen by the action of renin and subsequently different forms of angiotensin (Ang) peptides upon cleavage by angiotensin converting enzyme (ACE) and a homolog of ACE (ACE2)

components of RAS as well as enzymes such as renin, ACE and ACE2 is shown in Fig. 1.1. Although the local RAS has been identified in diverse tissues and organs such as heart, brain, liver, kidneys, intestine, and pancreas, it is not clear whether some of its components such as angiotensinogen and renin are synthesized endogenously in all organs and tissues or taken up from the circulation. The local RAS functions in an autocrine, paracrine, or intracrine manner to cause diverse cellular and physiological effects [18–21].

It is noteworthy to emphasize that both systemic and local RASs play role in cell growth, proliferation, differentiation, apoptosis, inflammation, fibrosis, hormone secretion, and production of reactive oxygen species [13, 19–22]. The release of Ang II from the peripheral RAS considered to occur as a consequence of the decrease in blood pressure whereas that from the local RAS occurs due to an increase in hemodynamic stress. With a multitude of actions that are physiologically and clinically interrelated, both RASs interplay to affect the final outcome and serve as potent therapeutic targets in many diseases. It should be mentioned that the inhibition of ACE was identified as a suitable target for reducing the formation of Ang II under various pathological conditions and in fact, several ACE inhibitors have been developed for clinical use in the treatment of patients with hypertension or heart failure [3, 5, 23]. Although some work regarding the development of renin inhibitors for the therapy of hypertension has appeared in the literature, there is a real challenge for drug development to manipulate different targets such as ACE2 to stimulate the formation of Ang (1–7) [8, 10, 12].

It is now well established that the most prevalently expressed Ang II receptors in humans are the Ang II type-1 receptors (AT_1R); however, the Ang II type-2 receptors (AT_2R) are also found in various human body parts and in the fetal tissue [2, 24, 25]. The sodium- and fluid-retaining effects of Ang II are manifested by the activation of AT_1R as a consequence of actions such as enhancing the sense of thirst and releasing

the antidiuretic hormone (ADH) and aldosterone from the hypothalamus and the adrenal glands, respectively. The ADH and aldosterone promote renal fluid retention, sodium reabsorption, and potassium excretion. These downstream actions of Ang II combined with its effects on vasoconstriction and sympathetic nervous simulation result in raising the blood pressure (BP). The activation of AT_1R by Ang II has also been shown to exert inflammatory, pro-thrombotic, fibrotic, as well as oxidative stress and hypertrophic properties. Some of these effects of AT_1R activation are shown in Fig. 1.2. It is noteworthy that overexpression of the peripheral RAS components such as ACE, Ang II, and AT_1R is associated with cardiac muscle hypertrophy and fibrosis as well as the fibrotic processes in the diseased liver [13, 26, 27]. It is also pointed out that the activation of AT_2R by Ang II has been shown to produce actions which are antagonists to those elicited by the activation of AT_1R. Some of the effects of AT_2R activation are shown in Fig. 1.3.

Unlike Ang II which results in vasoconstriction and sodium retention, Ang (1–7) has been demonstrated to induce vasodilation and natriuresis in addition to producing anti-thrombotic, anti-fibrotic, antioxidant, anti-inflammatory and anti-hypertrophic effects by binding to Mas receptors [10, 11]. These alterations may be explained by the counter-balancing effects of the Ang II/AT_1R activation and that of Ang (1–7) on bradykinin, a protein that contributes to vasodilation, diuresis, lowering of blood pressure, and bronchoconstriction [28–31]. Degradation of bradykinin is mediated by ACE, an action that is shown to be antagonist by Ang (1–7) and thus rendering ACE inhibitors as potent anti-hypertensive compounds. In addition to its anti-hypertensive properties, the Ang (1–7) peptide exerts anti-trophic, anti-inflammatory, anti-fibrotic, and anti-thrombotic effects, whose actions have been reported to protect organs such as heart and liver [32–36]. Some of the actions of Mas receptor activation by Ang (1–7) are shown in Fig. 1.4. Clearly, several actions of Ang (1–7)/Mas receptor activation oppose those of Ang II/AT_1R activation and thus equalize each other via

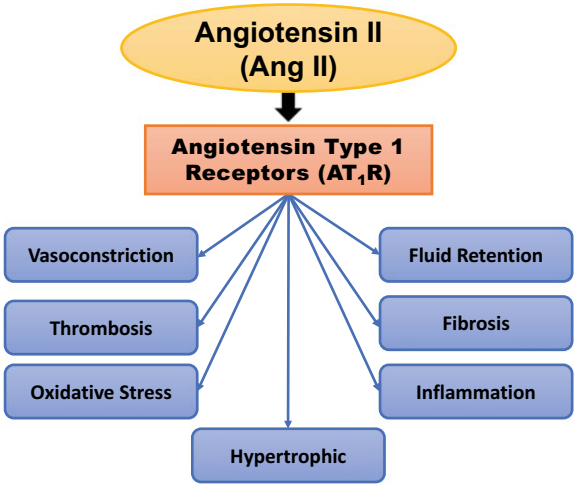

Fig. 1.2 Different actions of angiotensin II due to the activation of angiotensin type 1 receptors (AT_1R)

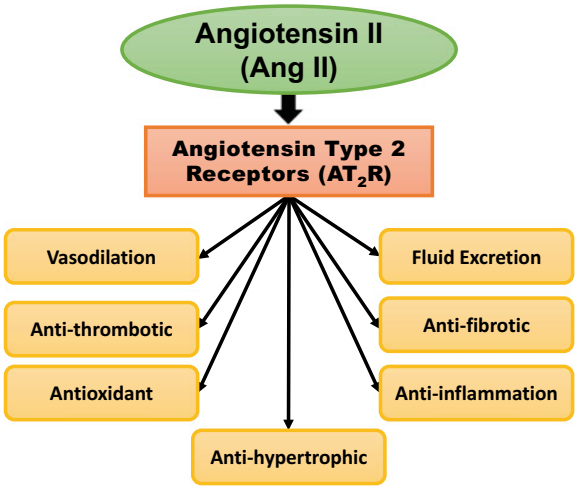

Fig. 1.3 Different actions of angiotensin II due to the activation of angiotensin type 2 receptors (AT_2R)

their counter-regulatory properties; however, the extent of this antagonists effect at different stages of any pathological condition remains to be investigated.

Since overstimulation of Ang II AT_1R system is known to exert deleterious effects in several diseases such as the cardiac and renal disease conditions, two potent blood pressure lowering interventions such as ACE inhibitors and angiotensin receptor blockers (ARBs) are considered to produce their anti-hypertensive effects by inhibiting Ang II synthesis and AT_1R binding, respectively. These medications are also shown to mediate their beneficial effects by upregulating the production of Ang (1–7) [13]. Moreover, several animal studies and a few clinical trials have documented the inhibition of fibrosis and tissue injury in heart and kidney disease upon

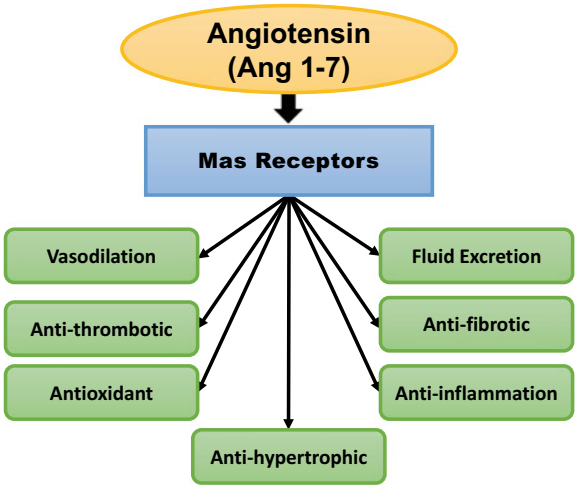

Fig. 1.4 Different actions of angiotensin (1–7) due to the activation of Mas receptors

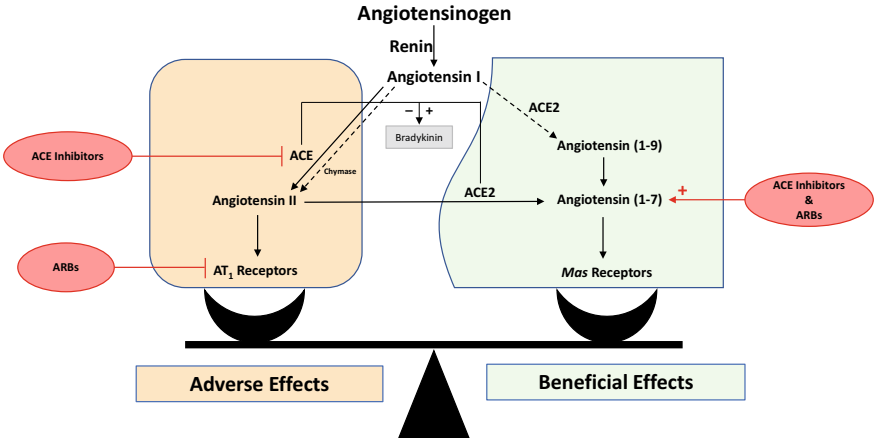

Fig. 1.5 Schematic representations of events showing a balance between the adverse effects of angiotensin II/AT_1R activation and beneficial effects of angiotensin (1–7)/Mas receptor activation. ACE—angiotensin converting enzyme; ARB—angiotensin receptor blocker

administration of these drugs [37–41]. A balance between the adverse effects of AT_1R activation and beneficial effects of Mas receptors activation is shown in Fig. 1.5. It appears that the beneficial effects of ACE inhibitors and AT_1R antagonists are not only elicited by their actions for the reduction of Ang II levels and blocking the Ang II receptor mechanisms respectively, but are also mediated through the stimulation of Mas receptors. Accordingly, Ang (1–7)/ Mas receptor system is an excellent target for developing drugs for improved combination therapy of pathological conditions associated with elevated levels of RAS.

Involvement of RAS in Liver Disease

In a diseased liver, the inflammation and fibrosis cause significant structural changes that increase hepatic resistance, obstruct the hepatic blood flow, and stretch the hepatic portal vein. Such changes result in portal hypertension but a compensatory mechanism of some vasodilators are released into the circulation to restore the blood volume. As the liver damage progresses, the compensatory mechanisms also continue to stay active which leads to further sympathetic stimulation and sodium and water retention. These deleterious effects result in the complications of advanced liver disease such as edema, ascites, portal hypertension, and hepatorenal syndrome. The circulating RAS and the local hepatic RAS both play role in liver function and liver disease. The overexpression of the RAS components, such as renin, ACE, Ang II, and AT_1 receptors, is detected in the diseased liver and is believed to contribute to the harmful inflammatory and fibrotic events [26, 27] In an attempt to compensate, the counteracting components, such as ACE2, Ang (1–7), and *Mas* receptors of RAS, are

also shown to be upregulated in the diseased hepatocytes to attenuate liver fibrosis, inflammation, and injury [26, 27]. The balance between the two opposing arms of RAS is critical in determining the net outcome in liver function and liver disease.

Angiotensinogen, which is a component of both the systemic and the local RAS and the precursor of all angiotensin peptides, is synthesized in the liver. Angiotensinogen and several other major RAS components, such as renin, ACE, AT_1R and AT_2R, are expressed in the hepatocytes [20]. The hepatic Kupffer cells do not contain the angiotensinogen and the AT_2R but express renin, ACE, and AT_1R [20]. The Kupffer cells are macrophages in the sinusoids of the liver that play an integral role in the immune system and host defense by removing bacteria, toxins, and other foreign debris that pass through the hepatic circulation. Additionally, the Kupffer cells are needed for the breakdown of red blood cells, recycling of hemoglobin, and metabolism of some nutrients. In response to an infection, injury, or inflammation, the Kupffer cells promote wound healing by producing cytokines, such as transforming growth factor beta (TGF-β), and the extracellular matrix (ECM) components, such as fibronectin [20, 42]. The excessive production of these substances and the dysregulated function of Kupffer cells are linked to the development of various liver diseases such as alcoholic liver disease (ALD), non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), liver fibrosis, cirrhosis, and intrahepatic cholestasis [20, 42–44]. Since the Kupffer cells lack angiotensinogen and AT_2R, the action of the Ang II via its AT_1R in the hepatic Kupffer cell cause local RAS to become prominent when mediating inflammation and fibrosis in liver injury. In fact, the Ang II-AT_1R interaction has shown to increase production of TGF-β and fibronectin and promote hepatic fibrogenesis [20, 45]. The overexpression of the AT_1R, also makes the cirrhotic liver hyper-responsive to Ang II which leads to vasoconstriction and the development of portal hypertension [26].

It is plausible to suppress the adverse hepatic events with potential treatments that exert either AT_1R antagonistic or Mas receptor agonistic properties. In animal models of liver failure and NASH, the blockade of AT_1R improved survival and delayed disease progression by suppressing the liver enzymes, oxidative damage, and fibrogenic factors such as TGF-β and collagen [13, 20, 46]. Additionally, suppression of the Ang II and AT_1R can alleviate liver fibrosis by deactivating the hepatic stellate cells which possess collagen depositing, abnormal ECM remodeling, and fibrogenic properties [20, 47, 48]. The two potent blood pressure lowering medications, ACE inhibitors and ARBs (angiotensin receptor blockers), exert their effects by inhibiting the Ang II synthesis and receptor binding, respectively, and by upregulating the production of Ang (1–7) [13]. Based on their inhibition of Ang II and stimulation of Ang (1–7), the antifibrotic effects of these medications have also been investigated in liver disease [13, 37–41]. Administration of the ARB Olmesartan, but not Losartan, significantly reduced fibrosis and progression of liver disease in an animal model of NASH [47–49]. In clinical trials, treatment with either the ARBs Losartan and Candesartan or the ACE inhibitor, Perindopril, significantly reduced the serum levels of hepatic fibrosis including the TGF-β, hyaluronic acid, and type IV collagen in patients with chronic hepatitis C and cirrhosis [41, 50–52]. The occurrence of hepatic

fibrosis was also reduced in the liver transplant patients with recurrent hepatitis C, who took medications that block RAS [40].

When weighing up benefits and risks associated with treatments, it is also important to take into consideration the simultaneous treatment-mediated effects on the systemic RAS as well as the locally expressed RASs in various organs. In the kidneys, the Ang II plays a critical role to maintain sufficient renal perfusion pressure, renal blood flow, and glomerular filtration rate (GFR). The overall reduction in the effective circulatory volume that is present in advanced liver disease, causes less-than-optimal renal perfusion and GFR. In response, the ACE and Ang II stimulate constriction of the efferent glomerular vessels to restore the renal perfusion pressure and increase the GFR. On the contrary, the ACE inhibitors and other RAS blocking agents suppress the GFR [53, 54]. Thus, the application of ACE inhibitors and RAS blocking agents to treat patients with advanced liver disease and portal hypertension can present a major drawback as these drugs which can cause renal impairment and a rapid drop in the GFR. Moreover, sustainability of the long-term treatment with ACE inhibitors and ARBs should be evaluated in patients with advanced liver disease due to the treatment-induced effects on some Ang II-forming enzymes, such as the hepatic chymase [13]. The ACE inhibition has shown to upregulate the hepatic chymase activity which, in turn, augments the formation of Ang II and the progression of liver fibrosis in advanced liver disease [55, 56]. Thus, the long-term sustainability of chronic ACE inhibition therapy in suppressing the levels of Ang II is questionable. It is based on the aforementioned drawbacks, such as induction of renal impairment and stimulation of some Ang II-forming enzymes, that the application of RAS inhibitors for the long-term treatment of advanced liver disease, particularly in presence of portal hypertension, remains disputable.

Involvement of RAS in Respiratory Syndrome Coronavirus Infection

The transmembrane protein of ACE2 has been identified as a high-affinity receptor site for the spike proteins of coronaviruses including the SARS (severe acute respiratory system) coronavirus [57]. The binding of coronavirus spike proteins to these functional ACE2 sites has been shown to facilitate infection of targeted cells [57]. The efficient replication of SARS coronaviruses has been documented in ACE2-transfected cells where the viral replication was subsequently blocked by anti-ACE2, but not anti-ACE1, antibodies in vitro [57]. Thus, SARS treatment strategies may involve inhibiting fusion of the coronavirus spike proteins to ACE2 by either blocking the functional sites or inducing unfavorable conformational changes in the ACE2 peptide [57].

Participation and Significance of RAS in Heart Failure

As discussed in our previous work, RAS gets activated during the early stages of pathological stimulus [23]. This activation of RAS along with sympathetic nervous system and natriuretic hormonal system release several hormones like Ang II, norepinephrine, growth factors and antidiuretic peptides in the circulation. The elevated levels of these hormones in the circulation induce changes to maintain cardiac function along with blood pressure maintenance and the integrity of crucial organs. These changes include increase in cardiac contractility, ventricular filling and peripheral vasoconstriction [58]. Although prolonged activation of these neurohormonal systems and elevated levels of these circulating hormones for longer duration leads to cardiac abnormalities and increase the hemodynamic overload in the left ventricle ultimately leading to development of fibrosis and apoptosis which eventually turns into heart failure [6].

There is a strong involvement of RAS in the pathophysiology of heart failure and the plausible role of ACE as a significant component of the system which converts Ang 1 to Ang II, the main effector of this system. Ang II elicits its response by interacting with AT_1R causing vasoconstriction, maintenance of peripheral blood flow and blood pressure. These compensatory mechanisms of RAS in the initial stages are favorable for improving cardiac function, although prolonged activation of RAS and elevated hormones are deleterious leading to worsening of cardiac function. On the other hand, activation of AT_2R has an antagonist action on AT_1R mediated effect. The signaling induced by AT_2R leads to protective vasodilatory and anti-hypertrophic effects in heart failure patients. The imbalance between opposing actions of AT_1R and AT_2R leads to progression of cardiovascular disease and cardiac dysfunction [59]. Furthermore, role of several ACE inhibitors in delaying the heart disease progression induced by RAS and AT_1R activation cannot be evaded. Although we don't yet understand their full mechanism of action, we do know their exhibit their protective effects either by changes in the collagen expression [60], energy metabolism [61], scavenging oxygen radicals [62], altering protein content or mRNA levels in heart failure [63]. The continued usage of ACE inhibitors is accompanied by some side effects such as dry cough but its beneficial effects in delaying the progression of heart failure over powers the negatives. These protective effects of ACE inhibitors may be of complex nature but these observations clearly support the role of RAS in inducing cardiac dysfunction and progression of heart failure.

Concluding Remarks

From the foregoing discussion, it is evident that the activation of RAS under acute conditions plays an adaptive role for maintaining cellular and metabolic homeostasis for different organ functions. On the other hand, the activation of RAS for a prolonged period induces adverse effects for the pathogenesis of various diseases in the body.

Table 1.1 Molecular targets in RAS for drug developments

Targets	Type of drugs indicated for treatment
A. Targets producing adverse effects	
Renin receptors	Inhibitors
ACE enzyme	Inhibitors
AT_1R receptors	Inhibitors
B. Targets producing beneficial effects	
AT_2R receptors	Activators
ACE2 enzyme	Activators
Mas receptors	Activators

ACE—angiotensin converting enzyme; AT_1R—angiotensin type 1 receptors; AT_2R—angiotensin type 2 receptor

Several components of RAS such as renin receptors, ACE, AT_1R, AT_2R, ACE2 and Mas receptor have been identified to be involved as molecular mechanisms for regulating the functions of RAS and are considered to serve as targets for drug development in manipulating this complex system (Table 1.1). It is pointed out that ACE is involved in the formation of Ang II whereas both ACE and ACE2 are involved in the formation of Ang 1–9. Furthermore, Ang II is known to produce the activation of two types of receptors namely AT_1R, which produce effects such as vasoconstrictions, fluid retentions, oxidative stress, inflammation, fibrosis, apoptosis, thrombosis, necrosis as well as hypertrophic effects, and AT_2R, which produces effects such as vasodilation, diuresis, anti-thrombotic, anti-fibrotic, anti-inflammatory, antioxidant and anti-hypertrophic effects.

It is noteworthy that Ang 1–7 produces actions upon activating Mas receptors, which are similar to those induced by AT_2R activation and in fact are also antagonists to those induced by AT_1R activation. Thus, the release of Ang II upon the activation of RAS is associated with the induction of both pathogenic and compensatory actions, which maintain a balance for regulating the functions of different organs. Any disturbance in such a balance in any organ can be seen to result in the development of disease in that particular organ. We have discussed this concept regarding the role of RAS in the genesis of liver and heart diseases and have not only emphasized the importance of reducing the formation of Ang II upon depressing the ACE activity by inhibitors but have also indicated the beneficial effects of using AT_1R antagonists. However, extensive research work remains to be carried out for developing the activators of both AT_2R and Mas receptors if we have to improve the treatment of RAS-induced organ dysfunction. Particularly, a combination therapy by using AT_1R antagonists and activators of both AT_2R and Mas receptors may prove most beneficial in this regard.

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Conflict of Interest The authors declare no conflict of interest.

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Chapter 2
The Interplay of the Renin-Angiotensin System and Solid Organ Transplantation

Kenneth J. Dery, Jerzy W. Kupiec-Weglinski, and Tien S. Dong

Abstract The renin-angiotensin system (RAS) plays a powerful role in controlling sodium balances, fluid body volumes, and arterial pressures. New studies linking the human microbial ecosystem to RAS may provide new insight and benefits to the field of organ transplantation. Ischemia–reperfusion injury (IRI) remains the primary obstacle limiting the success of solid organ transplantation (SOT) in patients with end-stage organ disease and those with tumor origin. The gut microbiota is responsible for shaping host immunity and metabolic activity. It acts as both a potent biological modulator of the RAS and in SOT. Bidirectional interactions of the intestinal community and the RAS activity may regulate gut-derived metabolites that may shape alloimmune responses necessary to mitigate allograft rejection. The current consensus is that reducing IRI syndrome might improve SOT outcomes and alleviate donor organ shortage. Given that treatment options to prevent or attenuate IRI-SOT are extremely limited, studying the underlying pathogenesis to uncover novel therapeutic targets is an area of intense investigation. The future challenge will be to understand better the physiological constraints of the RAS and the role of different microorganisms so that better technologies can be developed, ultimately to risk stratify patients.

Keywords Microbiome · Antibiotics · Renin · Organ transplantation · ACE2 · Angiotensin · Liver · Bacterial metabolites · Gastrointestinal system · RAS

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Abbreviations

ACE2	Angiotensin-converting enzyme-2
Abx	Antibiotic
COVID-19	Coronavirus 2019
FMT	Fecal microbiota transplantation
IRI	Ischemia-reperfusion injury
OLT	Liver transplantation
PT	Phage therapy
RAS	Renin-angiotensin system
SOT	Solid organ transplantation

Introduction

Interest in the renin-angiotensin (RAS) system has exploded in the past few years. This complex multi-organ endocrine (hormone) system is a key regulator of vascular resistance and tone, sodium, and water homeostasis. Although the birth of RAS research is credited to Finnish physiologist Robert Tigerstedt in 1898 [1], it took the onset of the coronavirus 2019 (COVID-19) pandemic to bring heightened awareness to the components of the RAS, especially how it relates to microbes. The virus responsible for COVID-19, SARS-CoV-2, uses angiotensin-converting enzyme-2 (ACE2) for cell entry and, therefore, directly affects the RAS [2]. The first report connecting the microbiome to RAS occurred in 2012, where it was linked to the regulation of intestinal amino acid homeostasis and the ecology of the gut microbiome [3]. Since then, 90 further articles have appeared in Pubmed using the keywords "renin-angiotensin" and "microbiome", with 67 occurring in only the last two years. This explosion of interest reflects both the influence of novel molecular techniques and highlights the infancy of our understanding of the gut microbiome/RAS axis.

Along a parallel track, considerable interest has been given to how the microbiota in mammalian hosts can alter the outcome of solid organ transplantation (SOT). New data indicate that different microbial community structures can affect inflammation processes, ultimately promoting or inhibiting allograft transplant survival rates [4]. Since post-transplantation hypertension is associated with decreased patient survival and allograft survival [5], understanding how the RAS affects solid organ transplantation remains a high priority. Compelling new studies reveal how bidirectional interactions of the intestinal community can modulate SOT and RAS activity, likely through the generation of gut-derived metabolites and supranormal activation of immune cells. Animal studies using inhibitors of the RAS system and conventionally raised mice show a less robust response in germ-free animals, thus implicating the microbiome [6]. Combined with the higher incidence of acute organ rejection associated with pre-transplantation diabetes mellitus and post-transplantation hypertension, it becomes increasingly important to understand how the intestinal microbiota

ameliorates the allograft rejection cascade in the background of the counterbalancing role of the RAS.

In this review, cellular and molecular mechanisms that govern SOT are highlighted, along with the current challenges that procured organs subjected to revascularization face. Next, research progress on how the gut microbiota modulates allograft rejection and the RAS are explored. The combinatorial effects on local immune activation and inflammatory cascades by the RAS on SOT are explored, with a final review of the status of clinical trials and novel therapeutic models. By presenting new insights into how tissue inflammation responses from SOT relate to the RAS, a rationale is presented for how understanding the structure of microbial communities might be harnessed therapeutically.

Complications of Solid Organ Transplantation

In 1954, a milestone was made when the kidney was the first human organ to be transplanted successfully between identical twins. Now it is the most transplanted organ in the United States [7]. Liver, heart, and pancreas transplantations were performed in the 1960s, while lung and intestinal organ transplant procedures began in the 1980s [8]. It has become an established and life-saving treatment that improves the quality of life, especially for patients with end-stage organ failure. Though SOT programs continue to grow and are essential for developed and mature health care systems, serious challenges remain. For one, transplant candidates and recipients in the modern era involve a growing proportion of elderly patients with comorbidities and impaired immune function. Second, organ shortages represent a system-related risk of allocated resources that reduce transplantation procedures in the United States and European countries [9]. Though efforts to increase the donor pool have included expanding the criteria into higher-risk categories, such as using older donors with more comorbidities and donation after cardiac death (DCD), initial enthusiasm remains tempered because suboptimal liver quality has often translated into inferior clinical outcomes [10]. The best strategy for addressing organ shortages should then be the investigation of cellular and molecular mechanisms to improve organ preservation during transport between donor and recipient and lengthening the survival of both organ and recipient following implantation.

Currently, ischemia–reperfusion injury (IRI) is one of the most understudied areas in clinical and experimental transplantation. It is an inevitable clinical consequence that results from the cessation of arterial blood flow after organ procurement (the ischemia component) and reestablishing blood flow in transplant recipients (reperfusion). IRI results in metabolic and oxidative disturbances and profound inflammatory immune responses involving direct and indirect cytotoxic mechanisms. It can lead to early graft nonfunction and contribute to acute and chronic graft rejection. Though advanced molecular biology has revealed the highly complex nature of this phenomenon, few definitive therapies exist. During the first phase of injury, hepatocellular damage develops in situ during liver transplantation, leading to local

inflammatory immune activation (Fig. 2.1). Reactive oxygen species produced by damaged hepatocytes initiate an immunological cascade that activates the release of danger-associated molecular patterns (DAMPs), such as high mobility group protein 1 (HMGB1). This is a key endogenous TLR4 ligand responsible for further immune activation in the liver during IRI. The boosted production of macrophage-derived pro-inflammatory cytokines and chemokines leads to increased expression of adhesion molecules and infiltration by circulating lymphocytes and monocytes, ultimately triggering necrotic and apoptotic cell death pathways.

Emerging data also suggests an equally robust adaptive immune response as mediators in renal and liver organs dependent on T cells. Our group was among the first to show that the sentinel TLR4 pathway provides the key triggering signal and a mechanistic link between innate and adaptive immunity to facilitate sterile inflammation in IR-stressed livers [11]. More recently, we showed that hepatic IRI is an exogenous Ag-independent innate immune-dominated sterile inflammation that requires activated CD4+ T cells to facilitate tissue damage [12]. This work adds to the volume of data that shows that liver IRI represents a continuum of processes in which TLR4

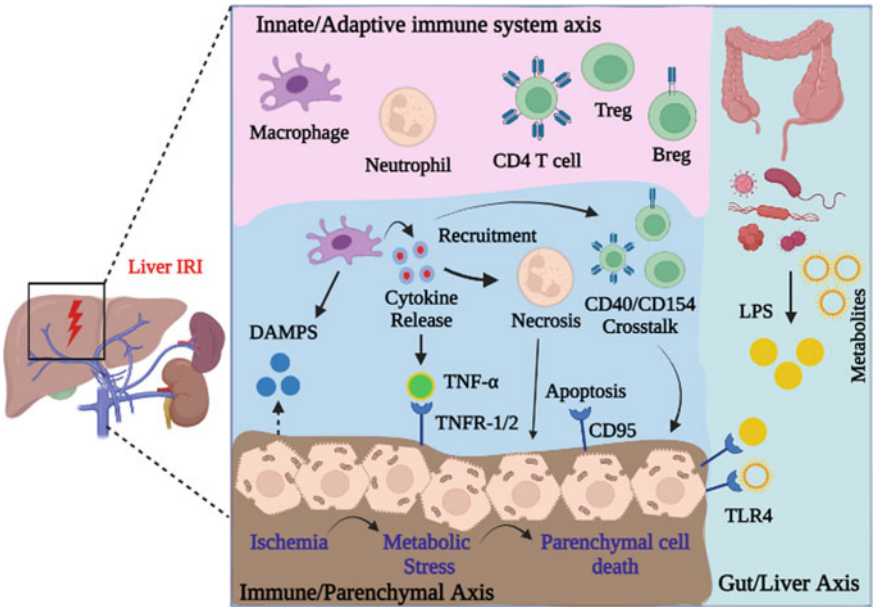

Fig. 2.1 Mechanism of hepatic IRI. Ischemic stress, caused in situ by transplantation, initiates hepatocellular damage leading to production of reactive oxygen species and release of DAMPS (e.g., HMGM1). During reperfusion, activated liver Kupffer cells and circulating macrophages produce cytokines and chemokines leading to the infiltration of circulating lymphocytes. As time progresses, the inflammation becomes an innate-immune-dominated response that is mediated by the sentinel pattern recognition receptor system (e.g., TLR4). Ultimately the metabolic stress leads to parenchymal cell death. In the gut, metabolites and LPS produced by the microbiota can have profound direct and indirect effects on ischemic liver grafts. Figure created with BioRender.com

activation triggers a "vicious cycle" of tri-partite crosstalk between infiltrating CD4+ T cells and macrophages, leading to hepatocyte death and ultimate organ failure [13].

Recent Efforts to Mitigate IRI

Strategies to attenuate reperfusion injury are an active area of research and include modulation of the gut microbiota, pharmacological therapy, regulation of genetic markers, and pre-and post-ischemic conditioning [14]. Most strategies are still at the stage of animal research, and as Table 2.1 shows, interest remains high in developing a cohesive therapeutic target, with the role of the microbiota playing a central role. When gut injury and mucosal barrier function is destroyed during the progression towards I/R injury, vascular permeability promotes inflammatory cells to release reactive oxygen species (ROS), pro-inflammatory chemokines, and protein kinases [15]. Penetration of the bacteria through intestinal barriers can lead to dysbiosis and colonization of human organs. This was demonstrated when 99mTc-labeled bacteria were administered prior to I/R treatment, and post-injury analyses revealed its presence in the serum, lung, liver, and mesenteric lymph nodes after mesenteric I/R injury in a time-dependent manner [16]. Not surprisingly, significant efforts have been made to understand the functional role of bacteria in organ I/R injury.

Our lab recently reported on the striking benefits of extended recipient antibiotic (Abx) pretreatment in mice and humans for improving early orthotopic liver transplantation (OLT) outcomes [17]. A retrospective analysis of a large human OLT cohort revealed that prolonged treatment with Rifaximin for more than 10 days in prospective recipients led to superior hepatic function and decreased incidence of early allograft dysfunction. Paradoxically, these beneficial effects were achieved despite parameters of preexisting severe acuity, i.e., higher MELD score, prolonged hospital/ICU stay, and the need for more blood transfusions, compared with controls (<10 days or no Abx pretreatment). The experimental component of this work identified the mechanistic underpinnings of Abx therapy using amoxicillin in an IR-stressed mouse OLT model. The data showed that Abx pretreatment of donor's livers augmented COX2—prostaglandin (PGE2) activity, attenuated endoplasmic reticulum (ER) stress, and enhanced autophagy signaling. In an interesting twist, fecal microbiota transplantation (FMT) from naïve mice recreated hepatic damage in Abx-treated otherwise IRI-resistant OLT recipients suggesting that the natural state of our gut–liver axis predisposes higher incidence of hepatic IRI. More recently, Zhang et al. reported the effect of *Lactobacillus acidophilus* on oxidative stress, inflammation, and intestinal flora in a mouse model of renal IRI [18]. *L. acidophilus* administered by intragastric gavage showed lower pro-inflammatory markers (e.g., MDA, TNF-α, IL-1β, and HMGB1) and altered abundance of Helicobacter *cultivated_bacterium*, and *k__Bacteria_ASV_3*. Another group showed the therapeutic potential of *Lactobacillus plantarum* in small intestinal I/R injury. Wang et al. reported that when used as a probiotic, *L. plantarum* could prevent intestinal barrier dysfunction [15]. Additionally, Ménard et al. demonstrated that metabolites from bacterial strains producing

Table 2.1 Recent representative milestones mitigating IRI, from 2019 to present

Year	Model	Mitigation method	Novelty	Source
2019	Liver	HuR overexpression	HuR-expressing clincal liver grafts showed lower sAST/ALT levels and improved LT survival	[105]
2019	Liver	Antibiotics prior to transplantation	Altered microbiome affected ER stress and autophagy regulation by the PGE2/EP4 axis	[17]
2019	Kidney	Shenhua tablet	Treatment mitigated renal injuries, suppressed degeneration induced by up-regulation of TLR2 and TLR4 expression levels in the MyD88-dependent signaling pathway	[106]
2019	Heart	Ethyl pyruvate	Treatment diminished inflammasome activation (NOD-like receptor 3 (NLRP3), apoptosis-associated speck-like protein, and caspase-1, and interleukin-1β	[107]
2020	Liver	CEACAM1	ROS and HMGB1 translocation, enhanced innate and adaptive immune responses, and inferior early OLT function	[108]
2020	Human dendritic cells	Senolytics, Dasatinib and Quercetin	Treatment lowers cell-free mitochondrial DNA associated with TRL9 activation and Th1/Th17 immunogenicity	[21]

(continued)

Table 2.1 (continued)

Year	Model	Mitigation method	Novelty	Source
2020	Liver	Notch1	Myeloid Notch1 signaling promotes heat shock transcription factor 1 and Snail activation, inhibits NLRP3 function and hepatocellular apoptosis	[109]
2020	Cardiomyocytes	Hyperbaric oxygen	Treatment prevented mitochondrial dysfunction and inhibiting autophagy	[110]
2021	Human coronary artery endothelial cell	Recombinant human thioredoxin	Protects against endothelial NO synthase chaperone-mediated autophagy	[111]
2021	Thromboembolism, skeletal muscle	Hydrogen sulfide (H_2 S)-loaded microbubbles	Treatment dissolved both white and red macrothrombi, alleviated skeletal muscle IRI through organ-specific release of H_2S	[112]
2021	Liver	miR122	HIF1α-dependent induction of miR122 enhances hepatic HIF responses through PHD1 repression	[113]
2021	Cerebral artery	Carthamin yellow	Treatment attenuated neurological deficits, brain water content and infarct area, and increased MAP-2 immunoreactivity in the cortex	[114]
2021	Kidney	Vitamin D	Treatment reestablished amounts of MCP1, CD68+, CD3+ cells and collagen type III, fibronectin, vimentin and α-SMA	[115]

(continued)

Table 2.1 (continued)

Year	Model	Mitigation method	Novelty	Source
2021	Kidney	Dexmedetomidine	Treatment inhibited mitochondrial damage and cellular inflammation by up regulating PGC-1α to affect STAT1 phosphorylation level and IRF-1 expression	[116]
2022	Macrophage	Gut microbial metabolite, 3,4-dihydroxyphenylpropionic acid	Treatment suppressed macrophage pro-inflammatory responses and lowered histone deacetylase inhibition in macrophages	[117]
2022	Heart	Adenosine, lidocaine, and magnesium	Treatment led to reduction in cardiac output and cardiac index, interleukin 2, interleukin 10, interleukin 4, and IL1RN, MTOR, and LAMP3 messenger RNAs	[23]
2022	Kidney	4-phenylbutyric acid	Treatment reduced ICAM-1 and VCAM-1 expression and lowered CypD, Cytochrome c, eIF2α, GRP78, and cellular oxygen free radicals and apoptosis	[20]

lactic acid inhibited the binding of lipopolysaccharide (LPS) to human monocytes and decreased NF-κB nuclear translocation (Fig. 2.1, [19]). For microbial studies to have much impact in future clinical trials, combinatorial interactions with routinely administered immunosuppressive agents (e.g., tacrolimus) will need to be evaluated to see if their bacteriostatic potential affects beneficial bacterial populations.

Pharmacological pre- and postconditioning have also translated into proof-of-concept studies to ameliorate I/R injury. Zhang et al. reported the therapeutic effect of using 4-phenyl butyric acid to suppress renal I/R injury in mice [20]. Lower levels of CypD, Cytochrome c, eIF2α and GRP78 were reported in the treatment group with reduced cellular oxygen free radicals, which alleviated the endoplasmic reticulum stress response. Another group investigated whether senolytics, a class of drugs, could selectively eliminate senescent cells that typically augment immunogenicity

by releasing cell-free mitochondrial DNA. The authors report that after I/R injury, a systemic increase of cf-mt-DNA promotes dendritic cell-driven, age-mediated inflammatory responses. When Dasatinib and Quercetin were administered, survival of old cardiac allografts increased, comparable to young donor organs, concomitant with a reduction of dendritic cell-mediated, age-specific inflammatory responses that promoted the survival of aged organs following transplantation [21]. Noble gases, such as Argon, helium, and xenon, have been investigated in cerebral, myocardial, renal, and hepatic IRI [22]. A meta-analysis review of published studies although concluded that xenon was the most neuroprotective in rodents, cardioprotective in rodents and pigs, and renoprotective in rodents in IRI models [22]. Finally, Granfeldt et al. recently used a combination of adenosine, lidocaine, and Mg2+ (ALM) to induce reversible hypotension in an endotoxemic porcine model [23]. That ALM infusion led to a hypometabolic state that caused attenuated tumor necrosis factor-α levels and improved cardiac and pulmonary function, showed the potential of pharmacologics to alter blood pressure levels, a prognostic indicator for worsened I/R outcomes. This is the case for patients with hearts that have hypertension and cardiac hypertrophy [24] and for the kidney, where hypertension causes renal perfusion impairment in CD1 mice, promoting progressive fibrosis [25]. In the liver, transient portal hypertension is a major confounding factor of peritransplant procedures, the result of portal vein occlusion, and can cause splanchnic vasodilation with subsequent intrarenal vasoconstriction [26]. Importantly, changes in blood pressure leads to activation of the RAS and a host of downstream consequences such as reduction of glomerular filtration rate, urinary sodium excretion, and free water excretion. Patients are ultimately at risk for renal tubular necrosis and renal dysfunction. Though mounting evidence reveals a role for the RAS in I/R, a role for mitigation therapy remains unexplored.

Historical Perspective of the RAS

The first of many milestones of the RAS era (summarized in Table 2.2) was the discovery in 1898 that extracts from rabbit kidney cortex contained a long-lasting pressor later named renin [1]. This proteolytic enzyme is the rate-limiting step that initiates a sequence of steps (discussed in the next section) in the RAS cascade. Interest in renin would wait another four decades until 1934 when Harry Goldblatt and colleagues demonstrated a new paradigm for hypertension [27]. Their studies in dogs showed that constriction of the right and left renal arteries caused sustained chronic hypertension. Further, no differences in hypertension levels were observed when maneuvers such as adrenalectomy were used to block the sympathetic nervous system. Only when the aorta above the kidney was constricted, hypertension levels increased, paving the way for a deeper understanding of the renal pressor system. The search for the pressor substance in plasma, that renin acted upon, led multiple groups to discover angiotensin in late 1930s. First, Kohlstaedt, Page and Helmer showed in 1938 that small amounts of blood, when added to a solution containing

purified renin, elicited strong vasoconstriction responses in the tails of dogs [28]. Then, Braun-Menendez et al. reported in 1940 a similar substance from venous blood that was thermostable and could elicit a sharp rise in blood pressure [29]. In the next decade, Skeggs et al. established the two forms that angiotensin takes, a decapeptide version called angiotensin I (Ang I) and an octapeptide version called angiotensin II (Ang II). He also discovered angiotensin-converting enzyme (ACE) [30]. The period of the late 1950–1970s further elucidated the role of Ang II as a potent regulator for adrenal aldosterone secretion [31–34], the discovery that a bradykinin-potentiating factor inhibits the conversion of Ang I to Ang [35], the identification of angiotensin receptors [36] and ACE inhibitors [37]. These milestones and others [3, 35, 38–44] are summarized in Table 2.2 (adapted from [45]), helped establish that the RAS is the body's most powerful hormone system for controlling sodium balance, fluid body volumes, and arterial pressure.

Renin-Angiotensin System (RAS) Molecular Mechanisms

The 'classical' RAS pathway is often depicted as a simple enzyme cascade that starts with the systemic release of renin into circulation by the juxtaglomerular apparatus in response to low blood pressure and adverse sodium concentrations (Fig. 2.2). Activation of specialized juxtaglomerular (JG) cells in the kidney cause the cleavage of the inactive precursor prorenin to renin. JG exhibit features of both smooth muscle cells and secretory epithelial cells and are found in the afferent arterioles of the glomerular capillary bed. Clinical symptoms of dehydration, hypotension, or septic shock caused by a decrease in renal perfusion cause the release of renin into the circulation to reestablish homeostasis. Other activators of JG include the macula densa (MD), the chief cells within the kidney that regulate body fluids, electrolyte homeostasis, and blood pressure. It has also been shown to respond to decreased sodium load in the distal convoluted tubule affecting hypertension levels [46]. MD regulation of neuronal nitric oxide synthase also plays a role in stabilizing glomerular filtration rates, natriuresis, and blood pressure levels in a diabetes model [47]. Renin-expressing cells also exist outside the kidney, but their role remains a mystery. An interesting new study reports that a unique type of B-1 lymphocyte, playing a role in defending organisms against infections, can synthesize renin, entrap and phagocyte bacteria and control bacterial growth [48]. After tracking the distribution, identity, and evolution of renin progenitors, B-1 lymphocytes expressing renin were shown to possess bactericidal activities against *Salmonella typhimurium*.

The next step in the enzyme cascade involves the systemic circulation of renin through the hepatic portal system. Once recognized by the liver-derived precursor angiotensinogen, conversion to physiologically inactive angiotensin (Ang-I) occurs. The enzyme angiotensin-converting enzyme (ACE), found primarily in the vascular endothelium of the lungs and kidneys, then plays the central role in converting precursor Ang-I into the metabolically pleiotropic hormone, angiotensin II (Ang-II). The importance of ACE in renal disease has been demonstrated for more than

Table 2.2 Selected milestones of the renin-angiotensin system (RAS) and microbiome axis

1898	1934	1939-1940	1954	1958	1959-1961	1968	1970	1977	2002	2007	2012	2017	2017	2020	2022
Discovery of renin from renal cortex extracts [1]	Renal artery constriction leads to hypertension [27]	Discovery of angiotensin [28-29]	Discovery of two forms of Ang I and Ang II [30]	Hypothetical relationship between Ang II and aldosterone articulated [38]	Demonstration that Ang II regulates aldosterone [31-34]	Discovery that Bradykinin-potentiating factor inhibits conversion of Ang I to Ang II [35]	Angiotensin receptors identified [36]	ACE inhibitors described [37]	Existence of proreinin receptor (PRR) identified [42]	Link established between patients with hepatitis C and allelic variants of ACE [40]	Discovery that ACE2 is a key regulator of dietary amino acid homeostasis, innate immunity, gut microbial ecology [3]	Short chain fatty acid acetate produced by gut microbes downregulated RAS in kidney [41]	Discovery that sodium butyrate suppresses Ang II-induced hypertension [44]	Remodeling of the RAS occurs after Kidney Tx [39]	Deletion of the angiotensin (1-7) receptor Mas leads to alterations in gut villi length modulating TLR4/PI3K/AKT and produces microbiome dysbiosis [43]	Demonstration that ACE inhibitory peptide VGINYW alleviates hypertension-associated intestinal microbiota dysbiosis [73]

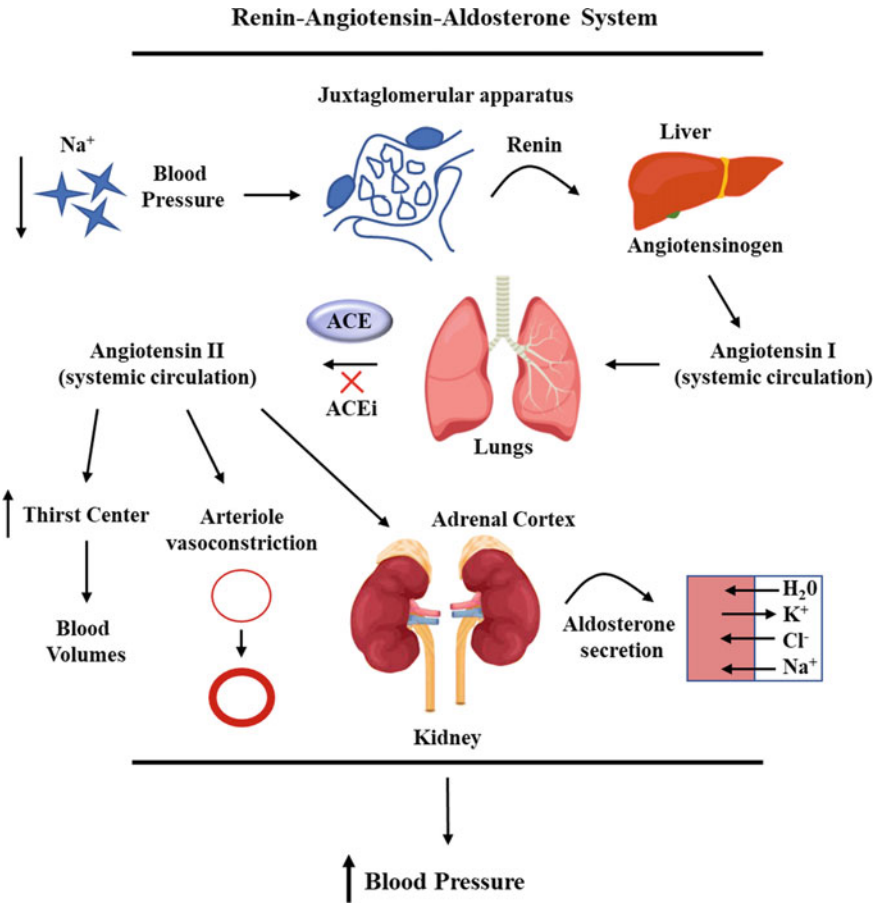

Fig. 2.2 Mechanism of the Renin Angiotensin System (RAS). Initiation of the RAS elevates arterial pressure in response to decreased renal blood pressure and/or decreased salt delivery to the distal convoluted tubule of the kidney. Activation of juxtaglomerular (JG) cells causes the cleavage of prorenin (precursor) to renin (final product). Renin is produced by the kidneys and its substrate, angiotensinogen, is primarily but not exclusively produced by the liver. Angiotensinogen is then modified by renin to produce angiotensin I, which is later converted by angiotensin-converting enzyme (ACE), a ubiquitously expressed protein, to form angiotensin II. Angiotensin II then binds to target organs leading to an increase in the thirst centers, arteriole vasoconstriction or aldosterone secretion from the adrenal cortex. Aldosterone acts as a steroid hormone to increase sodium reabsorption and potassium excretion at the distal tubule and collecting duct of the nephron

three decades by the therapeutic effectiveness of ACE inhibitors (Fig. 2.2) in treating hypertension, a significant risk factor for coronary disease, heart failure, stroke, and a host of other cardiovascular conditions [49]. Notably, several studies indicate that ACE mediates its role on blood pressure independent of the conversion of Ang-I to Ang-II. For example, Ang-II independent substrates like N-acetyl-seryl-aspartyl-lysyl-proline (AcSDKP), bradykinin, and Ang-(1–7), all processed by ACE, play

essential physiological roles in human disease [50]. When Zucker obese rats were fed a high salt diet and treated with AcSDKP, renal damage and inflammation, fibrosis, and systolic blood pressure were all reduced [51]. The potent endothelium-dependent vasoactive nonapeptide bradykinin was shown to lower blood pressure [52], and mice lacking the bradykinin B2 receptor displayed salt sensitivity, significant renovascular resistance, and decreased renal blood flow [53]. Finally, Ang-(1–7) exerts renoprotective effects in hypertension, diabetic nephropathy, glomerulonephritis, tubulointerstitial fibrosis, pre-eclampsia, and acute kidney injury [54].

Several studies also show a role for ACE in physiological processes related to immunity and metabolism. For example, macrophages or neutrophils genetically modified to force the expression of ACE show an increase of conferred resistance to methicillin-resistant *Staphylococcus aureus* and *Listeria monocytogenes* infections [55, 56]. The stability of Ang-II is relatively short; in plasma its half-life is 1–2 min, at which point peptidases degrade it into Ang-III and Ang-IV. Conversion to Ang-II by ACE causes extraordinary effects on the kidney, adrenal cortex, arterioles, and brain by binding to angiotensin type I (AT) and type II receptors [57]. Ang-II stimulates vasoconstriction in systemic arterioles, promotes sodium reabsorption in proximal convoluted tubules of the kidneys, and reduces the baroreceptor response's sensitivity to increased blood pressure so that this response would not counteract the effect of the RAS. It also induces the release of aldosterone from the adrenal cortex. Aldosterone then promotes sodium and water retention in the kidneys. In patients with hyperaldosteronism, called primary aldosteronism (PA), adrenal glands produce too much aldosterone affecting potassium and sodium balances in the body. Recently, gut microbiota alterations were investigated in 13 PA patients, 26 sex-matched primary hypertension patients, and 26 sex-matched healthy controls [58]. The study found that in addition to higher levels of body mass index and percentages of diabetes mellitus, the guts of PA patients had lower alpha diversity levels with far fewer short-chain fatty acid (SCFA)-producing genera (*Prevotella, Blautia, Coprococcus, Anaerostipes*, and *Ruminococcus*) and more inflammation-associated genera (*Megamonas, Sutterella,* and *Streptococcus*), as compared to healthy controls. These studies shed light on how disruptions of the microbiota can alter the interconnection between human cells and microbial communities affecting metabolic disorders regulated by the RAS.

RAS in Liver Disease

The effects of the RAS on SOT, in exerting immunomodulatory effects, extend to multiple target organs. To build a rationale for future therapeutic modulation of RAS in transplantation, the focus of this section will primarily be on the diseased liver, with the caveat that other pathophysiological diseased organs should be considered as well.

Common features shared in liver dysbiosis involve architectural distortions of the cellular matrix, capillarization of hepatic sinusoids, and elevated hepatic resistance resulting from inflammation and fibrosis. Deterrents to normal portal blood

flow, through hepatic resistance, can lead to portal hypertension [59]. Patients with advanced liver disease are affected by sodium and water preservation and inappropriate stimulation of the sympathetic nervous system, leading to the development of ascites, edema, hepatorenal syndrome, and hyperdynamic circulation [60]. Compensatory mechanisms for restoring functional blood volumes include stretching the portal vein and releasing the vasodilator nitric oxide, all of which the RAS plays an important role.

Although the intestinal microbiota and RAS axis share complementary functions, their role in liver disease is still far from clear. So far, it is known that components of the 'classical' RAS (e.g., renin, ACE, Ang-II and the AT_1 receptor) and 'alternative' RAS (e.g., ACE2, angiotensin Ang-(1–7) and the Mas receptor, MasR) are both upregulated in the liver [61]. These observations have led to the hypothesis that the 'classical' RAS axis contributes to the fibrotic process while the 'alternative' RAS components are necessary for restoring homeostasis [60]. Ang-(1–7) peptide is the main effector of the RAS counter-regulatory axis. It is known to have antihypertensive, anti-arrhythmic, and anti-trophic properties in vascular endothelial cells and has been shown to oppose the harmful activity of Ang-II [62]. It is formed by the ACE2 enzyme and acts through the G protein-coupled receptor encoded by the *mas* proto-oncogene [63].

Recent studies have exploited ACE2's potential to modulate Ang-(1–7) in animal studies and in the process hinted at its therapeutic potential. First, ACE2 overexpression led to the amelioration of biliary fibrosis in a mouse model that developed lesions resembling human primary sclerosing cholangitis [64]. This study was followed up by delivering ACE2 by gene therapy to mice fed a high-fat, high-cholesterol diet for 40 weeks in a model resembling nonalcoholic fatty liver disease (NAFLD) [65]. Their data showed that ectopic expression of ACE2 reduced pro-inflammatory cytokine expressions, hepatic stellate cell activation, collagen 1 expression cumulatively leading to less liver injury and fibrosis. Finally, when studies combined the role of Ang-(1–7) dependent MasR with the role the microbiota plays in liver physiology, it was demonstrated that genetic deletions of MasR led to increased TLR4, PI3K and AKT expressions that correlated with Bacteroidetes and decreased amount of Firmicutes. The treatment group showed increased glucose tolerance, impaired insulin sensitivity, and changes in intestinal microbiota, possibly due to lower neutral amino acids absorption by ACE2 [66]. Taken together, these studies on the microbiota, liver and the RAS axis show that considerations of a therapeutic benefit will need to first establish whether manipulation with antagonists of the 'classical' pathway or agonists of the 'alternative' pathway is the preferred treatment option.

OLT Modulates RAS

In OLT patients, the susceptibility of the graft to systemic hypotension and changes in metabolic liver function have been investigated. Before the transplantation procedure, the hemodynamics of advanced cirrhosis is characterized by increased systemic and

splanchnic vasodilation [67]. Often these patients have elevated circulating renin due to poor arterial blood flow to the kidneys in this setting of vasodilation [67]. The RAS can be altered significantly after transplantation, which is associated with the types of medications used for immunosuppression and the organ that was transplanted.

Hypertension is one of the most common cardiovascular complications after OLT [68]. While it is a common occurrence, the exact mechanism is still an area of research. In a study of 32 patients who underwent OLT, renin, aldosterone, and endothelin-1 (ET-1) were measured 3 times over a 6-month follow-up period [68]. All patients were normotensive before the transplantation. At 6-months, about 50% of the patients developed hypertension. There were no differences in demographic data, immunosuppression, rates of corticosteroid use, or rates of rejection in the groups that developed hypertension versus those that remained normotensive. In this study, they found renin levels actually decreased after OLT and that there were no differences in renin or aldosterone levels in the patients that developed hypertension after OLT versus those that remained normotensive after OLT. The authors, however, did find ET-1 to be significantly elevated in the hypertensive group as compared to the normotensive group post-OLT. These finding corroborate the research that is known with OLT and the RAS. As of yet, there is no evidence of stimulation of the RAS system after OLT within 1 year. In a study of 12 liver transplants, peripheral vascular resistance and cardiac index was normal after 2 weeks [67]. Furthermore, the elevation in renin, aldosterone and glucagon seen in liver failure were also lowered to more normal levels after transplantation [67]. However, studies have shown that the RAS system is elevated in patients as compared to control after 1 year [68, 69]. This suggests that the RAS may play an important role in the development of hypertension post OLT after 1 year, but within 1-year other mechanisms are likely at play, such as ET-1.

The type of immunosuppression also can have several effects on hypertension and the RAS system. Medications like azathioprine and mycophenolate mofetil have little to no effect on the RAS system [70, 71]. Glucocorticoids, which are often associated with hypertension, actually lead to lower RAS activation. The mineralocorticoid effect of glucocorticoids leads to sodium and fluid retention and increases the circulatory volume resulting in the suppression of the RAS system. Furthermore, steroid infusion in healthy controls have shown reduction in activated renin and in angiotensin II levels [71]. However, the main immunosuppressive drugs used in OLT are cyclosporin and tacrolimus. While both are calcineurin inhibitors, tacrolimus is associated with significantly fewer side effects than cyclosporin, including hypertension [70]. Cyclosporine is known to activate the RAS system through at least three mechanisms: (1) direct nephrotoxic effects that could lead to intrinsic renal ischemia, (2) directly leading to increase renin secretion by the renal cortex, and (3) increased sympathetic stimulation [70, 71]. While tacrolimus may have some of these effects on the RAS system, human and animal models have suggested that tacrolimus' main effect on hypertension is through the activation of sodium chloride cotransporter (NCC) [70, 72] and not necessarily through activation of RAS. Finally, a recent study demonstrated the effects of the novel hexapeptide VGINYW and its

effects on lowering blood pressure, oxidative stress and gut microbiota in spontaneously hypertensive rats [73]. Whether this peptide presents a therapeutic modality to immunomodulate SOT by lowering RAS-dependent hypertension remains to be determined.

RAS and Alterations in Immune Pathways and Gut Microbiome

The role of immunosuppressive medications on hypertension and the RAS system highlights the importance of the immune system in the development of hypertension and its effect on RAS. For example, animal models of hypertension have shown that blockade of the immune system by cyclophosphamide and mycophenolate leads to a reduction of blood pressure in spontaneously hypertensive rats [74]. Further studies in spontaneously hypertensive rats also showed that antibodies against toll-like receptor 4 (TLR4), a receptor involved in innate immunity, were able to ameliorate hypertension [75]. In renal infarct models, the suppression of antibody-mediated reactions as well as thymectomy led to improvements in hypertension [76]. Zhou et al. showed that complement deficiency prevented hypertension and renal injury by lowering the activation of the RAS system in a model of ureteral obstruction [77]. These data suggest that both inflammation and the immune system is important for the development and progression of hypertension.

An interesting area of research that connects the environment, the immune system, and hypertension have been the gut microbiome. The gut microbiome consists of trillions of cells and produces several highly active metabolites such as short-chain fatty acids (SCFAs) that have been implicated in many different diseases (Fig. 2.1). The gut microbiome is the nexus where the host immune system interacts with its environment. As mentioned earlier, TLR4 has been shown to progress hypertension in spontaneously hypertensive rats [75]. One of the key substrates to TLR4 is lipopolysaccharide (LPS), a significant component in the outer membrane of gram-negative bacteria. Immunosuppressants can directly alter the gut microbiome. In a large cohort of OLT patients, tacrolimus was associated with a decrease in *Bifidobacterium*, *Lactobacillus*, *Faecalibacterum prausnitzii*, and significantly higher *Enterobacteriaceae* and *Enterococcus* [78]. The direct effects of the gut microbiome and the RAS system are highlighted through the relationship between the RAS system and SCFAs. SCFA are produced from the fermentation of indigestible fiber by the gut microbiome. SCFAs have a multitude of effects, but their main effect is mediated by SCFA G-coupled protein receptors such as Gp41 and Gp43 [79]. These SCFA receptors are found in many different types of tissue, including the smooth muscle of renal arterioles [80]. SCFAs and their activation of Gp41 and Gp43 can directly promote inflammation in animal models [81]. However, animal models have also shown SCFAs to be directly related to renin release. Butyrate has been shown to suppress angiotensin II levels by inhibiting renal prorenin receptors and lowering

the activity of the renal RAS system [44]. Conversely, other SCFAs such as acetate and propionate have been shown to increase renin release through the activation of Gp41and other receptors such as SUCNR1 [82–84]. The effects of the gut microbiome on renin have also been highlighted in antibiotic-treated models and Gp41 knockouts [85].

But as much as the gut microbiome can affect the RAS system, the RAS system also has effects on the gut microbiome. For example, angiotensin II administration significantly alters the gut microbiome along with its circulating plasma metabolites [86]. Chronic infusion of angiotensin II in rats leads to hypertension, a decrease in bacterial diversity, and an increase in Firmicutes/Bacteroides ratio, a marker of dysbiosis seen often in patients with obesity and diabetes [86, 87]. This interconnection between the gut microbiome is highlighted by studies showing RAS activation leads to increased intestinal permeability and increased bacterial and metabolite translocation, which can further increase the RAS system [88].

RAS/Microbiome in Health and Disease

As mentioned earlier, hypertension is a common complication in post-transplant patients, and evidence suggests that the immune system and gut microbiome may be involved. The microbiome's role in RAS activation is highlighted in several disease states, including diabetic nephropathy, inflammatory bowel disease, and acute kidney injury. Several studies have linked the gut microbiome to the development of insulin resistance and diabetes [89–91]. A rat study performed by Lu et al. showed that SCFA production, specifically acetate, activates the RAS system leading to diabetic-induced early kidney injury [82]. Treatment of these rats with antibiotics led to lower acetate levels, inhibition of intrarenal RAS activity, and less kidney damage [82]. In recent animal studies, the addition of beneficial bacteria via probiotics, such as *Bifidobacterium longum*, was able to reduce insulin resistance, cholesterol, and increased expression of angiotensin-converting enzyme 2 (ACE2) and Mas receptor (MAS) in obese mice. This is important as activation of the ACE2-Mas axis can inhibit glucose uptake and improve insulin sensitivity in diabetes [92].

Like diabetes, inflammatory bowel disease also has a strong connection to intestinal dysbiosis and RAS. In general, an increased expression of renin activity increases the colon's susceptibility to inflammation [93]. Conversely, ACE inhibitor therapy can lessen chemically induced colitis [94]. The RAS system can promote inflammation by stimulating Th17 via JAK/STAT activation by angiotensin II [95]. Recent data have also suggested that the RAS system may promote autoimmunity through changes in Th1/Th17 [96]. In line with the connection of the RAS system and the microbiome, studies of a fecal transplant from inflammatory bowel disease patients induce a greater level of Th17 cells than a fecal transplant from normal donors [97]. In addition to changes in Th17 cells, activation of angiotensin 1 receptors can promote the translocation of leukocytes [85], and conversely, the blocking of

angiotensin II affects leukocyte adhesion by controlling the translocation of NF-κB into the nucleus, ameliorating colitis [98].

Lastly, while RAS activation may be harmful in chronic kidney disease, the inhibition of the RAS system is actually worse in acute kidney injury (AKI) [99]. This association between RAS inhibition and AKI has been highlighted recently with the COVID pandemic. In a recent meta-analysis, hospitalized COVID patients on RAS inhibitors had a higher increased risk of developing AKI than those not on RAS inhibitors. In line with these results, the administration of SCFAs, known activators of the RAS system, prevents AKI in an ischemia and reperfusion model [100]. However, this data does not mean that all bacteria are beneficial in AKI. Studies of both systemic antibiotics [101] and non-absorbable antibiotics [102] have shown to be of benefit in animal and human studies of AKI. This suggests that SCFA producing bacteria may have differing effects towards AKI as those bacteria that are more pro-inflammatory.

Future Considerations

As we learn more about the gut-organ axis (e.g., liver and kidney) and how it affects disease and even transplant outcomes, the gut microbiome may play an important role in prognostication and even treatment. If the associated changes in the gut microbiome precede RAS activation and disease outcomes, the gut microbiome could be a potential source of sensitive biomarkers that can predict outcomes such as transplant-related kidney injury or hypertension. And if these connections between the gut microbiome and RAS activity is causally related in disease outcomes, then altering the gut microbiome may present itself as an arena for novel therapeutics. However, the alteration of the gut microbiome can be complex. Commonly, the gut microbiome is altered either through dietary changes, antibiotics, probiotics, or fecal transplants. These methods usually have extensive effects on the entire microbiome community, changing the structure of both harmful and potentially beneficial bacteria. Research in bacterial phage therapy provides the opportunity of affecting the microbiome on a more specific approach. Phages are the natural predators of bacteria and are present in nearly every ecosystem [103]. While phage therapy has been successfully used in the treatment of multidrug-resistant bacterial infections [104], it is only recently has been examined as a tool for other diseases such as phage-directed immune response in allograft transplantation [103].

Conclusion

In conclusion, this review highlights the clinical importance of the RAS system on health and disease, including organ transplantation. Organ transplantation is a unique entity that connects the RAS to host immunity and the gut microbiome. It

shows the bidirectionality of the host immune system and the gut microbiome to the RAS system and vice versa, highlighting the important connection that the gut microbiome plays in the RAS system. This not only allows the gut microbiome to be a source of potential biomarkers to diseases related to the RAS system but also as a target for novel therapies, including targeted phage therapy.

Key points

- Efforts to increase the donor pool will depend on understanding cellular and molecular mechanisms that govern organ transplantation.
- Exploiting host-microbiota interactions with the RAS has great potential to improve transplantation outcomes.
- The effects of the RAS on organ transplantation have immunomodulatory effects that has not been well explored.
- Phage therapy is an emerging treatment option in organ transplantation.

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Chapter 3
Correlation Between Renin Angiotensin System and Infectious Disease

Antonio Vitiello, Francesco Ferrara, and Mariarosaria Boccellino

Abstract The renin- angiotensin system (RAS) is a physiological system that through a cascade of mediators and different mechanisms is able to regulate various functions of the body. The RAS is a cascade of mediators that includes two enzymatic pathways, the main or classical enzymatic pathway that involves renin converting angiotensinogen, released by the liver into angiotensin I (Ang I). Ang I is subsequently converted to angiotensin II (Ang II) by the angiotensin-converting enzyme (ACE). The other enzymatic pathway, termed nonclassical, consists of the ACE-2 enzyme that converts Ang II to angiotensin 1–7 (Ang 1–7) and Ang I to angiotensin 1–9 (Ang 1–9). The RAS exerts numerous local effects aimed at regulating tissue physiology and homeostasis. RAS can influence growth, proliferation, differentiation, and cell apoptosis, and also has effects on inflammation. RAS alterations are involved in many diseases, including atherosclerosis, diabetes mellitus, and viral infections, such as SARS-CoV-2. Therefore, it is crucial to investigate how the expression of the various components of RAS vary in tissues during infection disease and whether a change in their expression caused by drugs acting on RAS could have repercussions and correlations with infection at various stages.

Keywords Renin- angiotensin system · Angiotensin II · SARS-CoV-2 · Angiotensin 1–7 · Angiotensin 1–9 · Infectious disease

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Renin Angiotensin System (RAS)

The renin-angiotensin system (RAS) is a physiological system consisting of several enzymatic mediators with many important functions for the homeostasis of the body, such as regulation of blood pressure and electrolyte balance. The effects exerted in the body by RAS are both systemic and local. RAS can influence cell growth, proliferation, and apoptosis, and also has effects on modulation of inflammation. Alterations in RAS are involved in many diseases, including atherosclerosis, diabetes mellitus, and viral infections such as SARS-CoV-2. To date, two pathways of RAS activation are recognized, the so-called "classical" pathway and the "non-classical" pathway [1].

Classical Pathway RAS

The classic RAS pathway consists of successive enzymatic steps involving the conversion of the substrate Angiotensinogen (AGT) secreted by the liver, through several reactions, into the final active peptide product, namely Angiotensin-II (Ang-II), which exerts its biological effects by binding to its specific product, namely Angiotensin-II (Ang-II), which exerts its biological effects by binding to its specific membrane AT receptors. In the bloodstream, AGT is converted to angiotensin I (Ang-I) by renin, a proteolytic enzyme of the hydrolase class secreted by the iuxtaglomerular cells of the kidney, Ang-I is converted by the angiotensin-converting enzyme (ACE) into the peptide Angiotensin-II (Ang-II). The ACE enzyme has several other functions, such as the degradation and metabolization of vasodilator peptides, including bradykinin and kallikrein. Bradykinin is a peptide with several functions, including increasing vessel permeability, relaxing vessel muscle cells by giving vasodilation. Bradykinin also plays a very important role on pain and inflammation [2].

Non-classical Pathway RAS

The non-classical RAS pathway involves the conversion of Ang I to Angiotensin 1–9 (Ang 1–9) and the conversion of Ang-II to Angiotensin (Ang 1–7) through the action of ACE-2, which results in the formation of new peptide products with functional properties distinct from those of the ACE-Ang II-AT1R axis pathway normally associated with the maintenance of arterial pressure through hemodynamic and renal tubular mechanisms. Peptides formed from the nonclassical RAS pathway and by stimulation of AT7R or MASr receptors exert their biological effects. (Fig. 3.1).

In addition to Ang II and its receptors, the RAS has other important bioactive peptides and receptors, most of which have only recently been described, such as Ang

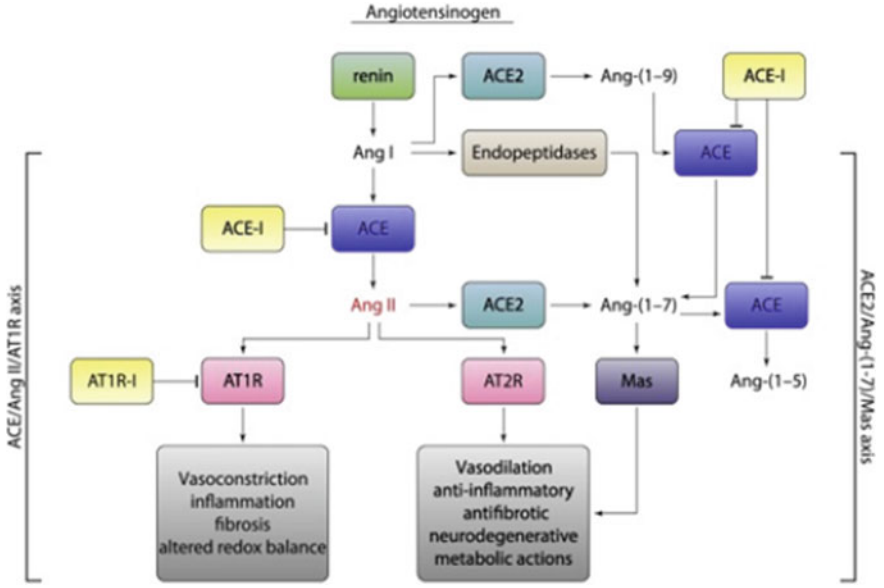

Fig. 3.1 Schematic representation of the RAS system and its components. The classical pathway through the ACE/Ang II/At1-r axis mediates inflammatory, vasoconstriction, and profibrotic effects, the nonclassical pathway through the ACE2/Ang (1–7)/MASr axis mediates opposite effects, anti-inflammatory, antifibrotic, and vasodilator effects

III, Ang IV, Ang- (1–7), Ang (1–9) the pro(renin) receptor, and the Mas receptor. Ang II and Ang III have the highest relative affinities for AT1R and AT2R, respectively, whereas Ang IV and Ang (1–7) bind only to AT2R. Ang III is the most potent endogenous AT2R agonist causing effects such as natriuresis [3].

Renin

Renin activates the first step of the RAS system, converting angiotensinogen, produced in the liver, into Ang I, which will finally be activated into the active molecule, Ang II, by the enzyme ACE. Renin is synthesized renally by the pericytes of the iuxtaglomerular apparatus near the afferent arteriole. The regulation of renin secretion is dependent on several factors, such as the reduction of renal perfusion pressure, the reduction of sodium concentration at the level of the distal tubule, the presence of noradrenaline secreted by sympathetic endings. The baroreceptors of the juxtaglomerular cells sense the increase or decrease in pressure by regulating renin secretion. The macula densa cells stimulate renin secretion by means of positive mediators such as prostaglandins or negative mediators such as adenosine, which stimulate or inhibit the juxtaglomerular cells, depending on the situation. Finally,

renin secretion occurs by direct stimulation of beta adrenergic receptors present in the kidney. Ang II acts in a feedback system leading to an inhibition of renin release. Interestingly, vitamin D3 and its receptor appear to play an important role in the complex regulation of renin transcription; it has been shown that there is an inverse relationship between plasma vitamin D3 levels and plasma renin activity and blood pressure. Furthermore, it has long been known that vitamin D3 supplementation lowers blood pressure in hypertensive subjects. Thus, the vitamin D3 receptor may constitute an important negative regulator of renin expression [4].

ACE

The angiotensin-converting enzyme (ACE) is an enzyme belonging to the carboxypeptidase family and plays a fundamental role in the regulation of the RAS system. Its main function is to convert Ang I into Ang II, in fact it catalyzes the cleavage of the decapeptide Ang I into the octapeptide Ang II, a potent vasoconstrictor. It is present both in the bloodstream and as a membrane glycoprotein localized on the endothelium of capillaries, mainly in pulmonary capillaries. Another important function of ACE is the degradation of bradykinin, a potent vasodilator and inflammatory mediator. ACE has another activity as an amyloid-degrading enzyme (ADE) that can hydrolyze beta-amyloid. Recent studies have suggested that the ACE gene is associated with a wide range of neurological diseases, including Alzheimer's disease, amyotrophic lateral sclerosis, multiple sclerosis, and Parkinson's disease. Some evidence has demonstrated a change in ACE expression during lung infections [5].

ACE2

The gene that transcribes the ACE2-converting enzyme is located on chromosome Xp22. The ACE2 enzyme is a carboxypeptidase, and is 40% identical in sequence with ACE. ACE2, hydrolyzes its substrates by removing a single amino acid from their respective C-termini. ACE2 thus has the ability to convert the decapeptide Ang I to Ang(1–9) and the octapeptide Ang II to Ang(1–7). Thus, the major role of ACE2 in angiotensin metabolism appears to be the production of Ang(1–7), whose actions oppose those of Ang II.

Several in vivo studies strongly support the concept that a major role of ACE2 is indeed the generation of Ang(1–7) from Ang II and that its conversion of Ang I to Ang(1–9) occurs under certain conditions that increase Ang II levels, such as during treatment with ACEi, with ARBs, or during pulmonary infections such as COVID-19. In addition to Ang I and Ang II, ACE2 also hydrolyzes apelin-13, neurotensin-(1–11), dynorphin A-(1–13), β-casomorphin-(1–7), and ghrelin. Still to be elucidated is the role of ACE2 on bradykinin metabolization. SARS-CoV-2 via the viral spike protein

and uses ACE2 for endocellular penetration. Studies show that ACE2 is present on endothelial cells and in smooth muscle cells of intra-myocardial vessels, in cardiac myocytes, and in pulmonary epithelium [6].

Angiotensin II

Ang II is an octapeptide hormone with multiple physiological functions such as pressor control and renal hydroelectrolyte control. Ang II is the most important effector component of the RAS system. Ang II acts through binding to two major classes of G-protein-coupled receptors, namely Ang II receptor subtype 1 (AT1R) and Ang II receptor subtype 2 (AT2R). The AT1R-type receptor mediates several biological effects, such as hypertension, endothelial dysfunction, vascular remodeling, hypertrophy, and inflammation. AT1R is a Gq protein-coupled receptor that after stimulation leads to activation of phospholipase C and phosphatidylinositol pathway. Although knowledge is constantly evolving, the activation of AT2R generally antagonizes the effects of Ang II binding on AT1R. At the cellular level, Ang-II modulates cell contraction, growth, differentiation, and apoptosis; it can also promote cytokine production, adhesion molecule expression, chemotaxis, and macrophage activation. Increased Ang-II formation induces inflammation, and Ang-II is itself a potent proinflammatory cytokine as well as a growth factor. It also activates the transcriptional factor nuclear factor (NF)-κb, a key factor in the nuclear transcription of proinflammatory and fibrotic molecules, including IL6 and IL1, which are responsible for the cytokine cascade [1].

Ang (1–9), Ang (1–7)

Angiotensin-(1–7) is a vasoactive peptide of the RAS, which is generated primarily by the ACE2-converting enzyme from Ang I and exerts its actions through activation of the Mas receptor. The Ang-(1–7)/ACE2/Mas axis is now considered a major mechanism, counterbalancing the vasoconstrictive actions of the classical RAS, which includes renin, ACE, Ang II, and its receptors AT1 and AT2 (Fig. 3.1). Activation of MASr induces vasodilating, antifibrotic, and anti-inflammatory effects. Ang (1–9) is formed via mediation of ACE-2 from Ang I, exerts different effects in vivo and in vitro independently of Ang 1–7 mediated MasR activation through stimulation of AT2 receptors (AT2-r) with antifibrotic, anti-inflammatory, vasodilator, and antiproliferative effects. The activation of MASr and AT2-R contributes to the reduction of endothelial dysfunction and to the reduction of Ang II-mediated atherosclerosis development. To date, a full understanding of the effects of Ang remains under investigation (1–9) [6].

RAS-Acting Pharmacological Agents

Therapeutic agents that act on the RAS system mainly belong to three pharmacological classes, ACE inhibitors (ACE-i), Angiotensin II receptor antagonists (ARB or Sartans) and direct renin inhibitors (DRi). These drugs have been used for many years with proven clinical efficacy and excellent tolerability for various cardiovascular diseases, such as hypertension, heart failure, ischemic heart disease. ACE-i inhibiting the ACE enzyme block the conversion of Ang I into Ang II. Decrease of Ang II concentration at AT1r receptors causes drop in blood vessel tone, decrease in blood pressure, reduction of aldosterone release from adrenal cortex. At the cellular level, a regression of the mitogenic effects, mediated by angiotensin II, on fibroblasts and myocytes of the heart can be observed, which especially after a myocardial infarction lead to unfavorable alterations (ventricular remodeling). ARBs or Sartans are AT1r receptor antagonist drugs, thus blocking the biological effects of Angiotensin II. Finally, direct renin inhibitors (Aliskiren) block the conversion of Angiotensinogen into Ang I. Recently, several pharmacological agents that modulate the RAS system with mechanisms of action different from the three pharmacological classes described, such as the administration of ACE-2 soluble, are being tested. The rationale for this line of research is the increased stimulation of the RAS pathway leading to the synthesis of Ang (1–7) and Ang (1–9) with effects opposite to the stimulation of AT1-r receptors by Angiotensin II [7].

RAS and Infectious Lung Disease

The circulating renin-angiotensin system (RAS) has a well-described role in circulatory homeostasis. Recently, local RAS at the tissue level have also been described and appear to play a key role in the injury/repair response from viral infections. The RAS system and its components are abundantly expressed in the respiratory tract. Evidence shows that in certain clinical situations, such as in cases of severe COVID-19 infection, modulation of RAS components, and in particular of ACE-2, occurs as a response to lung injury. Modulation of local RAS in the pulmonary pathways could play a key role in influencing the pathogenesis of lung injury through a variety of mechanisms directed at modulating vascular permeability, vascular tone, inflammation, fibroblast activation, and profibrotic mediators. The role of ACE2 as a counterregulatory mechanism of ACE may therefore be crucial in the lung. ACE2 is present in alveolar epithelial type I and II cells and to a lesser extent in bronchial epithelial cells. In addition, as in other organs, ACE2 is present in endothelial cells and arterial smooth muscle cells. With regard to the possible role of ACE2 in the lung in relation to acute lung injury in particular, the relationship to ACE2 expression at the alveolar capillary interface is of interest. We speculate that increased ACE2 may play a role in reducing the initial leakage at the alveolar capillary interface. This would slow the vicious cycle that often occurs after a damaging effect at this

interface and leads to the pathological clinical picture of diffuse alveolar damage with intra-alveolar edema and fibrin deposits. These data support a critical role of intrapulmonary RAS in the pathogenesis of acute lung injury and show that ACE2 is a key molecule involved in the development and progression of acute lung failure. Ultimately, the RAS system in the lungs is critical for the regulation of inflammation, oxidative stress, immunity, apoptosis, fibrosis, and other physiological activities [8].

RAS Dysfunction and Development of Acute Lung Injury (ALI)

RAS dysfunction is related in some aspects to the development of acute lung injury (ALI) and acute respiratory distress syndrome (ARDS), which can cause severe lung injury and lead to death. ALI can have several causes, such as viral infections caused by SARS-CoV, SARS-CoV-2, H5N1, H7N9. In particular, the SARS-CoV and SARS-CoV-2 Coronaviruses enter the host lung epithelial cells via binding to ACE2 membrane receptor. These viral infections can interfere with RAS function, leading to acute lung injury (ALI) and acute respiratory distress syndrome (ARDS). When the virus invades the respiratory tract, epithelial cells and vascular endothelial cells are first damaged and protein-rich edema fluid accumulates in the pulmonary interstitium and alveoli, triggering various immune cells such as macrophages and neutrophils to release a large number of inflammatory factors. When RAS homeostasis is disrupted, a storm of cytokines appears, eventually resulting in ALI. Some viral infections can also directly regulate the expression of RAS components by accelerating RAS imbalance and the appearance and development of ALI/ARDS. RAS dysfunction induces ALI primarily by triggering a cascade of downstream reactions. Ang II binding to AT1R activates NF-κB, AK2/STATs, and MAPK pathways by inducing inflammation and stimulating the production of reactive oxygen species (ROS), promoting cell apoptosis. Increased Ang II concentrations in the lungs thus induce increased numbers of cytokines, chemokines, and ROS that exacerbate lung damage caused by viral invasion. Pulmonary edema, pulmonary fibrosis, and lung cell apoptosis are related to the change in RAS [9].

Role of RAS in ALI Caused by Avian Influenza Virus Infections H5N1 e N7N9

Avian influenza virus subtype H5N1 is a highly pathogenic virus that was first detected in humans in 1997 in Hong Kong; in 2003 and 2004, this strain of avian influenza virus spread from Asia to Europe and Africa causing numerous deaths. The main risk factor for infection of the human species appears to be direct or indirect exposure to poultry (live or dead). In some categories of patients such as the

elderly or individuals with comorbidities, infection with this virus can have an unusually aggressive course, with rapid deterioration and a high mortality rate. Infections caused by avian influenza viruses often lead to severe ALI and can even progress to ARDS. H7N9 avian influenza virus infection was first detected in humans in 2013. In the case of N7N9, most patients go on to develop pneumonia or respiratory distress syndrome. Histological examination of patients who have died from avian influenza show features of ARDS and multiple organ dysfunction syndrome. Some studies have suggested that ALI caused by avian influenza virus is related to RAS dysfunction. Indeed, evidence shows that the level of ACE2 protein in lung tissue decreases rapidly after H5N virus infection and correlates with a substantial increase in Ang II in plasma. In contrast, no change occurs for the expression level of ACE. Evidence has shown that the RAS is deranged after H5N1 infection, and the level of Ang II in plasma can be used as one of the clinical indicators to assess the severity of H5N1 infection. The severity of avian influenza infection is associated with cytokine storm. H5N1 infection dysregulates the pulmonary RAS, decreases the concentration of ACE-2, and increases the expression of Ang II to stimulate the AT1 receptor, thereby inducing the activation of downstream inflammatory signals, forming a cytokine storm, and ultimately causing ALI, also ARDS [10, 11].

Hand, Foot and Mouth Disease (HFMD)

Hand-foot-mouth disease (HFMD) is an infection caused by several viruses, such as coxsackievirus A16, the enterovirus. The infection results in a vesicular rash on the hands, feet, and oral mucosa. Some patients may rapidly progress to severe HFMD, and develop encephalitis meningitis, neurogenic pulmonary edema (NPE) that can cause ALI. As with avian influenza, there appears to be a correlation with RAS dysfunction in severe HFMD. Indeed, the RAS may be involved in the systemic inflammatory response induced by HFMD virus infection. Some evidence shows that Ang II concentrations in the plasma of severely ill children with HFMD are substantially higher than those of the control group. The higher Ang II concentration and increased AT1-r receptor stimulation may also be associated with the formation of pulmonary edema. Indeed, increased Ang II activates downstream signals, increases water and sodium retention, promotes capillary contraction, and increases blood pressure, all of which are important in the development of HFMD in NPE by inducing lung damage. Furthermore, as in avian influenza, dysregulation of RAS may be associated with the hyperinflammatory state with massive release of proinflammatory mediators that occurs in severe forms of HFMD [9, 12].

Role of RAS in ALI Caused by SARS-CoV Coronavirus Infections

In 2003, Severe Acute Respiratory Syndrome (SARS) was first reported in China and then spread around the world causing many deaths and many infected. The coronavirus called severe acute respiratory syndrome coronavirus (SARS-CoV) is the pathogen of this outbreak. SARS-CoV infection may have an asymptomatic course, or in some cases cause severe forms of infection particularly in immunocompromised patients. The ACE2 protein is the functional receptor of endocellular penetration of SARS-CoV. Evidence shows that the concentration of ACE-2 is dramatically reduced in the viral replication stages due to virus-receptor interaction. Meanwhile, Ang II level in lung tissues increases considerably. Ultimately, SARS-CoV infects people through the combination of its S protein and pulmonary ACE2, reducing the level of ACE2 in the lungs, increasing Ang II expression, and thereby hyperactivating AT1 receptor-mediated biological effects causing inflammation and fibrosis. The unregulated immune/inflammatory response causes the expression of proinflammatory, prooxidant, and profibrotic mediators. Inflammatory pathways, apoptosis, oxidative stress, and fibrosis are all hyperactivated in patients with SARS. Finally, many cytokines, chemokines, and proteases are released, resulting in a cytokine storm and eventually severe ALI/ARDS or even death [13, 14].

Role of RAS in ALI Caused by Coronavirus Infections SARS-CoV-2

Coronavirus Disease 2019 (COVID-19) caused by SARS-CoV-2 infection is responsible for the current global COVID-19 pandemic. To date, data indicate approximately 6,225,063 Mln deaths. The infection can have an asymptomatic, mild symptomatic, and severely symptomatic course with severe lung injury and lead to death. Severe lung injury is primarily caused by the cytokinetic storm that occurs, leading to a hyperinflammatory state and multi-organ dysfunction. Similar to SARS-CoV, the S protein of SARS-CoV-2 utilizes the cell membrane ACE2 for endocellular penetration. ACE2 is distributed widely in the human body; in fact, it is present in the testes, brain, vessels, lungs, and intestines. Single cell sequences showed that alveolar type II (AT2) cells are the major cell type expressing ACE2 in all human lung cells and show functions in viral replication and transmission compared with AT2 cells not expressing ACE2. This phenomenon may partially explain the severe alveolar damage of SARS-CoV-2 infection. ACE-2 is expressed both on the cell surface (in bound form) and in plasma and urine (soluble form). Evidence shows that membrane ACE-2, during SARS-CoV infection, is internalized into the cytoplasm upon virus binding. Evidence shows that decreases in ACE-2 occur in severe COVID-19 patients. This variation could likely be one of the causes of the damage caused by COVID-19. As described, decreased expression of ACE-2 leads to a decrease in

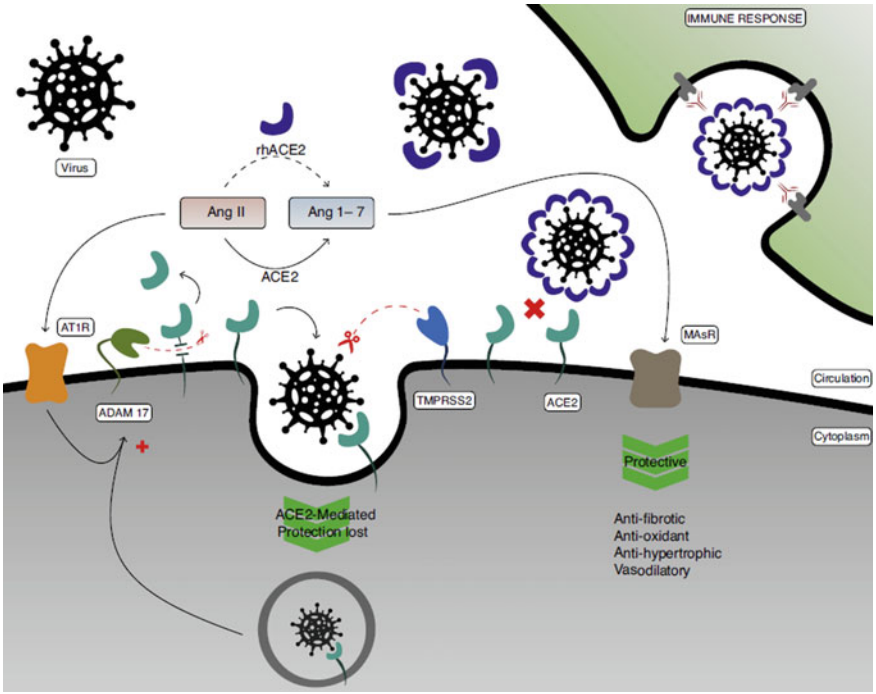

Fig. 3.2 Pathogenesis of the renin-angiotensin system in acute lung injury caused by viral infections. SARS-CoV and SARS-CoV-2 bind to angiotensin-converting enzyme 2 (ACE2) membranes, facilitating fusion of viral and cellular membranes. Occupation of ACE2 by the virus leads to reduced levels of ACE2 on the cell surface. When the virus enters the cell, not only SARS-CoV and SARS-CoV-2, but also H5N1, H7N9, angiotensin II (Ang II) levels are significantly increased, a large amount of Ang II binds to the angiotensin II type 1 receptor (AT1R), activating proinflammatory,prooxidant and profibrotic signaling pathways

the ACE2-Ang (1–7)-MasR axis and an increase in Ang-II (Fig. 3.2). The imbalance between Ang 1–7 and Ang II leads to the prevalence of biological effects of Ang II such as profibrotic, proinflammatory vasoconstriction, and procoagulation. Biological effects may be responsible for the pulmonary and cardiac injury that can occur in severe COVID-19 patients [15, 16].

Could A Pharmacological Approach That Modulates the RAS be of Clinical Benefit to Combat Lung Damage from Respiratory Infections?

The three different pharmacological classes modulating the RAS have different effects on the regulation and enzymatic expression of the RAS and its components.

In fact, based on in vitro studies, it appears that the use of ACEIs, with a blockade of the ACE enzyme, increases the expression of the ACE-2 enzyme (by stimulating other synthesis pathways). The use of ARBs appears to be linked to a slight increase in the expression of both ACE and ACE-2. Finally, the use of DRIs, by inhibiting the upstream RAS cascade, appears to inhibit expression and consequently decrease ACE and ACE-2 concentrations. This shows that there are agents available that can act with different modulation of the expression of RAS and its components. This evidence suggests that the RAS could be modulated by a pharmacological approach to avoid pulmonary complications of the viral infections described above. Indeed, an increase of ACE-2 in the most severe stages of viral infection could be of benefit, with stimulation of Ang 1–7 and Ang-1–9 synthesis with vasodilator, anti-inflammatory, and antifibrotic properties [17].

Conclusions

Pneumonia caused by viral infection is a major threat to human health. The RAS plays an important role in lung damage caused by viral infection, and RAS dysfunction is key to the occurrence and development of ALI/ARDS in patients with viral infections. Dysregulation of the RAS can lead to a dysregulated inflammatory/immune system and the massive synthesis of many cytokines, chemokines, and proteases that act together to induce ALI or even ARDS. Larger epidemiological studies should be designed to investigate the true correlation between RAS and pulmonary viral infections such as COVID-19, in particular to also clarify whether an increase in ACE-2 induced by administration of pharmacological agents in the most severe stages of infection may play a protective role.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish The authors consent to the publication of the manuscript.

Authors Contributions AV: Conceptualization, Writing—Original Draft.
FF: Writing—Review & Editing, Supervision.
MB: Validation, Reviewing and Editing.

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Chapter 4
Emerging Contributions of Endocrine Pathophysiology in Virus-Related Infectious Disease: Focus on the RAAS in COVID-19 and HIV

Teressa S. Thomas and Suman Srinivasa

Abstract Aside from its traditional role in maintaining vascular hemodynamics and sodium balance, the renin–angiotensin–aldosterone system (RAAS) is established to have novel roles in inflammatory pathways, adipose biology, and cardiometabolic derangements. Hormone systems facilitate system-wide cross-talk in biologic processes, and in this way may have key functions in non-endocrine related diseases. Knowing that the RAAS may influence inflammation, it is not surprising that this system may be implicated in infectious diseases in which inflammation is inherent to the pathogenesis. In this regard, the RAAS may have important contributions to viral-related infectious diseases, in particular SARS-CoV-2 and HIV. Many parallels can be drawn between the complications of these viruses, especially related to inflammatory and cardiovascular sequelae, and one potential mechanism linking the two viruses could be RAAS dysregulation. In SARS-CoV-2, the virus is dependent on the ACE2 working as a functional receptor for viral entry, and following viral entry, downregulated ACE2 may contribute to a pro-inflammatory milieu and worsened respiratory disease. In HIV, excess RAAS activation has been linked to visceral adiposity, insulin resistance, inflammation, and reduced natriuretic peptides. In this review, we discuss what is known about the RAAS and the pathogenesis of SARS-CoV-2 and HIV, highlight potential RAAS-directed therapeutic strategies in both SARS-CoV-2 and HIV-associated complications, and consider future directions to enhance our understanding of RAAS dysregulation in infectious disease.

Keywords Renin–angiotensin–aldosterone system · Virus-related infectious disease · SARS-CoV-2 · HIV

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Introduction

The renin–angiotensin–aldosterone system (RAAS) is an important endocrine feedback system that maintains homeostasis of intravascular volume and sodium balance. The RAAS interacts with other important networks, such as kinins and natriuretic peptides, that are integral to vascular hemostasis and natriuresis. Preclinical studies support an additional role of the RAAS as a mediator of inflammatory and metabolic complications. Emerging clinical studies have examined the relationship of the RAAS in HIV infection and demonstrate unique physiology. With the onset of the coronavirus disease 2019 (COVID-19) pandemic, additional clinical studies are emerging that highlight the relationship of the RAAS to SARS-CoV-2. Many parallels can be drawn between the complications of HIV and SARS-CoV-2, especially related to inflammatory and cardiovascular sequelae, and one potential mechanism linking the two viruses could be RAAS dysregulation. RAAS blocking agents are well-known to be cardio- and reno-protective in populations such as those with reduced ejection fraction post-myocardial infarct or those with diabetes. Expanding our knowledge of RAAS-mediated cardiometabolic and inflammatory complications in both SARS-CoV-2 and HIV may provide evidence for the additional utility of RAAS blockade in infectious disease. Herein, we outline what is known about both HIV and SARS-CoV-2 with regards to RAAS-related mechanisms and therapeutic strategies and future implications of this knowledge.

Understanding the RAAS in Health and Dysregulated States

The renin–angiotensin–aldosterone system (RAAS) is a complex hormone network important to the homeostatic control of intravascular volume and blood pressure. A cascade of substrates is sequentially upregulated or downregulated and work to counterbalance one another through both the classical and alternative pathways (Fig. 4.1). The RAAS is also closely integrated with other systems in regulating physiologic processes. These systems extend to natriuretic peptides (NPs), the kallikrein kinin system (KKS) and glucocorticoids, which may impact the homeostasis of processes, including coagulation, inflammation, fibrosis and glucose and lipid metabolism. Perturbation of the RAAS and these related systems may result in diseased states. Drawing upon knowledge of this dysregulation, physiological manipulation to modify this dysregulation has the potential to improve adverse consequences.

The initial substrate in the RAAS is renin, a protease secreted by the juxtaglomerular apparatus of the kidney. Renin initiates the sequence by cleaving angiotensinogen, derived by hepatic production, to angiotensin I (Ang I). Angiotensin converting enzyme (ACE) is ubiquitous, though abundantly expressed in the endothelium particularly the pulmonary vascular bed and the small intestine and converts Ang I to the octapeptide angiotensin II (Ang II) in the classical pathway. The primary

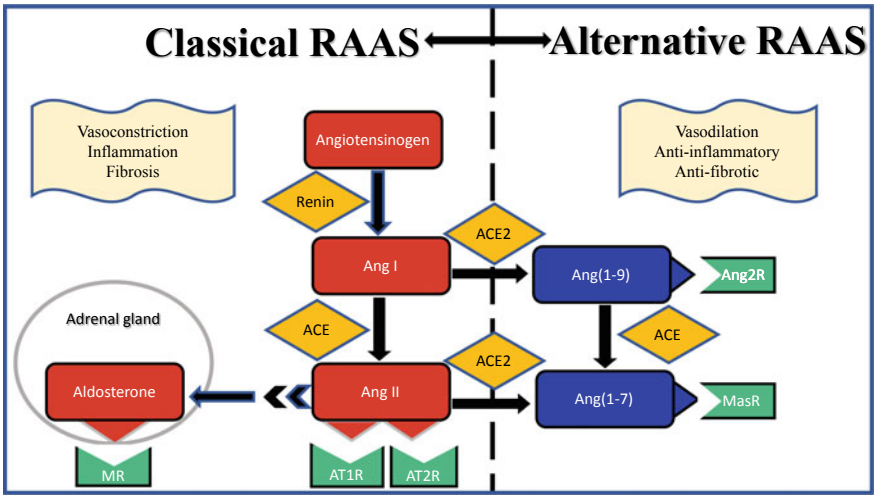

Fig. 4.1 Cascade of substrates forming the classical and alternative RAAS pathways. The RAAS is composed of the classical and alternative pathways. In pathologic states, excess RAAS activation may contribute to deleterious processes along the classical pathway, including vasoconstriction, inflammation, and fibrosis. If substrates are shunted towards the alternative pathway, this may contribute to protective processes, including vasodilation and anti-inflammatory and anti-fibrotic effects. Color coding [red- classic pathway substrates, blue- alternative pathway substrates, yellow- co-factors/co-enzymes, green- receptors]. RAAS = renin–angiotensin–aldosterone-system, Ang I = angiotensin I, Ang II = angiotensin II, Ang(1–9) = angiotensin (1–9), Ang(1–7) = angiotensin (1–7), ACE = angiotensin converting enzyme, ACE2 = angiotensin converting enzyme 2, AT1R = angiotensin II type 1 receptor, AT2R = angiotensin II type 2 receptor, MasR = Mas receptor, MR = mineralocorticoid receptor

actions of Ang II binding to the angiotensin II type 1 receptor (AT1R) are vasoconstriction to maintain vascular tone and stimulation of the adrenals to produce aldosterone. In excess, Ang II can result in oxidative damage, cellular proliferation, fibrosis, and inflammation [1]. Aldosterone itself, in pathological states, through activation of the mineralocorticoid receptor (MR) has been linked to cellular hypertrophy and fibrosis. These changes of hypertrophy and fibrosis have been demonstrated in rodent models following infusion of Ang II to renin/angiotensin double transgenic mice with MR blockade reversing the adverse remodeling [2, 3]. Taking all of this into account, when the classical pathway predominates, an excess of Ang II and/or aldosterone mediates vascular and myocardial damage.

The discovery of the zinc metalloprotease ACE2 was pivotal in our understanding of the RAAS and more recently, the pathophysiology of COVID-19. With 42% homologous identity to ACE, ACE2 is also found to be widely distributed in endothelial tissues (myocardium, lung, vascular smooth muscle, kidney, testis and the gastrointestinal tract) [4]. The predominant role of ACE2 is the conversion of Ang(1–9) from Ang I, but ACE2 has a higher magnitude of enzyme efficiency for

catalysis of Ang II to Ang(1–7). ACE2 is a key regulator against Ang II overexpression. ACE2 can exist as membrane-bound or in secreted form. This protein spans the cell membrane with an intracellular COOH-terminal tail and an extracellular NH_2-terminal domain which can be truncated at its catalytic site by ADAM-17, producing soluble ACE2 (sACE2). sACE2 has similar function, but more attenuated function compared to membrane-bound ACE2 within the alternative RAAS pathway [5].

The cornerstone of the counterregulatory response or alternative pathway is the heptapeptide Ang(1–7). Ang(1–7) binds to the G-protein coupled Mas receptor (MasR) forming the Ang(1–7)/MasR complex, the dominant effects of which are vasodilatory, anti-inflammatory and anti-proliferative. Ang(1–7) is formed by two catalytic reactions: cleavage of Ang II by ACE2 and conversion of Ang(1–9) to Ang(1–7) by ACE. Ang(1–9) binds to the angiotensin II type 2 receptor (AT2R) stimulating Ang II counterregulatory effects (vasodilation and anti-proliferative effects), although it remains to be elucidated if this role occurs in normal health and/or primarily as a compensatory action in pathological states [6].

Aberrant RAAS signaling may promote inflammation and fibrosis of the cardiovascular system. Increased aldosterone and MR expression can lead to myocardial fibrosis and vascular remodeling in animal and human studies, translating clinically to phenotypes of vascular stiffness and diastolic and systolic heart failure [7]. Infusion of aldosterone leads to increases in circulating monocyte chemoattractant protein-1 (MCP-1), osteopontin, plasminogen activator inhibitor-1(PAI-1) and ICAM causing myocardial injury in rats [8]. In further preclinical studies, AT1R activation has been found to propagate inflammation and fibrosis through NF-κB and TGF-β pathways [9, 10]. Angiotensin receptor blockers (ARBs), by inhibiting TGF-β, may be clinically beneficial in several conditions for their anti-fibrotic properties. ARBs have been utilized to reduce progression of aortic dilatation in Marfan syndrome, to prevent renal interstitial fibrosis in a variety of renal diseases including after renal transplant, and as anti-remodeling agents in heart failure with reduced ejection fraction [11–13].

There is evidence to suggest that the RAAS has local effects, in addition to systemic functions. Individual components of the RAAS can be produced within adipose tissue. The adipose tissue RAAS through autocrine signaling may drive adipocyte differentiation, modulation of the secretion of proinflammatory adipokine and localized inflammation [14]. Ang II can be independently secreted in the adipose depot, derived from angiotensinogen overexpression or driven by leptin production within adipose tissue. Obesity and excess visceral adipose tissue (VAT) are associated with overactivation of Ang II and aldosterone, which may contribute to obesity-induced hypertension and other cardiometabolic sequelae [15, 16]. Furthermore, RAAS components have been found on immune cells. The RAAS may influence the immune system, as MR and AT1R are expressed on macrophages and T lymphocytes and can promote pro-inflammatory M1 and T helper-1/T helper-17 phenotypes upon activation, respectively [17].

Associations with other systems, including the KKS, glucocorticoids and NPs, are worth noting to enhance understanding of RAAS dysregulation in infectious disease. The KKS consists of biologically active kinins that are activated by kallikreins.

Bradykinin, the main kinin of the KKS, potentiates vascular permeability and vasoconstriction which can mediate tissue damage and is upregulated by proinflammatory cytokines [18]. ACE is responsible for the degradation of bradykinin to bradykinin(1–5), a thrombin-induced platelet aggregation-inhibitory peptide [19]. Des-Arg^9 bradykinin (DABK), another kinin with similar function to bradykinin, can be inactivated by ACE2. However, in the presence of reduced ACE2, increases in DABK may propagate lung inflammation and injury [20]. Kallikreins are associated with further coagulation-promoting effects, which require factor XII for their activation, and then in turn potentiate factor XII-induced inflammation and intrinsic coagulation pathway activation [21].

Glucocorticoids also interact with the RAAS and modulate MR activation. Cortisone is converted to cortisol by 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1), and cortisol is reciprocally inactivated by 11β-HSD2. 11β-HSD1 expression is enhanced by pro-inflammatory cytokines and by hyperglycemia. The type 1 enzyme has been associated with elevated lipids, fat redistribution, atherosclerosis and insulin resistance. Cortisol has a high affinity for the MR. Cortisol-induced activation of the MR is protected by the conversion of cortisol to cortisone. On the contrary, in states of compromised 11β-HSD2 activity, cortisol may oversaturate the MR.

Important endocrine hormones of the heart are secreted in the form of two natriuretic peptides, atrial NP (ANP) and B-type NP (BNP), which have significant natriuretic and kaliuretic properties. The NP system is engaged in cross-talk with the RAAS, and the two systems feedback on each other. NPs suppress the classical RAAS pathway by downregulating renin and aldosterone production and inhibiting Ang II/AT1R functions. Furthermore, in the alternative pathway, Ang(1–7) promotes ANP secretion [22]. In this way, NPs may be cardioprotective and oppose cardiac remodeling through anti-fibrotic and anti-proliferative effects. Dysregulation of these important interactions between the RAAS and related systems have implications in COVID-19 and HIV infection, which will be subsequently discussed.

Traditional Use of RAAS Blockers

A dysregulated and overactive RAAS is central to many metabolic disorders. Breakthroughs in treating cardiovascular and renal diseases have occurred with the advent of therapeutics aimed at RAAS blockade. These RAAS blocking agents demonstrate both traditional and non-traditional actions including lowering of blood pressure, natriuresis, and anti-proteinuric, anti-proliferative and anti-fibrotic effects [23].

Angiotensin-converting enzyme inhibitors (ACEis) block ACE and reduce Ang II levels directly. ACEis are first line therapy for hypertension and heart failure with reduced ejection fraction, and have been shown to reduce left ventricular hypertrophy and all-cause mortality in hypertension [23]. Angiotensin receptor blockers (ARBs) block the AT1R and increase Ang II, which shunts Ang II into the alternative counterregulatory pathway [Ang(1–7)/MasR], another mechanism providing

benefit [1]. ACEis and ARBs have non-specific antiproteinuric effects, which enable their use in several renal diseases to preserve renal function and delay initiation of renal replacement therapy. Spironolactone, eplerenone and the newer non-steroidal finerenone are MR antagonists that are used in hypertension, heart failure to prevent adverse cardiac remodeling, and ascites due to liver cirrhosis. They exhibit beneficial effects on blood pressure reduction and natriuresis, and other pleomorphic effects as an anti-fibrotic and anti-inflammatory agent [24, 25].

Non-traditional uses of RAAS blockers are emerging, particularly in inflammatory and infectious etiologies, and is the focus of emerging research. Concurrent manipulation of the related systems (KKS, NPs, and glucocorticoids) could be synergistic and are also being explored [23]. The increase in cardiovascular disease (CVD) in HIV and COVID-19 may be uniquely linked to the RAAS and will be the focus of discussion of this chapter.

Where SARS-CoV-2 and HIV Meet

Many parallels can be drawn between HIV and SARS-CoV-2 infections. Both viruses have given rise to devastating pandemics that have impacted the world globally and have prompted numerous investigations to understand mechanisms of virulence and effective treatment strategies. Aside from acute illness, both viruses present with chronic long-term sequelae, even after viremic resolution of SARS-CoV-2 or virologic control of HIV, respectively. HIV and SARS-CoV-2 infections both promote an inflammatory milieu, and persistence of this inflammation serves as a key mediator of organ dysfunction and mortality. In particular, long-term cardiovascular sequelae may remain after the initial infection. While several mechanisms are being investigated, one potential mechanism for these CVD complications is RAAS dysregulation (Fig. 4.2).

Insights into the mechanisms of cardiometabolic disease in HIV have been ongoing and may identify RAAS dysregulation as a driver of this persistent inflammation. A common pathophysiologic mechanism may be an ACE2 deficiency, which has been identified in COVID-19 and needs to be explored further in HIV [7]. RAAS blockers have been investigated in both viruses to understand their potential utility as a physiologically-targeted therapeutic strategy. Understanding the specific perturbations in RAAS physiology within each viral infection may broaden our insight into potential therapeutic strategies. We will focus on the available literature supporting the role of the RAAS in infectious disease, specifically related to SARS-CoV-2 and HIV.

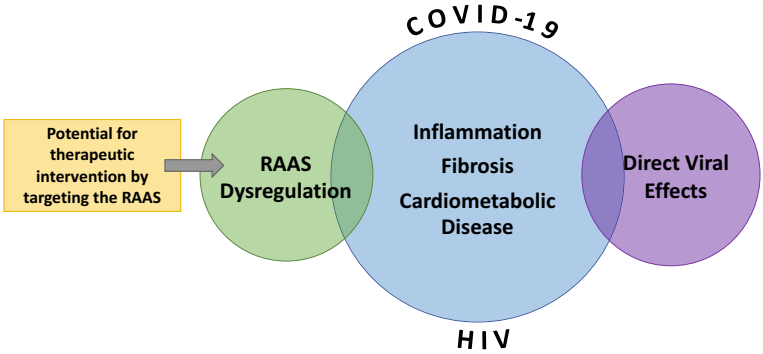

Fig. 4.2 Pathophysiological overlap between COVID-19 and HIV includes an important endocrine system. Individuals infected with COVID-19 or HIV are similarly at risk for inflammation, fibrosis and cardiometabolic disease. Potential underlying mechanisms may include direct cytopathic viral changes and/or RAAS dysregulation in both HIV and COVID-19. If RAAS dysregulation is implicated in HIV or COVID-19 infections, therapeutics which target the RAAS may be of clinical utility to decrease inflammation, fibrosis, and cardiometabolic disease. RAAS = renin–angiotensin–aldosterone-system

SARS-CoV-2 and the RAAS

RAAS Dysregulation and Organ Dysfunction in COVID-19

Preceding the COVID-19 pandemic, available literature described a link between viral pneumonias and the RAAS. Meta-analyses found an association between ACEi use and an attenuated severity of pneumonia [26, 27]. Avian influenza A (H7N9), initially discovered in 2013 after causing a lethal respiratory illness was declared an epidemic by 2016. Preclinical mouse models revealed that ACE2 deficiency resulted in severe acute respiratory distress syndrome (ARDS) in H7N9 [28]. A retrospective review of H7N9-affected patients in China showed that Ang II plasma levels correlated with mortality, better than other indices, such as C-reactive protein and $PaO2/FiO_2$ ratio (partial pressure arterial oxygen/fraction of inspired oxygen), in this cohort [29]. Respiratory syncytial virus (RSV)-induced acute lung injury was found to be similarly associated with ACE2 deficiency and could be alleviated by recombinant ACE2 (rhACE2) infusion in mouse models [30]. Furthermore, losartan, an ARB, reduced lung injury in Avian influenza A (H5N1)-infected mice [31].

Similarities exist between SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV) to the novel coronavirus SARS-CoV-2. These similarities include identical genetic sequence of SARS-CoV-2 (80% and 50% for SARS-CoV and MERS-CoV, respectively), parallel modes of viral entry, and secondary cardiovascular comorbidities accounting for worsened morbidity and mortality [32, 33]. Infectivity and mortality with SARS-CoV-2, however, far outweigh the prior pandemics. The coronaviruses are so-named for the projections on their cell surface,

the spike glycoproteins (S proteins) [34]. Analogous to SARS-CoV, SARS-CoV-2 establishes viral entry through binding of the N-terminal domain of the S protein to membrane-bound host ACE2 receptor. MERS-CoV also utilizes the S protein, but binds to a different receptor, dipeptidyl peptidase-4 (DPP4) [33].

The COVID-19 pandemic has been unprecedented and left many countries struggling with an outbreak of severe disease since the onset in December 2019 in China. Efforts are ongoing to identify therapeutics that block direct viral entry and cytotoxicity. As SARS-CoV-2 infection continues, additional efforts are aimed at targeting downstream pathways that contribute to disease progression. A possible dual target of both infection and disease progression could relate to the RAAS and ACE2 [32, 35]. ACE2 is integral to permitting viral entry. ACE2 may be subsequently downregulated by SARS-CoV-2 following endocytosis and has been linked to disease severity with worsening of lung and tissue injury [36]. Attachment of the S protein requires priming by transmembrane serine protease 2 (TMPRSS2) to expose fusion peptides, with inhibition of TMPRSS2 or TMPRSS2-KO mice resulting in reduced capability of viral entry [35, 36]. Increases in SARS-CoV viral load are related to the magnitude of downregulation of ACE2 mRNA and protein expression in target tissues of the virus. It remains unclear whether ACE2 suppression is from a direct viral effect or secondary to a pro-inflammatory cascade with IL7 and IL2, cytokines that have been linked to suppression of ACE2 in vitro [36].

Vaccines have been developed that activate neutralizing antibodies against S protein production or that stimulate anti-S responses against a modified adenoviral vector [37]. Even with continued downregulation of ACE2 after viral entry, SARS-CoV-2 continues to disseminate. This suggests alternative pathways of entry and spread, which some postulate may be a syncytium, a hybrid membrane with host and viral particles that can escape antibody detection [18, 38] or the existence of other receptors that enable entry (aminopeptidases and DPP4) [39, 40]. ACE2 expression varies in many tissues. The oral cavity and specifically the tongue demonstrate an increased expression of ACE2, which may facilitate an important route of entry for SARS-CoV-2. Type II alveolar epithelial cells have higher ACE2 tissue vs. plasma activity, and the lungs exhibit increased RAAS activity [5].

Genetic factors may account for a large proportion of variability of ACE2 expression, but specific disease states can either up- or downregulate levels. Aging, hypertension, diabetes mellitus and arterial plaque formation have been associated with reduced ACE2 expression, which may confer a worsened COVID-19 disease outcome [41, 42]. ACE2 levels are highest intrapartum and decline with age, providing some context for the observation that children exhibit milder COVID-19 symptoms [43]. Disease presentation also has gender differences, with males exhibiting greater severity and mortality compared to women in studies of COVID-19. This could be linked to epidemiological differences: higher rates of some comorbidities and risk factors such as hypertension, coronary heart disease, smoking and alcohol consumption in males [44–46]. In addition, the ACE2 gene is located on the X chromosome, which could account for sex-specific difference in ACE2 biology [47].

Patients at risk for COVID-19-related mortality often present with severe pneumonia, cytokine storm, and disseminated intravascular coagulation. All of these perturbations have been related to RAAS imbalance. Activation of the AT1R by Ang II stimulates signal transduction via several pathways (PI3K, JAK/STAT, MAPK) with resultant vasoconstriction, vascular permeability, macrophage activation, pro-inflammatory cytokine expression and fibrosis [48, 49]. The magnitude of Ang II release correlates with severity of COVID-19 illness [50]. Patients with severe COVID-19 who require ICU admission are found to have higher concentrations of cytokines (MCP-1, TNF-α, GCSF, IL2 and MIP1A) compared to those not requiring ICU admission [51]. In persons with ARDS, depletion of ACE2 enhances IL-1β, IL-6 and TNF-α secretion [18]. Greater cytokine release can depress myocardial function. SARS-CoV and SARS-CoV-2 are known to cause myocarditis with autopsy specimens showing lymphocytic infiltrates, necrosis and viral RNA in the myocardium [36]. Perturbations in the KKS may contribute to the cytokine storm, as bronchoalveolar lavage specimens from patients with COVID-19 demonstrate a downregulation of enzymes that degrade bradykinin and an overactivity of bradykinin receptor stimulation [52].

Activation of ADAM-17 by SARS-CoV-2 viral entry leads to cleavage of the extracellular portion of ACE2 liberating sACE2. sACE2, in turn upregulates ADAM-17 and a vicious cycle of AngII/AT1R activation through reduced ACE2 activity perpetuates [53]. ADAM-17 also promotes TNF-α release, increasing macrophage recruitment and activation [54]. sACE2 retains enzymatic activity, but has markedly lower efficiency compared to membrane-bound ACE2 in catalyzing Ang II and activating the alternative pathway [18]. Triggering of the coagulation cascade by SARS-CoV-2 occurs as a result of widespread endothelial damage exposing tissue factor in disseminated infection. Excess bradykinin and its metabolites, as a consequence of ACE2 downregulation contributes to enhanced coagulation through activation of factor XII and plasmin [35, 55, 56]. The activation of the KKS may be excessive in COVID-19, with an overproduction of inflammatory cytokines, hyperalgesia and vascular leakage [55, 56].

There is a bidirectional link between SARS-CoV-2 and cardiometabolic disease, such that individuals with a prior history of CVD may suffer worsened outcomes when infected, and SARS-CoV-2 can result in both acute and long-term cardiometabolic disease. Mechanisms for the development long-term cardiovascular sequelae of COVID-19 are under investigation and include residual damage from initial viraemia and alteration of homeostatic systems, including the RAAS, complement cascade, cytokines, and the autonomic system [57].

Therapeutic Targets and Trials in COVID-19

Choosing effective physiologically targeted therapy for COVID-19 is essential to combat severe and progressive disease. Given that the RAAS is intertwined in the

pathophysiology of all the phases of COVID-19 (viral, pulmonary, and hyperinflammatory phases) [18], many trials have leveraged RAAS blockade and manipulation of the other interconnected systems to test potential treatments. ACE2 levels measured sequentially in patients with severe COVID-19 in Vienna showed a sevenfold increase from initial to later stages in their disease, with a simultaneous increase in Ang(1–7) representing a likely protective mechanism. Markedly higher ACE2 levels are present among those patients with COVID-19 when compared to patients with influenza with similar disease severity indices (Sequential Organ Failure Assessment score) [58].

In preclinical and clinical scenarios with viral pneumonia (secondary to SARS-CoV or RSV) or ARDS, rhACE2 has shown promise in improving physiological parameters [59, 60]. Monteil et al. demonstrated in vitro infection of human tissue with SARS-CoV-2 can be prevented by sACE2 [61]; however, in vivo human studies in COVID-19 have shown mixed results [18]. Differing results between human and preclinical studies may be explained by the relatively reduced efficacy of sACE2 in Ang II catalysis compared to membrane-bound ACE2. rhACE2 may analogously be subject to limited efficacy similar to sACE2. Furthermore, due to the larger size of rhACE2 vs. sACE2, rhACE2 may also be limited in tissue penetration. Therefore, some suggest rhACE2 may instead function as a competitive docking site for SARS-CoV-2 S proteins [18]. Further complexities exist such that emerging studies suggest that physiological doses of rhACE2 could in fact promote SARS-CoV-2 viral entry, with supraphysiological doses of rhACE2 being protective [62].

ACEis and ARBs are postulated to provide benefit by shifting the RAAS to favor the Ang(1–7)/MasR over the Ang II/AT1R pathway. ACEis work upstream by inhibiting ACE, preventing conversion of Ang I to Ang II. In addition, ACEis block inactivation of kinins, enhancing vasodilatory effects. ARBs block action of Ang II at the AT1R, allowing activation at the AT2R to predominate [18]. The majority of observational literature to date do not provide evidence that RAAS blockers affect ACE2 expression or activity despite prior pre-pandemic data suggesting an effect [63–67]. While some have suggested that ACEi/ARBs may on the contrary promote viral entry of COVID-19 through the upregulation of ACE2, two large databases reviewing the current literature conclude that ACEi/ARB use does not relate to the acquisition of COVID-19 infection by increasing ACE2 expression [68–70]. A large population-based cohort presented similar evidence that RAAS blockers do not enhance risk of COVID-19 infection or hospitalization [71].

ACEis and ARBs are clinically used in patients with hypertension, chronic kidney disease, cardiovascular disease and diabetes, and interrupting a stable drug regimen may exacerbate these underlying co-morbidities [72]. Three randomized controlled trials (RCTs) evaluating the impact of continuing vs. discontinuing RAAS blockers in hospitalized patients with COVID-19 revealed no differences in outcome (days alive and length of hospital stay) [73–75]. From these data, the conclusion was drawn that ACEis/ARBs can be safely continued during hospitalization for COVID-19 if a prior indication exists. A cohort study of nearly 2 million persons with uncomplicated hypertension from a French database found that ACEis and ARBs conferred a lower risk of hospitalization, intubation and death from COVID-19 [76]. Another observational cohort of 8.2 million patients from a UK registry showed a decreased

incidence of COVID-19 diagnosed by RT-PCR in those exposed to ACEis and ARBs within 90 days of data collection [77]. A systematic review highlighted that ACEi or ARB use was linked to more severe COVID-19 disease, but not mortality. However, the results could be confounded by an increased risk due to underlying comorbidities rather than being directly related to mechanistic actions of the drugs themselves [78]. Furthermore, in a meta-analysis which controlled for relevant confounders, those using RAAS blockers demonstrated reduced mortality [79]. Robust evidence for or against the use of ACEi/ARBs in COVID-19 is not present, and on this basis, many expert committees recommend against discontinuation of these RAAS-related drugs as there may be continued benefit to treatment of underlying co-morbidities [36, 72].

Evidence of MR antagonist effects on ACE2 levels are conflicting. A RCT found that spironolactone, an MR antagonist, did not affect ACE2 levels [80]. Contrary to this, one study showed that MR antagonists decrease soluble ACE2, but not membrane-bound ACE2 levels. In addition, the study showed that spironolactone may interfere with entry co-factor TMPRSS2 through its anti-androgenic actions [81]. A single blinded trial evaluated add-on therapy with spironolactone, sitagliptin or a combination of the two versus standard care in hospitalized patients with COVID-19 in Iran. Any group that received intervention had better clinical scores on day 5 of admission, and individuals receiving spironolactone tended to have lower mortality [82].

Future Directions in COVID-19

It would be important to discern the exact RAAS-related mechanisms that are contributing to both acute and chronic complications in COVID-19, as this could highlight potential drug targets to either reduce SARS-CoV-2 infection or mitigate severity of disease. Several other drug targets have already been suggested based on pathophysiological evidence of SARS-CoV-2 virulence. Suppression of renin release will reduce Ang II and aldosterone. The use of beta-blockers may prevent the ‘sympathetic storm’ that is postulated to occur in some patients as a result of hypoxia, proinflammatory cytokines and sympathetic activation. In addition, beta-blockers will reduce renin release from the kidneys [83]. No data from RCTs of beta-blockers in COVID-19 are currently available.

Vitamin D has been shown to inhibit renin expression. A recent meta-analysis showed improved outcomes with administration of vitamin D following COVID-19 infection [84]. A systematic review evaluating nearly 2 million patients found associations between vitamin D deficiency and both risk and severity of COVID-19 infection. Bias and heterogeneity were assessed as high within studies, potentially affecting results [85]. Further clarity is needed with regards to the role of vitamin D in COVID-19 infection and whether beneficial effects may relate to the RAAS.

Activators of ACE2 have been investigated as a potential therapeutic approach for administration following the COVID-19 viral phase, after which ACE2 becomes

downregulated. Diminazene aceturate (DIZE), an antiparasitic drug, is a potent ACE2 activator in vitro and additionally reduces inflammation via NF-κβ mechanisms. However, use of DIZE may be limited by concerns for cyto- and genotoxicity at therapeutic doses [86, 87]. In vitro studies have identified ursodeoxycholic acid (UDCA) derivatives, BAR107 and BAR708, as having equivalent ACE2 activation to DIZE and may suggest potential utility of these therapeutic compounds against COVID-19. Further potential strategies include prevention of S protein-ACE2 interaction though peptides, antibodies, antibody fragments or small molecule inhibitors [88].

The COVID-19 pandemic has highlighted the clinical necessity to rapidly acquire epidemiological data to enhance our understanding of disease severity and progression. The benefit of observational studies and their ability to provide immediate information must be weighed against limitations in interpretation due to potential confounders. For example, associations between ACEis/ARBs use and increased morbidity and mortality in a study population that includes hospitalized patients or those with severe illness only may be complicated by selection bias if those on ACEi/ARBs have underlying cardiovascular comorbidities [20]. In this regard, prospective studies may offer useful information. Additional prospective studies evaluating the use of ARBs in COVID-19 and related pulmonary and cardiac complications are currently underway. Ultimately, RCTs which are rigorously designed can offer the most robust evidence and may direct management if RAAS blockers are shown to provide benefit.

HIV and the RAAS

RAAS Dysregulation and Organ Dysfunction in HIV

HIV infection has transformed to a manageable chronic disease with the advent of highly active antiretroviral therapy (ART). Experienced ART-users have a longer life expectancy, and the cause of HIV morbidity and mortality has shifted primarily to non-communicable from communicable disease among those well-treated with ART. In this regard, a twofold increase in cardiovascular disease remains in well-treated persons with HIV (PWH) compared to individuals without HIV, after controlling for traditional cardiovascular risk factors. Several pathophysiological factors may account for this excess risk, including residual inflammation, viral persistence, ART adverse effects and more recently explored, RAAS dysregulation. RAAS activation is linked to several processes that could mediate cardiometabolic disease, including insulin resistance, redistribution of fat, and inflammation in PWH [7].

RAAS dysregulation in PWH has been demonstrated. ACE levels are increased in PWH compared to HIV negative controls [89]. Furthermore, the increased RAAS activation has been linked to VAT, an inflamed, dysfunctional ectopic adipose tissue depot which has been found to accumulate in PWH [90]. HIV-infected women demonstrate a higher 24 h urine aldosterone excretion than women without HIV.

Further analyses among these women with HIV found urine aldosterone to be independently associated with VAT and hemoglobin A1c after controlling for potential confounders [91]. Using a controlled low sodium diet to simulate activation of the RAAS, PWH demonstrated increased RAAS activation when compared to persons without HIV [90, 92]. Persons with HIV and increased VAT showed the greatest RAAS activation (plasma renin activity (PRA) and aldosterone concentrations). Other anthropometric indices (BMI, subcutaneous adipose tissue (SAT)) did not relate to RAAS activation, which suggests these relationships were unique to the visceral depot [90].

HIV and its treatment may have effects on mediating the RAAS. The structure of renin is similar to HIV-1 protease, which may have important implications. HIV CD4+ T cells have been shown to promote renin mRNA expression [93], and this may be a potential endogenous source of RAAS activation. Renin may be implicated in HIV viral replication. In vitro studies have corroborated a threefold increase in HIV protein (p24) production when human T cells are incubated in a renin-rich media, and complementary to this, there is suppression of protein production (Agt and Gag) when treated with aliskrein, a direct renin inhibitor [94]. Protease inhibitors have been shown to activate the RAAS in adipocytes, which could provide a RAAS-related mechanism for their metabolic side effects [95].

Numerous factors may drive inflammation in HIV, including HIV viral persistence, translocation of microbiota across compromised mucosal barriers, and co-infection of other pathogens (hepatitis B and C viruses, herpes viruses) [96]. RAAS activation could play a key role in inflammation, as an increase in levels of CCL-2 and soluble CD163 were demonstrated in PWH under dietary sodium restrictive conditions simulating RAAS activation. CCL2 and CD163 are both markers of monocyte and macrophage activation that have been linked to the unstable and inflammatory type of coronary plaque found in HIV [92], as well as to arterial inflammation and insulin resistance [7]. Under conditions of RAAS activation, IL-6 and high-sensitivity CRP (hsCRP) levels are also increased in PWH, but not in those without HIV [90]. HIV viral persistence may be driven by a shift in Th17 cells, with MR activation driving this phenotype and MR blockade downregulating it [97–99]. Ang II promotes TGF-β1 signaling and resultant fibrosis. Lymphoid tissue fibrosis, driven by TGF-β1, may prevent complete CD4+ T cell recovery following treatment with ART to achieve virological control [11].

Glucocorticoids and mineralocorticoids can lead to immune dysfunction despite virological control, and activation of these steroid pathways may promote insulin resistance. The acquired cortisol resistance found in PWH may be an adaptive response to inhibit cortisol's suppression of the adaptive immune system and may occur at the expense of losing its anti-inflammatory properties [95]. Persistent inflammation leading to cardiovascular disease has been investigated in many chronic diseases, including rheumatoid arthritis, chronic obstructive pulmonary disease in addition to HIV, and glucocorticoid resistance is a commonality found in many of these [95]. Pro-inflammatory cytokines (IL-1, IFN-γ, IFN-α,TNF-α) are increased in ART-experienced PWH, with the latter two stimulating cortisol release and favoring a pro-inflammatory (Th1) rather than anti-inflammatory (Th2) phenotype

[95]. Chronic inflammatory states, like HIV infection, have been associated with an imbalance in 11β-HSD1/11β-HSD2 activity, which may promote excess MR activation and subsequent metabolic disease [95]. Cytokine release activates 11β-HSD1 activity in HIV [95], and this has been linked to adverse metabolic outcomes including reductions in insulin sensitivity, lipid derangements, fat redistribution, hypertension and atherosclerosis in preclinical models [100]. 11β-HSD2 protects against detrimental cortisol- or aldosterone-induced activation of the MR by shunting cortisol to cortisone, but conversely 11β-HSD2 itself has also been associated with insulin resistance [95].

RAAS activation and insulin resistance may be linked in several ways. Insulin signaling may be interrupted by aldosterone-induced release of cytokines. Ang II may induce insulin receptor phosphorylation. Furthermore, modulators of insulin sensitivity (translocation of glucose transporter-4, adiponectin and PPAR-γ expression) are dampened by MR activation [90]. Adiponectin is an adipokine that has protective effects on insulin sensitivity. During a RAAS activated state, adiponectin was demonstrated to be lower in PWH with excess VAT [90] and insulin resistance worsens [92]. Moreover, aldosterone is an independent predictor of insulin resistance, after controlling for adiponectin and other relevant factors (VAT, specific ART exposure, age, sex) [90]. Furthermore, during a state of RAAS activation, markers of inflammation, hsCRP and IL-6, were increased and adiponectin decreased relative to a non-RAAS activated state [90] (Fig. 4.3).

Natriuretic peptides are released from the myocardium and downregulate the RAAS, which may serve as a protective feedback system. In primary aldosteronism, a condition of autonomous aldosterone production, NPs are found to be increased during of states of aldosterone elevations. In contrast, reduced NPs have been shown to contribute to metabolic disease in obesity [7]. Relatively lower levels of BNP were demonstrated in PWH compared to persons without HIV during RAAS activation. Stratification by BMI (above and below a BMI of 25 kg/m^2) showed lowest BNP in individuals with BMI $\geq$ 25 kg/m^2 [101]. Reduced NP may provide a mechanism for increased metabolic risk as a result of reduced RAAS suppression. Lipocalin-2 is a protein secreted by various cell types, including immune cells. Lipocalin-2 may be targeted by MR activation and mediate adverse cardiac remodeling [7]. Cardiac and inflammatory biomarkers during a RAAS activated state were related to increases in serum lipocalin-2 in PWH [102].

Therapeutic RAAS blockade in preclinical studies may reverse cardiometabolic sequelae induced by activation. Treatment of obese rats with ARBs reduced markers of adipocyte differentiation (leptin, PPAR-γ and glycerol-3-phosphate dehydrogenase) [103]. Tat-dependent HIV transcription was uniquely inhibited by spironolactone, but not eplerenone, in permissive CD4+ cell lines suggesting an effect independent of MR antagonism given the divergent effects of the medications [104]. Preclinical obese db/db mouse models demonstrate that MR blockade reduces inflammatory indices (TNF-α, MCP-1, CD68) [105] in the adipose depot and lowers HOMA-IR and triglycerides [106]. These mice demonstrated increased MR mRNA expression

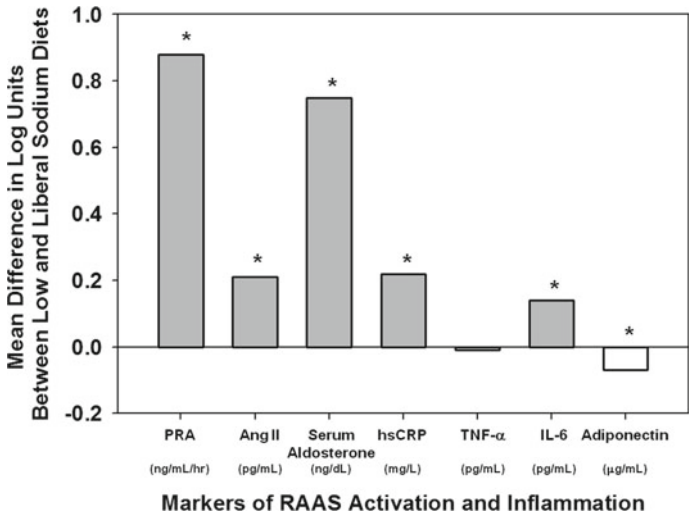

Fig. 4.3 RAAS Activation and Relationship to Inflammatory Markers and Adipokines in the HIV. Mean difference in markers of RAAS activation and inflammation among persons with HIV between a low and liberal sodium diets. A low sodium diet simulates a RAAS activated state, and a liberal sodium diet simulates a RAAS suppressed state. *P value < 0.05 low versus liberal sodium diet. Bars above the zero line represent analytes increased during the low sodium diet relative to the liberal sodium diet. Reprinted from Srinivasa et al. [90] with permission from the Endocrine Society/OUP

in white adipose tissue, and suggest MR blockade may have targeted the adipose [106].

Therapeutic Targets and Trials in HIV

There are no currently approved therapies uniquely targeting CVD in HIV. Traditional CVD-risk reducing medications do not completely mitigate the increased risk of CVD in HIV. Limited therapeutic options specific to HIV are available, a growth hormone releasing hormone analog may target excess VAT in HIV lipodystrophy, and at this time its effects on CVD outcomes are unknown. MR blockade remains an attractive therapy, as it is mechanistically linked with metabolic and inflammatory perturbations in HIV, may have many other potential beneficial effects already described in preclinical models and limited human studies, and are approved therapies for the general population [90]. The phenomenon of 'aldosterone breakthrough' [107] often seen with ACEis/ARBs, which results in feedback-induced increases in aldosterone after initiation of therapy may also be avoided with MR antagonists [90]. Despite evidence linking MR activation to insulin resistance in HIV, a 6 month RCT assessing eplerenone vs. placebo did not show MR blockade improves

insulin sensitivity measured by the euglycemic-hyperinsulinemic clamp technique in PWH [108]. These findings correlate with a trial of obese persons without HIV, in which spironolactone did not improve insulin sensitivity evaluated through an oral glucose tolerance test [109]. However, in the same trial among PWH, treatment with eplerenone did improve inflammatory markers (MCP-1) and lipid parameters (high-density lipoprotein and intramyocellular lipids) over six months [108].

The pleomorphic effects of ACEis and ARBs have also been explored in HIV. Lisinopril use in a group of predominantly male PWH showed significant reductions in hsCRP and TNF-α [96]. hsCRP is elevated in association with CVD in both persons with and without HIV [96]. ACEis may increase 11β-HSD2 expression, which may effectively reduce substrates for MR activation [95]. Telmisartan, an ARB with additional PPAR-γ agonist properties that may reverse ART-induced adipocyte toxicity [110], has been evaluated in several studies in HIV. PPAR-γ agonists have been proposed to have antifibrotic effects and in animal models have reversed histological fibrosis [11, 111]. Trials in persons without HIV show that telmisartan may improve insulin resistance [110]. In the MATH trial, telmisartan failed to show a reduction in VAT, metabolic or inflammatory indices in well-controlled PWH [112]. No improvement in HOMA-IR with telmisartan administration in HIV was reported in the TAILoR trial [110], although two preceding smaller trials did show a benefit of telmisartan on insulin resistance [113, 114]. Telmisartan was also evaluated in older PWH and did not demonstrate improvements in endothelial function measured by brachial artery flow-mediated dilation [115].

ACEis and ARBs, as an add-on to ART among PWH, have failed to show reduction in fibrosis in small clinical trials. Rectal lymphoid fibrosis as well as gut viral reservoirs were unchanged following lisinopril use in PWH [116]. Telmisartan provided no additional benefit over ART in reducing lymph node and adipose tissue collagen deposition [111]. Losartan, another ARB with PPAR-γ agonist properties, did not show improvements in markers of inflammation, fibrosis and myocardial function in an older cohort with HIV (aged 50 years and above) [11]. In contrast, RCTs in the general population selected for high CVD risk have shown reductions in inflammatory and fibrotic indices with ACEi and ARB use [11].

ACEis and ARBs as reno-protective agents have been tested in HIV-associated renal disease, based on their known anti-proteinuric effects from studies of other chronic renal diseases such as diabetic nephropathy [117]. ACEis have shown benefit in PWH with microalbuminuria in one small trial, with telmisartan improving microalbuminuria in a small cohort with HIV and stage 1 hypertension [113]. HIV associated nephropathy (HIVAN) is the most common HIV-related chronic kidney disease. The underlying renal benefit of ACEis may be linked to the RAAS's permissive role in TGF-β-mediated fibrosis and NF-κβ-mediated inflammation, both which are upregulated in persons with HIVAN [118, 119]. Renal survival, defined as time from diagnosis to initiation of renal replacement therapy, is prolonged by ACEis in HIVAN [118, 120, 121] Mortality benefit was also demonstrated among persons with HIVAN [118].

Albuminuria has been linked to neurocognitive impairment in the general population, and proteinuria has been associated with neurocognitive impairment in PWH [122]. Considering RAAS blockers (ACEis and ARBs) effectiveness at lowering

albuminuria/proteinuria, these therapeutics were investigated in clinical trials to understand their impact on cognitive decline. The ONTARGET and TRANSCEND trials showed a lower odds of cognitive decline in the general population among those with baseline macroalbuminuria treated with ACEi or ARB vs. placebo [123]. In contrast, one study evaluating PWH initiated on RAAS blockers did find consistent effect on neurocognitive function [122].

Future Directions in HIV

The source of renin resulting in downstream RAAS activation in PWH warrants further exploration and could present another therapeutic target. Independent renin secretion from adipose tissue or excess β-adrenergic stimulation in the visceral depot stimulating renin release have been proposed as potential mechanisms, but further evaluation in human studies is needed [90].

An additional RCT, The *MIRACLE HIV* Study (NCT02740179), is currently ongoing in PWH, which will be able to test the efficacy of eplerenone on cardiovascular endpoints, such as coronary flow reserve, myocardial inflammation and fibrosis, and arterial inflammation. Given that initial studies demonstrated eplerenone provided some benefit to inflammation and lipids among PWH, there may be favorable effects on CVD. Moreover, to address a potential perturbation in the NP-RAAS system in HIV, *The ENCHANTMENT HIV* Study (NCT04153136), will test the efficacy of sacubitril-valsartan on myocardial inflammation and fibrosis and structure and function. It is postulated that sacubitril-valsartan, a combination neprilysin inhibitor and ARB, may increase NP and block aldosterone, which could modify unique physiology demonstrated among PWH.

The current RAAS physiology studies may also direct future dietary guidance. Individuals with hypertensive disorder and other CVD are often prescribed a low sodium diet to prevent progression of disease. In contrast, low sodium diets stimulate excess RAAS activation in HIV and are associated with metabolic derangements and a pro-inflammatory milieu. Therefore, further studies may need to discern the benefits and risk of sodium restriction unique to the HIV population [90].

Conclusion

Aside from its traditional role in maintaining vascular hemodynamics and sodium balance, the RAAS is established to have novel roles in inflammatory pathways, adipose biology, and cardiometabolic derangements. Hormone systems facilitate system-wide cross-talk in biologic processes, and in this way may have key functions in non-endocrine related diseases. Following the classical pathway, excess production of renin or stimulation of Ang II and aldosterone production could result in

overactivation of the MR and promote a pro-inflammatory environment. In the presence of ACE2, excess Ang II and aldosterone production may be shunted towards the Ang(1–7)/MasR alternative pathway, instead directing substrates towards an anti-inflammatory environment. Knowing that the RAAS may influence inflammation, it is not surprising that this system may be implicated in infectious diseases in which inflammation is inherent to the pathogenesis. There is current evidence to suggest that unique RAAS physiology may be implicated in two important infectious diseases—HIV and SARS-CoV-2. In the absence of a cure, both viruses are complicated by long-term metabolic sequelae even in the presence of aviremia. If it is determined that the RAAS contributes to these sequelae, RAAS blockers may be ideal treatment strategies. In SARS-CoV-2, the virus is dependent on the ACE2 working as a functional receptor for viral entry, and following viral entry, downregulated ACE2 may contribute to a pro-inflammatory milieu and worsened respiratory disease. Since the recent emergence of SARS-CoV-2, RCTs are lacking, but initial data of observational studies overall suggest ACEi/ARB use are not associated with worse outcomes in COVID-19 and some data may even suggest a mortality benefit, which needs more rigorous testing to confirm. rhACE2 is not an approved therapeutic and may be important to investigate in the context of reduced ACE2 physiology. In HIV, excess RAAS activation has been linked to visceral adiposity, insulin resistance, inflammation, and reduced natriuretic peptides. Studies of RAAS blockade using ARBs have shown mixed results on inflammatory and metabolic endpoints, whereas MR antagonists have shown initial utility as an anti-inflammatory and lipid-improving agent. Further studies are necessary to understand the full potential of RAAS blockers to reduce metabolic disease in HIV. Enhancing our understanding of a physiologic connection between the RAAS and SARS-CoV-2 and HIV should prompt us to consider whether other connections of the RAAS exist with other infectious diseases or inflammatory processes.

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Chapter 5
Understanding the Renin-Angiotensin System in Coronavirus Disease 2019

Prithiviraj Nagarajan

Abstract At the beginning of the COVID-19 pandemic, there was increasing concern that therapy with renin-angiotensin-system (RAS) inhibitors, such as angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blockers, might increase the risk of infection, severe illness, or death from COVID-19. The renin-angiotensin system (RAS) plays a significant role in the fibrogenesis and inflammatory destruction of numerous organs, including the liver and lungs, in COVID-19 patients. However, the clinical fate of these individuals is unknown. ACE2 catalyzes the conversion of angiotensin II to angiotensin-(1–7), and the ACE2/angiotensin-(1–7)/MAS axis counteracts the detrimental effects of the renin-angiotensin system (RAS), which plays a key role in maintaining physiological function. As well as the pathophysiological equilibrium of the organism. In addition to the direct viral impacts and inflammatory and immunological factors involved in the pathogenesis of COVID-19, ACE2 down regulation and an imbalance between RAS and ACE2/angiotensin-(1–7)/MAS the following infection may also contribute to multiple organ damage in COVID-19. Is. -19. The SARS-CoV-2 spike glycoprotein, which binds to ACE2, is a possible target for creating particular medicines, antibodies, and vaccines. Restoring the equilibrium between the RAS and ACE2/angiotensin-(1–7)/MAS may aid in the prevention of limb injuries. In this chapter, we'll look at the role of ACE2 in COVID-19 and possible therapeutic targets for it to learn more about how to treat the pandemic.

Keywords SARS-CoV-2 · COVID-19 · Angiotensin-Converting enzyme 2 · Multi-organ injury

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Introduction

A new Coronavirus (2019-nCoV) outbreaks was reported in Wuhan, Hubei province of China, in late 2019 [1, 2]. The outbreak has developed into a worldwide pandemic. The virus appears to be more contagious than the coronaviruses responsible for Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS) (MERS-CoV). Full-length genome sequencing and pairwise protein sequence analysis showed that 2019-nCoV has 79.5 percent of the same sequence as SARS-CoV and is in the same coronavirus family as SARS [3]. The same receptor, angiotensin-converting enzyme 2 (ACE2), is used by both 2019-nCoV and SARS-CoV to enter host cells [3]. As a result, this virus was named SARS-CoV-2. The angiotensin-converting enzyme (ACE) homolog ACE2 is found in various human organs and tissues. It has many biological functions and may lessen the harmful effects of diseases caused by the renin-angiotensin system (RAS) [4].

The spike protein of SARS-CoV-2, like SARS-CoV, interacts with ACE2, so COVID-19 may have a pathogenic mechanism similar to SARS. Most SARS-CoV-2 infections have mild clinical symptoms such as fever, dry cough, myalgia, increased sputum production, loss of smell, and diarrhoea [5, 6]. However, the elderly and those with concomitant diseases such as asthma or respiratory problems, cardiac abnormalities, diabetes mellitus, and liver and kidney disorders are more prone to developing pneumonia due to lower respiratory tract infections [7, 8]. In addition, an increase in blood lactate dehydrogenase, a measure of lung tissue destruction, was observed in COVID-19 patients (8) and was associated with a greater risk of serious illness (7). In addition, age and lymphopenia are potential risk factors for severe COVID-19 infection (8). We will look at the function of ACE2 and its possible therapeutic targets in COVID-19 to learn more about how to stop epidemics.

Angiotensin-Converting Enzyme

The renin–angiotensin–aldosterone system (RAAS) is a key regulator of arterial blood pressure, electrolyte balance, and water balance [7, 9, 10]. Classical and local or tissue RAS are two distinct RAS systems that have been identified in new research on the renin-angiotensin system (RAS) [7, 11]. Although the tissue RAS regulates all functions of the body tissues in question, the classical RAS and aldosterone are primarily responsible for maintaining systemic arterial blood pressure and body fluid balance [7, 12]. Renin is released into the circulatory system in response to a decrease in arterial pressure, where it acts on angiotensinogen (produced in the liver) to convert it to angiotensin I (Ang I) [13, 14]. Endothelial cells of the circulatory system contain angiotensin-converting enzyme (ACE) receptors and lung endothelial cells where ACE is secreted and converts Ang I to Ang II [15]. A potent vasoconstrictor, angiotensin II elevates blood pressure to normal [7, 11, 16]. However, low systemic arterial blood pressure requires the renal system's additional homeostatic

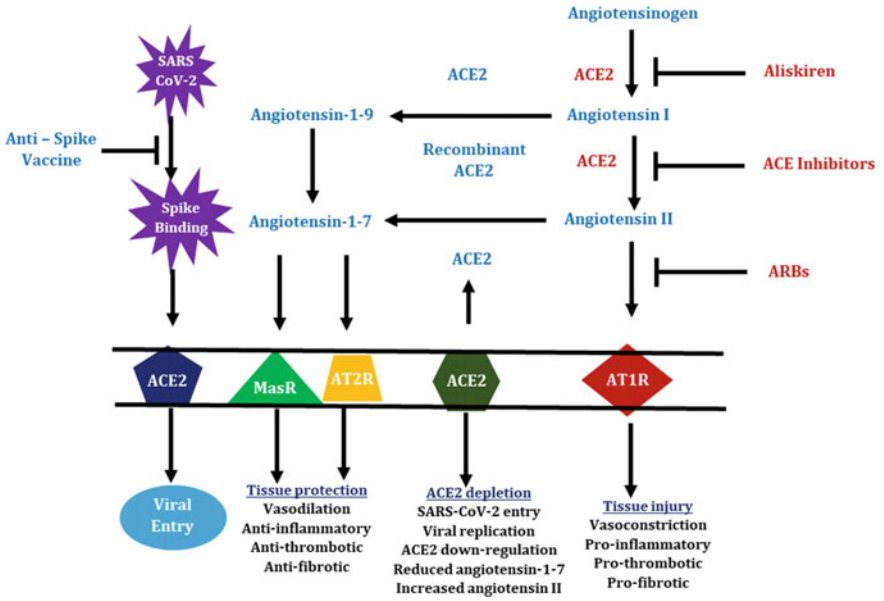

Fig. 5.1 COVID-19 and the renin-angiotensin system. Diagram demonstrating the dual ACE- and ACE2-dependent pathways as well as the possibility for therapeutic treatments employing RAS-inhibiting agents, recombinant ACE2, and vaccination strategies

regulation of body fluid and electrolyte levels [11]. Renin, angiotensinogen, ACE and ACE inhibitors are among the tissue-specific RAS present in most body tissues [17]. Aliskiren's direct inhibition of upstream renin may assist in restoring the angiotensin imbalance brought on by SARS-CoV-2 cell invasion by inhibiting the conversion of angiotensinogen to angiotensin I. Figure 5.1 demonstrates that discussions on RAS inhibitors in COVID-19 have mostly centred on the more frequently used ACEIs and ARBs, as opposed to aliskiren, the sole renin inhibitor approved for clinical use.

RASS Pathway

Three overlapping stages, including the viral, pulmonary, and hyperinflammatory phases, compensate for COVID-19's natural history [18]. The RAAS is a large neurohormonal network that spans all three stages of COVID-19 and may be used as an additional therapeutic target. The RAAS system is exploited by COVID-19 to obtain entry, spread, and harm several organ systems, including the respiratory system. According to studies, the angiotensin-converting enzyme 2 (ACE2) is a doorway for the entrance of SARS-CoV-2 into tissues. [19]. Angiotensin II (AII), whose production is triggered by the cleavage of angiotensinogen by renin into angiotensin I (AI) and subsequently converted to AII by ACE (20), has been used

as the major effector peptide in conventional RAS (Fig. 5.1). Although this is the primary pathway for making AII, additional enzymes may also be involved (30). The interaction of AII with three receptors is responsible for most of its functions (AT1, AT2, and AT1nonAT2). G protein-coupled receptors classify AT1 and AT2 (20), whereas nonAT1 and nonAT2 are more likely to be angiotensin clearance receptors or angiotensinase (21). The AT1 receptor can induce vasoconstriction, increase catecholamine release, and increase aldosterone production (22). Additionally, AT1 receptors may increase the expression of mitogen-activated protein kinase (MAPK), collagenase activity, fibrosis, and inflammatory processes (2, 5). These receptors exert pro-inflammatory effects through several pathways, including decreased NADPH oxidase expression in smooth muscle cells, increased reactive oxygen species (ROS) production, and pro-inflammatory effects such as nuclear factor-kappaB (NF-B). Transcription involves the increased activity of nuclear factors. kB) and release of various cytokines, including E26 transformation-specific sequence (ETS) (23), TNF-a, IL-6, and MCP-1 (24), and macrophrenic shift (25). Instead, activating the RAS by stimulating AT2 receptors has a protective effect by causing anti-inflammatory, anti-oxidative, and anti-fibrotic effects (20). Other peptide mediators and enzymes are involved in the non-classic RAS. The key mediator is angiotensin 1–7 (A1–7), which two different pathways can produce. One carboxypeptidase is triggered by ACE2 splitting AII into A1-7, while the other is triggered by ACE2 converting AI to angiotensin 1–9 (A1–9) and then by ACE (5) to A1–7 (Fig. 5.1). Today, two versions of ACE2 are known, one soluble and one transmembrane, both of which contribute to the production of A1-7. The G protein-coupled receptor MAS1 is activated by A1–7, leading to increased nitric oxide production (26), Akt activation (27), and anti-inflammatory actions (28), Searching the "ClinicalTrials.gov" website has revealed three clinical trials currently underway, evaluating rhACE2 in COVID-19 patients are illustrated in Table 5.1. Furthermore, activation of MAS1 receptors, which are present on the surface of macrophages, suppresses the inflammatory macrophage phenotype and the production of pro-inflammatory cytokines (30). Consequently, A1–7 is a favorable RAS axis component with cardiac and renal consequences in contrast to the ACE/AII/AT1 axis (29). Table 5.2 shows six clinical studies evaluating Ang-(1–7) infusion in COVID-19 patients.

Although ACE2 possesses enzymatic activity on Ang I, it targets Ang II as a substrate with 400-fold better efficiency. The Ang II/AT-1 axis requires less substrate to activate its adverse effects by metabolizing and decreasing plasma Ang II. The primary consequence of ACE2-Ang II metabolism is Ang 1,7. Ang 1,7 peptides show action on both the MrgD and Mas receptors, producing anti-inflammatory, antifibrotic, vasodilatory, and natriuretic effects that directly contradict the activity of the Ang II/AT-1 receptor (Fig. 5.1) [20].

Table 5.1 Clinical trials using recombinant ACE2 in the treatment of COVID-19

S. No.	Title	Interventions	Registry identifier
1	Evaluate the safety, tolerability, pharmacodynamics, pharmacokinetics, and immunogenicity of HLX71 (recombinant human angiotensin-converting enzyme 2-FC)	HLX71	NCT04583228
2	Recombinant human angiotensin-converting enzyme 2(rhACE2) as a treatment for patients with COVID-19 (APN01-COVID-19)	RhACE2 APN01	NCT04335136
3	Recombinant bacterial ACE2 receptors-like enzyme of B38-CAP could be promising COVID-19 infection and lung injury-preventing drug better than recombinant human ACE2	Recombinant bacterial ACE2 receptors-like enzyme of B38-CAP (RBACE2)	NCT04375046

Table 5.2 Clinical trials using angiotensin-(1–7) in the treatment of COVID-19

S. No.	Title	Interventions	Registry identifier
1	Angiotensin-(1–7) for the treatment of COVID-19 in hospitalized patients	Intravenous angiotensin-(1–7) infusion	NCT04570501
2	Treatment of angiotensin peptide (1–7) for COVID-19	Angiotensin-(1–7) derived plasma	NCT04375124
3	TXA127 for the treatment of severe COVID-19	Intravenous angiotensin-(1–7) (TXA127)	NCT04401423
4	Angiotensin 1–7 as a therapy in the treatment of COVID-19	Subcutaneous angiotensin- (1–7)	NCT04605887
5	Use of angiotensin-(1–7) in COVID-19	Intravenous supplementation of angiotensin-(1–7)	NCT04633772
6	Angiotensin-(1, 7) treatment in COVID-19: the ATCO Trial (ATCO)	Intravenous angiotensin-(1–7) infusion	NCT04332666

Hypertension

Blood pressure is influenced differently by angiotensin-converting enzyme (ACE) and angiotensin-converting enzyme 2 (ACE2) receptors. While ACE produces Ang II, ACE2 produces Ang 1–7 that have hypotensive effects via acting through Mas receptors, which are primarily present on blood vessels and cause vasodilation.

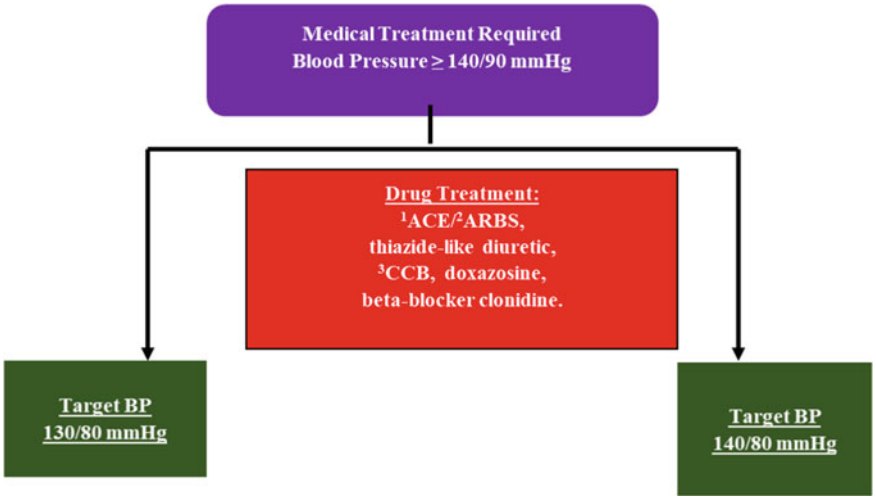

Fig. 5.2 Treatment of chronic kidney disorders, diabetes mellitus, hypertension, and cardiovascular disease (Guidelines International Society of Hypertension, 2020), [1]Angiotensin-Converting Enzyme Inhibitors (ACEIs), [2]Angiotensin II Receptor Blockers (ARBS), [3]Calcium Channel Blockers (CCBs)

However, some people continue to have hypertension. Primary, whose precise etiology is unclear, and secondary are the two major categories [16]. Primary hypertension is known to be influenced by lifestyle and genetic variables, and some populations react to ACEIs/ARBs and other hypertensive medicines [21], while others-primarily those of African ancestry-might only respond to diuretics and calcium channel blockers [21, 22]. Therefore, it is standard practice to provide ACEIs, ARBs, calcium channel blockers, diuretics, and alpha or beta-blockers to patients with hypertension, diabetes, or cancer. Based on the 2020 recommendations of the International Society of Hypertension (ISH) [23], Fig. 5.2 depicts how to manage hypertension.

SARS-COV-2 and ACE_2 in Lungs

A membrane envelope surrounds the betacoronavirus's single-stranded positive-sense RNA genome, known as SARS-COV-2 [24]. The glycosylated spike(s) protein, a key indicator of the host immunological response, is one of several structural proteins the genome specifies. Because it binds to the receptor protein ACE2, found on the surface of lung alveolar epithelial cells (host cells), the S protein is important for mediating host cell invasion by SARS-COV-2 [25]. According to estimates, the extracellular domain and S protein binding region of ACE2 has a 15 nM affinity [26]. Human androgen-sensitive transmembrane serine protease type 2 (TMPRSS211)

facilitates activation of the S protein, which is essential for invasion [24]. Specifically, TMPRSS211 breaks down the S protein to produce the S1 and S2 subunits. Given that both subunits are essential for viral entry into host cells, this is an important step [26]. S2 is the component that induces membrane fusion and viral internalization in the pulmonary epithelium, whereas S1 is the subunit that is recognized by ACE2 and allows viral attachment [27]. The increased affinity of the S1 subunit for ACE2 was attributed to the increased virulence of SARS-COV-2 compared to SARS-COV-1 [24, 26]. A cryo-EM structural study showed that the S protein of SARS-COV-2 has an affinity for ACE2 that is approximately 10–20 times larger than that of the SARS-S COV-1 protein [25].

Another important thing to consider is that SARS-CoV-2 may cause ACE2 to be taken inside the cell. This would mean that there would be less ACE2 on the cell's surface, making it more difficult to break down AII and make A1–7. If the levels of AII and A1–7 are out of balance, the lung damage caused by SARSCOV-2 can worsen. Therefore, a decrease in ACE2 may affect how COVID-19 affects tissue fibrosis, inflammation, and pulmonary function [26]. This mechanism was previously observed concerning SARS-CoV-1 infection, which was associated with lower levels of ACE2 on the cell membrane and more severe lung damage [28]. Since both SARS-COV-1 and SARS-COV-2 use the same cellular invasion pathway, their causes and symptoms of lung damage may be similar [28].

SARS-COV-2 and ACE_2 in Heart

The heart and other cardiovascular tissues might potentially get infected by the SARS-CoV-2 after it has entered the bloodstream [26]. According to the evidence, individuals with COVID-19 often had cardiovascular symptoms in addition to respiratory ones, and patients without underlying cardiovascular disorders also reported experiencing similar symptoms [29]. In some verified instances, the SARS-CoV-2 infection began with cardiovascular symptoms (such as heart palpitations and chest tightness), according to the National Health Commission of China (NHC). Additionally, the 11.8% of individuals who passed away with COVID-19 but had no underlying cardiovascular disorders suffered severe cardiac damage [29]. These findings indicate that cardiologists must be involved in treating COVID-19 patients [30]. However, it is unclear how much SARS-CoV-2 contributed to the onset of cardiac damage [31]. It is known that cardiovascular illnesses and the development of cardiovascular problems may be directly impacted by the infection itself [32]. Careful consideration must also be given to the expression of TMPRSS211 or other proteases that may facilitate viral entry into the tissue; Another idea for the development of heart damage considers the reduction in ACE2 caused by SARS-CoV-2, which may exacerbate symptoms in patients with preexisting cardiovascular disease [26, 33] This may result from an imbalance between classic and non-classic RAS that favours AII, which, in addition to the viral infection, may further impair cardiac

function [26]. A preclinical study indicates that animal models lacking ACE2 exhibited inferior left ventricular remodelling in response to the acute insult generated by AII, demonstrating a protective role for nonclassical RAS in myocardial recovery [34]. This finding may explain the cardiac damage seen in COVID-19 people without cardiovascular disease [29]. This theory was backed by a study demonstrating a linear correlation between viral load and lung damage and a notably elevated amount of AII in plasma samples from SARS-CoV-2-infected people [31]. A separate study also found that the presence of viral RNA was linked to a decrease in the production of the ACE2 protein in the hearts of 35% of SARS patients [35]. The systemic inflammatory response and immune system difficulties during disease development may be responsible for the myocardial damage. The cytokine storm [31] is an alternative idea for why myocardial injury develops [29]. In this scenario, the typical RAS cascade may have had a minor role in promoting inflammation in addition to the viral infection. In addition, it should be considered that a number of the anti-COVID-19 drugs now under investigation may pose a risk of cardiac toxicity [30]. Lastly, evidence revealed that COVID-19 might produce a kind of disseminated intravascular coagulation (DIC) since microthrombi were discovered in the autopsies of COVID-19 patients [36]. There are still several unidentified causes of DIC. Here are some possible speculative mechanisms: IL-6, for example, promotes the formation of fibrinogen [37]; the virus may directly adhere to endothelial cells; or DIC and cytokine storm are connected (where one exacerbates the other).

Other Organs and Tissue Injuries

Pancreas

The high expression of ACE2 in pancreatic cells implies that COVID-19 may influence the pancreas [38]. According to studies, up to 16% of people with severe COVID-19 have elevated amylase and lipase levels in their blood, and 7% of these patients have significant pancreatic abnormalities seen on CT scans [39]. Patients with COVID-19 have been recorded as presenting with acute pancreatitis [40]. ACE2/angiotensin-(1–7) prevents diabetes by improving pancreatic cell survival, boosting insulin production, and lowering insulin resistance [41]. According to studies, significantly more SARS patients who had never had diabetes before and did not get steroid medication during their hospital stay developed insulin-dependent acute diabetes than patients with pneumonia that was not caused by SARS [42, 43].

Moreover, plasma glucose levels and diabetes are independent predictors of death in SARS patients [43]. The majority of pancreatic islets in the autopsies of a subset of SARS patients exhibited shrinkage and amyloid degradation, suggesting that the virus may be harmful to the islets [44]. Consequently, COVID-19 can impact pancreatic function similarly to SARS, and glucose levels should be closely monitored, especially in people with diabetes and those taking glucocorticoids.

Skeletal Muscles

More than 30% of SARS patients had muscle weakness and high blood creatine kinase (CK) levels [45]. Upon admission, there was also a modest to moderate increase in CK values in individuals with COVID-19 [46]. Despite myofiber necrosis and atrophy being observed in skeletal muscle tissue, electron microscopy failed to find any SARS-CoV particles [47]. Recent research has shown that the RAS contributes significantly to the pathophysiology of many skeletal muscle problems and that the ACE2/angiotensin (1–7)/MAS axis has anti-atrophy effects [41]. Uncertainty exists regarding the association between SARS-CoV-2 attack on muscle and myopathy and the downregulation of ACE2.

Central Nervous System

ACE2 is broadly distributed in the brain, mainly in the neurons, and participates in the neural control of various physiological processes, including stress response, neurogenesis, and metabolic and cardiovascular activity [48, 49]. The SARS-CoV entered the mouse brain via the olfactory bulb and propagated transneuronal to other regions [50]. Numerous COVID-19 patients have reported olfactory and gustatory dysfunctions, which raises the possibility that the olfactory bulb is involved in transmitting the SARS-CoV-2 virus [51, 52]. SARS-CoV was discovered in human brain tissue samples [52]. According to autopsies, the brains of SARS patients had edoema and localized neuronal degeneration [53]. SARS-CoV-2 was found in the cerebral fluid of a patient with encephalitis, and 78 out of 214 individuals (about 78%) showed neurologic symptoms of COVID-19 [54, 55]. Because SARS-CoV-2 and SARS-CoV have a much stronger affinity for the same receptor (ACE2), SARS-CoV-2 may be able to infect and hurt the central nervous system.

Blood Vessels

Small and big blood vessels have endothelial cells that produce angiotensin-(1–7) and express ACE2 [41]. Because of the ACE2/angiotensin-1–7/MAS axis, vascular dilation, antiproliferation, and antithrombotic actions are shown in the vasculature [41]. SARS RNA may be discovered in numerous organs' endothelium of small veins [56]. D-dimer levels are significantly elevated in COVID-19 patients who are critically ill [57], and DIC is common in the early stages of the disease. Endothelial damage can be caused by viral infections and inflammatory responses, which can increase permeability and mess up the microcirculation of COVID-19.

Potential Targets and Drugs

As both SARS-CoV and SARS-CoV-2 are receptors for ACE2, several transmembrane proteinases, such as ADAM17 and TMPRSS, are involved in membrane fusion and binding processes; these locations may be prospective targets in the development of antiviral medicines for the treatment of COVID-19. Examples include the ability of serum from convalescent SARS patients to block the spike-driven entrance of SARS-CoV-2 into host cells, indicating the potential for vaccines that target the spike protein [27]. According to research, SARS-CoV-specific monoclonal antibodies and recombinant ACE2-Ig can effectively neutralize SARS-CoV-2, and a hexapeptide from the spike protein's receptor-binding domain binds to ACE2 to prevent SARS-CoV entry [58, 59]. The local equilibrium between the RAS and ACE2/angiotensin-(1–7)/MAS axis is disturbed in organs following viral infection due to the downregulation of ACE2, which may be linked to organ damage. According to animal studies [60], ARBs can increase the plasma levels of both Ang-II and angiotensin-(1–7) as well as the cardiac expression and activity of ACE2, while ACE inhibitor (ACEI) therapy can increase plasma angiotensin-(1–7) levels, decrease plasma Ang-II levels, and improve cardiac ACE2 expression. To prevent organ damage, the renin-angiotensin system must be blocked and/or angiotensin levels raised by using ACEIs/ARBs, renin inhibitors, and angiotensin-(1–7) analogs [61]. Other animal investigations have shown that the administration of ARBs might treat ALI caused by the influenza virus or a SARS-CoV spike in mice [62]. A population-based trial found that ACEIs and ARBs dramatically lowered the 30-day death rate of pneumonia patients who needed to be hospitalized [63]. As ACE2 expression levels rise in the target organs of ACEI/ARB therapy, there are worries that this may promote infection and raise the likelihood of developing severe and deadly COVID-19 [64]. However, two sizable cohort studies revealed that the use of ACEIs/ARBs was not linked to a higher risk of SARS-CoV-2 infection but rather to a decreased risk of all-cause death in hospitalized patients [65, 66]. The protective effects of ACEIs and ARBs in COVID-19 need more investigation.

Inaccuracies and a Strong Urge to Act

The effect of RAAS on COVID-19 and pulmonary physiology illustrates the complex link between SARS-CoV-2 and ARDS development. Even though histology and animal models present intriguing pharmacological targets, it is questionable if RAAS inhibitors can mitigate lungs damage. Most animal models and tissue samples are used in non-pulmonary research [67]. In addition, nothing is known regarding the use of RAAS inhibitors in individuals who have not yet established a chronic condition. An expanded study must be conducted on the effects of RAAS inhibition in ARDS using readily available medications and novel agents (Table 5.2). Finally, we suggest that the case has been established for undertaking randomized controlled

trials with RAAS inhibitors for viral pneumonitis and that these trials would likely yield critical information for treating inflammatory disorders that manifest as ARDS [68]. Until then, stopping RAAS medication should be discouraged because cardiovascular comorbidities and COVID-19 are associated with an increased risk of death [69]. The importance of RAAS inhibition in keeping cardiovascular homeostasis is shown by the fact that when neurohormonal medications are stopped, Ang II levels go back to where they were before treatment, and end-diastolic volumes go up within 4 and 15 days, respectively [70].

Conclusion

The RAS and ACE2/angiotensin-(1–7)/MAS axis play crucial roles in several physiological and pathological conditions. ACE2 acts as the receptor for SARS-CoV-2 and SARS-CoV to enter host cells. SARS-CoV-2 infects not just the lungs, but other organs with high ACE2 expression since ACE2 is widely expressed in various organs and tissues. The pathophysiology of COVID-19 is quite complex and includes many factors. In addition to the direct effect of the virus and inflammatory and immunological factors, the downregulation of ACE2 and imbalance between the RAS and ACE2/angiotensin-(1–7)/MAS axis may further contribute to the extensive organ damage in COVID-19. The spike glycoprotein of SARS-CoV-2 is a potential target for developing specific drugs, antibodies, and vaccines. The ACE/Ang II/AT1 axis of the system has a role in developing fibrotic diseases, still other counter-components may provide innovative therapy options. By restoring the appropriate balance between the RAS and ACE2/angiotensin-(1–7)/MAS, organ damage caused by COVID-19 might be mitigated. In this chapter, the authors discuss the connection between and relevance of lung diseases. Future studies are necessary to completely appreciate the role of RAS in lung and other organ damage, it has been hypothesized that RAS regulation by ACE-2 may one day serve as a therapy for lung disease.

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Chapter 6
Metabolomics Approach in Differentiating RAS Responses in ARDS and SAR-CoV-2

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Abstract Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) utilizes host angiotensin converting enzyme 2 (ACE2) for viral entry. This interaction can lead to viral-induced dysfunction of renin angiotensin system (RAS) that has been ascribed to SARS-CoV-2 mediate morbidity and mortality. This chapter highlights the role of how bioactive RAS metabolites and their alteration in SARS-CoV-2 and sepsis correlates with disease pathogenesis. Similarly, alteration of RAS metabolite levels has been associated with poorer clinical outcomes, and thus may be a good biomarker to measure disease. LCMS based metabolomics approach is specific, highly sensitive, and precise in quantifying RAS metabolites. This approach can provide a systemic and comprehensive evaluation of the RAS metabolome which may explain specific defects. Additionally, this type of approach can also identify therapeutic targets for the development of effective therapeutic intervention. Accordingly, we discuss the use of LCMS-based RAS metabolomic to study SARS-CoV-2, sepsis, and acute respiratory distress syndrome (ARDS). The overarching purpose of this chapter is to explicate the role of RAS and its bioactive metabolites in viral infections and related immune response. The ability to determine the overall changes and compensatory mechanism may provide insights as to disease severity and highlight potential therapeutic targets within the RAS pathway for the amelioration of lung fibrosis associated with SARS-CoV-2 infection.

Keywords Renin angiotensin system · Metabolomics · SARS-CoV-2 infection · Acute respiratory distress syndrome angiotensin converting enzyme 2

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Introduction

The renin angiotensin system (RAS) is a well-established modulator of hemodynamics homeostasis. However, RAS is less known for its critical role in regulating tissue reparative processes. In this regard, bioactive RAS peptides have been shown to regulate (1) stem and progenitor cell mobilization and recruitment to the affected site, (2) migration and proliferation of cells within the site, and (3) promote tissue regeneration and revascularization. With these biological activities, it is not surprising RAS is a central regulator in the response to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) mediated lung injury.

SARS-CoV-2 is a coronavirus with strong binding affinity for angiotensin converting enzyme 2 (ACE2) found on cellular membranes of various epithelial cells lining the mucosal tracts. Similar to SARS-CoV-1, this nascent viral strain binds onto this monocarboxypeptidase, where its attachment can disrupt enzymatic activity. Because ACE2 is central in the formation of pro-resolving angiotensin metabolites, disruption of this enzyme, may explain some of the clinical presentations associated with SARS-CoV2 infection.

To clearly understand the complex role of RAS in response to tissue injury, it is necessary to comprehend the biosynthesis and metabolism of various bioactive angiotensin peptides. The RAS metabolic pathway begins with hepatic biosynthesis of penultimate precursor, angiotensinogen (Agt), which undergoes a series of metabolic alterations to form the various bioactive angiotensin peptides highlighted in Fig. 6.1. Renin catalyzes the metabolism of Agt to form Angiotensin (1-10) (Ang(1-10) or Angiotensin I (AngI)), an inactive peptide. AngI is a promiscuous peptide precursor and substrate capable of undergoing further metabolism mediated by angiotensin converting enzyme I (ACE1) and ACE2. AngI metabolism by ACE1 and ACE2 form key bioactive RAS peptides like angiotensin II (AngII) and Ang(1-9), respectively. In addition, AngI is also a substrate for chymase which is found primarily in cardiac cells, where this enzyme can also metabolize AngI to form AngII. Furthermore, AngI can form Ang(1-7) through non-classical RAS enzymes like neprilysin (NEP), prolyl endopeptidase (PEP), and thimet oligopeptidase (THOP) as highlighted in Fig. 6.1 [1]. In this context, AngI can be metabolized into Ang(1-9), AngII, or Ang(1-7) depending on the enzyme(s) involved. Currently, there is a lack of clarity as to how these metabolic enzymes are regulated.

AngII is a key bioactive peptide that can bind onto various types of G-protein coupled receptors (GPCRs). When AngII binds onto the angiotensin type 1 receptor (AT1R), this interaction can trigger vasoconstrictive, inflammatory, and pro-fibrotic wound healing mechanisms. The vasoconstrictive activity can be partially mediated through AngII/AT1R ability to inhibit baroreceptor reflex facilitating noradrenaline release at synaptic clefts. Other biological effects ascribed to AngII/AT1R include the ability to promote migration and proliferation of vascular smooth muscle cell into damaged tissues. In addition, wound healing properties such as the activation of the collagen biosynthesis pathways leading to profibrotic tissue remodeling can also be attributed to AngII/AT1R mediated activity. The transactivation of the AngII/AT1R

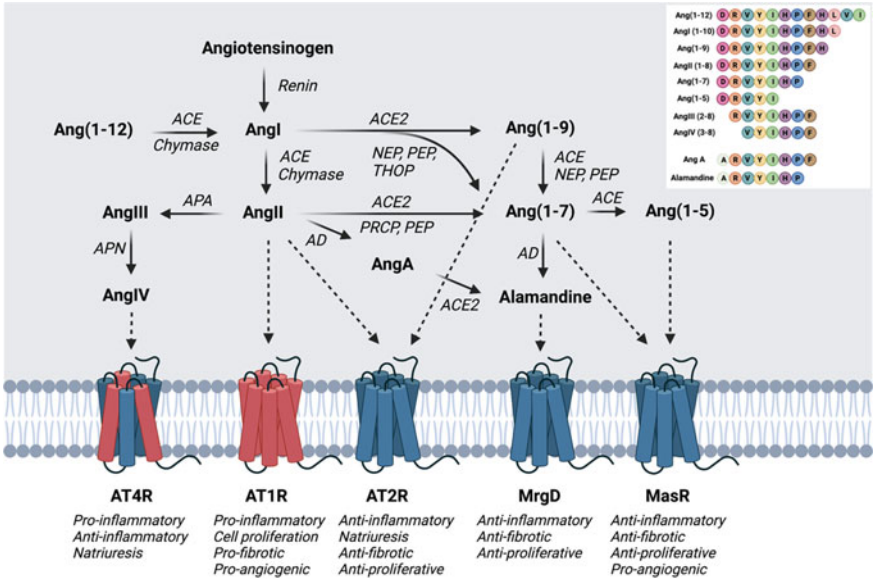

Fig. 6.1 Renin Angiotensin System (RAS) Pathway. ACE (angiotensin converting enzyme); ACE2 (angiotensin converting enzyme 2); NEP (neprilysin); PEP (prolyl endopeptidase); THOP (thimet oligopeptidase); PRCP (pro-X carboxylpeptidase); APA (aminopeptidase A); APN (aminopeptidase N); AT1R (angiotensin II receptor type 1); AT2R (angiotensin II receptor type 2); AT4R (angiotensin II receptor type 4); MasR (MAS receptor); MrgD (MAS-related G-protein coupled receptor). Created with BioRender.com

axis has been closely linked to the ability to promote TGF-β expression. Additionally, profibrotic healing is accompanied by AngII-mediated increased expression of NADPH oxidase (NOX) which increases production of reactive oxygen species (ROS). The inability to scavenge reactive metabolites can cause tissue damage subsequent to triggering a vicious cycle of tissue regenerative responses. These biological effects have led some to refer to the AngII/AT1R/ACE axis as key members of the "classical" or "pathogenic" arm of the RAS.

Although AngII can activate a host of biological activities, it can also be a substrate for additional metabolism. AngII can be metabolized by ACE2 or proplyl oligopepitidase (POP), where either enzyme can remove a single amino acid from the carboxyl terminal, to form Ang(1-7) [2]. Despite only one amino acid difference when compared to AngII, Ang(1-7) binds onto its own specific RAS receptor, MasR. Ang(1-7)/MasR counterbalances the biological activities associated with AngII/AT1R. In particular, Ang(1-7) binding onto MasR can promote anti-fibrotic and anti-inflammatory wound healing. These biological effects have led to the classification of Ang(1-7)/Mas/ACE2 as key components of the "protective" or "non-classical" arm of RAS. Another member of the protective arm of RAS includes Ang(1-9), a metabolite formed through ACE2 cleavage of AngI and is a ligand

for AT2R. Similar to Ang(1-7)/Mas, Ang(1-9)/AT2R binding can also promote non-fibrotic wound healing (Fig. 6.1).

There are additional angiotensin metabolites that have been identified. Similar to AngII, Ang(1-7) can be further metabolized by ACE to form Ang(1-5), which is also a ligand for MasR [3]. Ang(1-5)/MasR was found to increase production of atrial natriuretic peptide (ANP), where the addition of MasR antagonist, A779, was able to abolish this activity. Ang(1-7) can be decarboxylated by asparate decarboxylase (AD)- mediated metabolism to form alamandine, a RAS metabolite that binds onto Mas-related G protein-coupled receptor D (MrgD) [3]. The activities of alamandine/MrgD include antithrombogenic, anti-inflammatory, and antifibrotic properties. Similar to Ang(1-7), the administration of alamandine can ameliorate pulmonary fibrosis through activation of MrgD, and has propelled some to suggest the use of this compound for the treatment of SARS-CoV-2 associated lung fibrosis [4].

AngII is also susceptible to N-terminal amino acid metabolism, where aminopeptidase A (APA) can catalyze the formation of AngIII (Ang(2-8)) which is a ligand for both AT1R and AT2R subtypes [5]. Aminopeptidase N (APN) can further metabolize AngIII by removing an addition N-terminal amino acids to form AngIV, a ligand for AT4R, which is highly expressed in the neocortex, hippocampus, cerebellum, basal ganglia structures, and various peripheral tissues. Currently, AngIV/AT4R is proposed to regulate memory acquisition and retrieval, the regulation of blood flow, neurite outgrowth, angiogenesis, and kidney function [5]. As expected other angiotensin peptides such as Ang(1-4), Ang(3-7), Ang(5-7), and Ang(3-4) have been isolated via the enzymatic cascades of Ang-(1–7) and AngII, however their biological activity has not yet been elucidated.

SARS-CoV-2 Interaction with the RAS

SARS-CoV-2 has been implicated as the causative pathogen leading to COVID-19. Similar to SARS-CoV-1, host cell entry of SARS-CoV-2 is mediated through spike (S) protein receptor binding domain (RBD) interaction with human ACE2 [6–8]. In comparison to SARS-CoV-1, SARS-CoV-2 has significantly higher RBD-ACE2 affinity, which partially explains the higher transmissibility of this coronavirus variant [9]. Cellular entry of SARS-CoV-2 is also facilitated by the membrane protease transmembrane protease serine 2 enzyme (TMPRSS2), which cleaves and activates the viral S-protein at the S1/S2 boundary. Activation of the furin cleavage site (PRRAR) in the S-protein is crucial for both viral replication and survival in the presence of neutralizing antibodies, where the loss of furin cleavage site was found to attenuate SARS-CoV-2 disease pathogenesis which has been a target for treatment strategy [10].

Viral S-protein binding onto ACE2 will trigger the ACE2 ectodomain to detached from the cell membrane through ADAM17 mediated activity [11]. ADAM17 is also known as TNF - α converting enzyme (TACE), a metalloprotease expressed in the

lungs, kidney, small intestine, and heart, which is able to cleave membrane-anchored proteins [11]. ADAM17 promotes ACE2 shedding which leads to reduce ACE2-enzymatic activity; this may subsequently lead to increased AngII accumulation and bioavailability for AT1R activation. AngII/AT1R activation will further lead to NFkb activation and thus explains the increase in pro-inflammatory cytokines interleukin-1β (IL-1β), IL-6 and TNF-α, where high expression has been seen in SARS-CoV-2 mediated cytokine release syndrome (CRS) [12]. These cytokines can also upregulate NADPH oxidase activity which can further increase overall oxidative stress; ROS generation is thought to be one factor leading to permanent lung tissue damage [13].

Increased expression of inflammatory cytokines such as IL-1 β, IL-8, and CXCL2 have additional biological activities including the promotion of neutrophil activation and T cell response [14]. When the inflammatory activation is not attenuated, patients with severe SARS-CoV-2 may progress to develop CRS, which is closely linked to development of acute respiratory distress syndrome (ARDS), where acute and diffuse alveolar inflammation and damage is evident [15].

Application of RAS Components in Relation to SARS-CoV-2-Related Morbidities

Patients infected with SARS-CoV-2 have been linked to detrimental physiological alterations including pulmonary and cardiac fibrosis. This may be attributed to reduced cell- associated ACE2, and thus allows accumulation of AngII to promote pro-fibrotic healing. In addition, the loss of ACE2 can reduce Ang(1-7) levels that are necessary to counterbalance AngII-mediated pulmonary and cardiac fibrosis through sustained AT1R activation via the MasR [16]. Preclinical studies have demonstrated the administration of Ang(1-7) or alamandine can improve oxygenation and reduces pulmonary fibrosis in mice models [17]. Alamandine/MrgD activates adenyl cyclase to increase cAMP and thus trigger protein kinase A activation. Like Ang(1-7), alamandine can stimulate eNOS and induce vasodilation [18].

ACE2 knockout mice were shown to have increased lung edema and neutrophil accumulation. This also shows ACE2-mediated activity can attenuate immune activation and lung damage [19, 20]. These findings point to the importance of ACE2 in regulating systemic and tissue levels of Ang(1-7) in response to virally induced lung injuries as seen with SARS-CoV-2 infection. Clinical trials are underway to determine Ang(1-7) efficacy in patients with severe viral-induced respiratory distress.

In Prospective Urban Rural Epidemiology (PURE), a case cohort study involving 10,000 patients, high levels of soluble ACE2 (sACE2) were correlated with higher risk for cardiovascular (CV) events that were independent from traditional CV risks [21]. Although ACE1 and ACE2 have been closely related to the RAS pathway, these enzymes can also catalyze the metabolism of other susceptible peptides. ACE2 catalyzes the metabolism of des-Arg^{9}-bradykinin to form the inactive bradykinin

(1–7), where reduced ACE2 activity can lead to enhanced bradykinin B1 activation, which has been implicated in the cause of pulmonary vascular leakage and edema [22].

RAS Components as Clinical Biomarkers in SARS-CoV-2

One meta-analyses evaluated the impact of RAS components in patients infected with SARS-CoV-2. This study found there was a 74% increase of AngII, but a reduced level of AngI, corresponding to a reduction in ACE activity. Interestingly, circulating Ang(1-7) levels were tenfold higher in the virally infected patients as compared to controls. When stratifying the patients for COVID19 severity, patients with severe COVID19 had a six-fold increase in Ang(1-7) levels when compared to non-severe patients [2].

The increase in Ang(1-7) was an unexpected finding; it was suggested the peptidases from different tissues, such as NEP or THOP, may be able to compensate for the loss of ACE2 activity. Both NEP and THOP can form Ang(1-7) through AngI catabolism. Additionally, Ang(1-7) can be produced through POP mediated metabolism of AngII. This hypothesis is supported by the observation that, in the circulation and in the lungs, the conversion of AngII to Ang(1-7) is much more POP-dependent than ACE2-dependent [23, 24]. However, one study showed no difference in POP activity when comparing COVID19 patients and controls [25]. These studies point to the contribution of non-classical RAS metabolism of these bioactive peptides.

Despite SARS-CoV-2 interaction with cellular ACE2, the overall ACE2 activity was 158% higher in SARS-CoV-2 infected patients as compared to controls. It is notable that disease severity has been associated with poorer clinical outcomes. Not only were levels of sACE2 different between COVID19 and controls, but the enzymatic activities were significantly different. One study showed that control versus COVID19 infected patients had ACE2 enzymatic activities of 0.06 pmol/min/mL and 5.8 pmol/min/mL, respectively. Enhanced ACE2 activity continued even 114 days post-infection [26]. Increases in ACE2 activity have been hypothesized to be a consequence of conformational modifications following receptor binding subunit S1 of the viral spike protein [27]. In addition, it is also conceivable that elevated Ang(1-7) may be a consequence of non-ACE2 mediated metabolism. As aforementioned, AngI metabolism via NEP or THOP can form Ang(1-7). Alternatively, Ang(1-7) can be produced through POP mediated metabolism of AngII, where the expression of POP is higher in the lungs.

Although systemic Ang(1-7) is upregulated in a number of studies, it is important to recognize that this may be a compensatory reaction to deficits in the affected tissue. These systemic findings are interesting but additional molecular dissection at the affected site may be warranted.

Metabolomic Approaches to Study SARS-CoV-2

Metabolomics is the measure of the small molecule substrates, intermediates, and by products as a consequence of cellular metabolism. Although focusing primarily on small molecules, advancement in metabolomics has included peptides and lipids, which can characterize how the biological system is functioning in a disease state. When these measures are compared with findings from a non-disease control cohort, the etiology can be identified by evaluating cellular or host changes in metabolism.

Metabolomics utilizes technological advancements in nuclear magnetic resonance (NMR) or liquid chromatography-mass spectrometry (LCMS) to simultaneously identify and quantify a large number of metabolites/analytes. The ability to measure these analytes reliably, accurately and precisely is at the center of why this approach has evolved to become a comprehensive diagnostic and even prognostic tool to monitor disease progression and outcome prediction [28]. This approach can provide metabolic insights when correlated with clinical outcomes [29]. Metabolomics of the RAS can broaden our understanding of several RAS-mediated diseases and its response in relation to disease progression and/or resolution.

This powerful tool is emerging as a precise guide to treatment strategies and providing feedback as to the effectiveness of these interventions. Additionally, metabolomics has been used to determine the timing as when to initiate therapeutic interventions. This approach can also identify the limitations of the therapeutic modality as a consequence of compensatory mechanisms that may not be evident when using a focused analyte approach.

Metabolomics approaches can be classified as either untargeted or targeted. An untargeted metabolomics approach is typically used to perform comprehensive analyses of all measurable analytes in a sample. This is normally based on the chemical characterization of various unknown analytes. In contrast, targeted metabolomics can measure pre-defined biochemically annotated metabolites in a given sample [30]. If available, the metabolite analytes can be measured to provide a quantitative measurement or threshold that lends context to the overall system.

One study evaluated 120 hospitalized COVID19 patients where blood samples were collected at the time of hospital admission. The study intent was to determine whether disease severity (i.e., mild or severe) and clinical outcome (i.e., discharged or deceased) could be predicted using an untargeted metabolomic approach [31]. Small molecules such as deoxycytidine and ureidopropionate (indirectly reflecting viral load), kynurenine (reflecting host inflammatory response), and multiple short chain acylcarnitines (energy metabolism) were used as potential predictors for clinical outcomes. Kynurenine, a metabolite of tryptophan, was significantly increased in patients with increased disease severity. This approach was able to predict for clinical outcome and severity when employing a Monte Carlo cross validated area under the Receiver Operating Characteristics ROC curve of 0.792 (SD 0.09) and 0.793 (SD 0.08), respectively. This translated to increase mortality in the group with higher kynurenine, which was 1.5-fold higher. A blind validation study on an additional

90 patients was able to predict outcome and severity at ROC area under the curve (AUC) of 0.83 (CI 0.74–0.91) and 0.76 (CI 0.67–0.86).

Metabolomics of RAS in SARS-CoV-2

Despite a surge of data regarding SARS-CoV-2, there is still a dearth of understanding as to how SARS-CoV-2 infection can impact the RAS pathway, and its link to disease severity and duration. New information has shown Ang(1-7) levels are an important determinant of SARS-CoV-2-related clinical outcomes. Unfortunately, most studies use ELISA-based tests which may cross react with both AngII and Ang(1-7), and thus confound their findings [32]. The inability to differentiate whether the levels are associated with AngII and Ang(1-7) may lead to inaccurate conclusions. Unlike ELISA-based assays, liquid chromatography mass spectrometry (LCMS) based metabolomics may provide more reliable and specific levels of AngII and Ang(1-7). More importantly, this approach can provide a more comprehensive approach as to the dynamic changes within the RAS pathway arms.

A phase I/II clinical trial used a targeted RAS peptide approach showed AngII accumulation is accompanied by reduced levels of Ang(1-7) (30). This study evaluated blood samples from severe SARS-CoV-2 infected patients ($N = 13$) and compared with non-SARS-CoV-2 infected healthy volunteers with no prior history of cardiac disease ($N = 6$). Arterial blood was collected using guanidine HCl and TFA containing tubes to prevent further ex vivo metabolism. RAS peptides (e.g., AngI, AngII, Ang(1-7) and Ang(1-5)) were quantified using tandem LCMS with a dynamic range of 10–1000 pg/mL.

This study found statistically lower blood concentrations of AngII (6.03 ± 1.18 versus 10.7 ± 1.87 pg/mL; $p = 0.0381$) and Ang(1-5) 3.43 ± 0.75 versus 19.3 ± 5.80 pg/mL; $p = 0.0084$) in SARS-CoV-2 infected patients as compared to controls [33]. This study affirms SARS-CoV-2 infected patients had higher circulating levels of Ang(1-7) (14.0 ± 2.32 versus 7.49 ± 1.42 pg/mL; $p = 0.0214$). These findings were in contrast to findings by other groups who utilized ELISA-based assays. It is important to note that the Ang(1-7) levels for both SARS-CoV-2 and controls patients were lower than previously reported in non-disease patients, where the mean levels were about 100–300 pg/mL. This may be due to the blood treatment with guanidine and THF treatment where these peptides were susceptible to acid degradation. In this analysis, there was no significant difference between Ang I levels (31.2 ± 6.23 versus 40.8 ± 9.54 pg/mL; $p = 0.3959$).

Additional analyses removed patients who were on RAS modulating therapies (e.g., ARBs and ACEi). Three (3) patients from the SARS-CoV-2 and control groups were previously on ACEi therapy, where there was no treatment interruption during the course of this study. When the impact of ACEi therapy was also evaluated, the authors did not find any significant alterations with patients receiving ACEi. When the comparisons excluded patients receiving ACEi treatment: AngI 34.5 ± 7.07 versus 45.2 ± 10.8 pg/mL, $p = 0.4023$; AngII 6.00 ± 1.33 versus 11.2 ± 2.07 pg/mL, p

= 0.0407; Ang-(1–7) 12.9 ± 2.02 versus 6.47 ± 1.03 pg/mL, p = 0.0080; Ang(1-5) 3.75 ± 0.86 versus 17.5 ± 6.29 pg/mL, p = 0.0330 were identified.

RAS Changes in Patients with Sepsis and ARDS

Sepsis or septicemia occurs as an inflammatory response to infectious processes. Irrespective of the cause, sepsis is characterized by excessive inflammatory responses leading to organ dysfunction and potentially septic shock and severe complications, including acute kidney injury (AKI), acute respiratory distress syndrome (ARDS), sepsis-induced myocardial dysfunction (SIMD), liver dysfunction, cerebral dysfunction, and skeletal muscle atrophy. Disease progression has been linked to increased expression of various inflammatory cytokines that mobilize the immune system to respond to viral or bacterial intrusion.

The cytokine storm has been attributed to an accumulation of AngII, which triggers inflammatory pathways involving NF-κB and IL-6-STAT3, particularly in non-immune cells including endothelial cells and epithelial cells. This pathway forms a positive feedback loop, named IL-6 amplifier, resulting in its excessive activation and therefore the subsequent cytokine storm as well as other complications [34]. Besides maintaining normal systemic osmotic performance, the accumulation of AngII can also activate TGF-β 1, which promotes pro-fibrotic healing. However, fibrotic wound repair leads to organ scarring and loss of elasticity, thus impacting normal physiological functions. Thus, the ability to prevent the fibrotic cascade has been shown to be beneficial to the survival of sepsis patients [35].

One study evaluated whether changes in RAS components were good prognostic markers of clinical outcomes in patients with sepsis [36]. Patients diagnosed with severe sepsis had blood drawn on days 1 and 3 after diagnosis. Levels of AngII, ACE, and other clinical values were compared at each timepoint in relation to clinical outcomes. Patients' clinical course was monitored for a total of 28 days. This study compared whether RAS components were precise as average Acute Physiology and Chronic Health Evaluation II (APACHE II) and Sepsis-related Organ Failure Assessment (SOFA) score in predicting overall outcomes. Mean APACHE II and SOFA scores were 22.2 and 6.1, respectively, for patients upon study entry. Logistic regression analysis revealed that mortality-associated variables included the APACHE II score on day 1, the SOFA score on day 1, and high lactic acid levels on day 3. In addition, low systemic AngII and ACE levels on day 1 and day 3 were all predictive for clinical outcome. Specifically, AngII levels below 86.1 ng/ml at study entry had a sensitivity of 88.2% and specificity of 77.3% for predicting mortality. Accordingly, ACE levels < 39.2 ng/ml on day 1 had a similar sensitivity of 88.2% and specificity of 72.7% for predicting mortality. When compared to APACHE II and SOFA scores, both AngII and ACE levels were better predictors for mortality. This study showed an understanding of the RAS pathway components such as AngII and ACE, may be important in biomarker identification to better predict for mortality and other clinical outcomes for severe sepsis.

Animal studies using ARDS models have exhibited reduced Ang(1-7). In contrast, upregulation of Ang(1-7) was able to reduced ROS and pulmonary fibrosis. These findings suggest that Ang(1-7) may be able to counterbalance the effects associated with AngII-related increases in ROS. Molecular studies have shown that Ang(1-7) can increase expression of extracellular regulated kinase-1/2 (ERK1/2). In addition, the administration of Ang(1-7) has been found to be cardioprotective in heart failure mice models [17].

RAS Metabolomics for ARDS

In a non-interventional study involving 39 consecutive patients, where 20 survived (51%) and 19 succumbed to their ARDS (49%), circulating RAS peptides levels were measured using a quantitative RAS metabolomics assay [37]. Blood samples were collected at study entry (24 h), 48, and 72 h, where the results are summarized in Table 6.1. The data was stratified based on survival status (i.e., survivors (S) versus non-survival (NS)). Although patients who survived had a five-fold higher Ang(1-7) and two-fold higher Ang(1-8) or AngII at study entry as compared to patients who succumbed to ARDS, the difference for both peptides were not statistically different. In contrast, the Ang(1-10) or AngI levels were significantly elevated in NS patients, which was statistically higher across all time points ($p < 0.05$). This suggests that patients succumbing to ARDS were unable to metabolize the inactive Ang(1-10) into bioactive peptides such as Ang(1-9) and Ang(1-8); this activity is mediated by ACE2 and ACE1 enzymes respectively.

To determine whether enzymatic activities are altered in patients who succumb to ARDS, we then evaluated RAS enzymatic activity by analyzing product/reactant ratios. This analysis showed that the metabolism of RAS peptides was reduced in the NS group as compared to S. Using Ang(1-12) as a precursor peptide for ACE1 conversion to Ang(1-10), the NS group had a 15-fold higher Ang(1-12) → Ang(1-10) ratio as compared to S (p-value < 0.001) (Table 6.2). Biologically, Ang(1-12)

Table. 6.1 Median RAS peptide concentrations stratified by patient outcome across timepoints

RAS peptide	Median concentration (ng/mL)					
	24 h		48 h		72 h	
	NS	S	NS	S	NS	S
A(1–12)	0.05	0.18	0.57	0.06	1.04	0.05
A(1–10)*	5.12	1.43	12.15	1.92	4.64	1.24
A(1–9)	0.89	0.98	0.97	1.54	3.46	1.42
A(1–8)	0.31	0.68	0.59	0.78	0.77	0.61
A(1–7)	0.21	1.08	0.83	0.97	0.81	0.88

S survivors; *NS* non-survivors

* statistical significance is $p<0.05$ for all time points

administration can increase blood pressure, which is blocked when either the ACE inhibitor captopril or the AT1R antagonist candesartan was administered. This finding suggests that Ang(1-12) exerts its vasoconstrictive activity through AT1R binding. In contrast, no biological activity has been ascribed to Ang(1-10); thus increased Ang(1-10) may reduce blood pressure support. The current understanding is that Ang(1-12) conversion into Ang(1-10) will rapidly form Ang(1-8) or AngII via ACE1 metabolism. Our study showed that Ang(1-10) → Ang(1-8) ratio was lower in NS patients (median 0.06 [0.01–0.11]) as compared to S (median: 0.75 [0.39–1.09]), p-value < 0.001, which is mediated by ACE1. This finding was further substantiated by Ang(1-10) → Ang(1-7) ratio where S (median: 0.48 [0.14–0.97]) is higher than NS group (median: 0.03 [0.01–0.08]), p-value < 0.001. This suggests S patients were more readily able to form Ang(1-7) from AngI compared to NS, where ACE1 and ACE2 activities are preserved in S than NS patients. Another unexpected finding was the importance of an alternative pathway for Ang(1-10) metabolism. We found that ACE2-mediated metabolism of AngI can form Ang(1-9), a AT2R agonist, which was higher in the S (median: 1.00 [0.51–2.05]) versus NS group (median: 0.08 [0.04–0.27]) where the difference had a p-value < 0.001. These findings suggest ACE1 and ACE2 metabolism was higher for the S versus NS group.

This study also evaluated Ang(1-7) breakdown, where no difference between S versus NS groups (data not shown) were observed for a majority of the ratios. However, Ang(1-7) → Ang(1-5) ratio trended toward significance where the NS was threefold higher than the S group(p-value = 0.07) (Table 6.2). Others have shown that Ang(1-7) can be metabolized by ACE1 to form Ang(1-5), a recently discovered MasR agonist. We evaluated whether patients with renal failure, as defined by the need for dialysis, would have differences in RAS peptide metabolism. Renal failure patients (Table 6.3) were compared to ARDS patients without renal failure. Higher

Table. 6.2 RAS peptide ratio correspondence to patient outcome

*Ratio: precursor → product	Median ratio (interquartile range)		P-value
	Non-survivors	Survivors	
A(1–12) → A(1–10)	60.0 (2.05–315.99)	4.05 (0.89–29.93)	< 0.001
A(1–10) → A(1–9)	0.08 (0.04–0.27)	1.00 (0.51–2.05)	< 0.001
A(1–10) → A(1–8)	0.06 (0.01-0.11)	0.74 (0.39–1.09)	< 0.001
A(1–9) → A(1–7)	0.27 (0.04–1.09)	0.4 (0.09–0.64)	0.692
A(1–10) → A(1–7)	0.03 (0.01–0.08)	0.48 (0.14–0.97)	< 0.001
A(1–7) → A(1–5)	1.00 (0.27–2.6)	0.29 (0.08–2.44)	0.070

* p values are there to identify statistical significance

Table. 6.3 RAS peptides and peptide ratios in patients stratified according to renal status

Variable	Median (interquartile range)		P-value
	No renal failure (N = 17)	Renal failure (N = 22)	
Ang(1-5) (ng/mL)	0.05 (0.05–0.14)	0.44 (0.09–0.73)	0.003
Ang(1-9) (ng/mL)	1.88 (0.63–3.50)	0.69 (0.05–1.83)	0.025
Ang(1-5)/Ang(1-7)	0.18 (0.12–1.25)	1.00 (0.46–3.11)	0.020
Ang(1-9)/Ang(1-10)	0.64 (0.14–2.06)	0.10 (0.05–0.52)	0.023

circulating levels of Ang(1-5) were seen with patients with renal failure (median 0.44 ng/mL [0.09–0.73]) as compared to no renal failure (median: 0.05 ng/mL [0.05–0.14], p-value = 0.003). In contrast, patients without renal failure had significantly higher Ang(1-9) as compared with the renal failure group (p-value = 0.025). The Ang(1-7) → Ang(1-5) ratio in patients with renal failure (median: 1.00 [0.46–3.11]) were higher than those without renal failure (median: 0.18 [0.12–1.25], $p < 0.02$) suggesting higher ACE1 activity in renal failure patients. Our finding is consistent with those reported by Mitani et al. who showed increased ACE1 expression in patients with overt renal tissue damage (38). This study cohort also showed patients with renal failure had a significantly lower Ang(1-10) → Ang(1-9) ratio versus no renal failure ($p < 0.023$) suggesting that ACE2 activity is reduced in patients with kidney damage (Table 6.3). These two observations were consistent with others who have shown that there is upregulation of ACE and downregulation of ACE2 in diabetic patients with overt nephropathy.

RAS Modulators in the Treatment of Diseases

AngII in Sepsis

A number of studies have shown Ang II and renin levels were altered in sepsis correlating with disease severity. Specifically, serum ACE concentration in patients with pulmonary sepsis were lower when compared to healthy volunteers, where reduced levels were associated with increased sepsis-related mortality [36, 39]. These findings suggest that reduced levels of AngII may contribute to reduced vasoconstrictive properties. Studies have shown exogenous infusion of AngII reduce the dosage of vasopressor to maintain blood pressure [38].

During sepsis, AngII upregulation can induce ROS generation and endothelial structural changes, both of which are pivotal physiologic responses to infection. However, AngII can be a "double-edged sword" where its persistent levels are central in the pathogenesis of its most severe presentation, namely septic shock. This may be in response to inadequate vascular tone to maintain blood pressure.

A large observational study did not support the downregulation of the classical RAS would have a protective effect against sepsis [39]. In the ATHOS-3 trial, the vast majority of patients had confirmed or suspected sepsis. Ang II infusion initiated during the acute phase of vasodilatory shock may contribute to an unknown degree of inflammatory enhancement and bacterial clearance. These clinical findings concurred with in vitro Ang II-mediated enhanced phagocytosis and inhibited abscess formation during experimental murine peritonitis [40].

In addition, this study reported a significant improvement in mean arterial pressure (MAP) and a reduction in the dosage of vasopressor for patients who received AngII infusions [39]. Post hoc analyses of this clinical study showed dramatic mortality benefit in subgroups with baseline Acute Physiology and Chronic Health Evaluation II score > 30, acute kidney injury requiring renal replacement therapy (RRT), ACE dysfunction in relation to renin, and rapid response to AngII [41, 42]. Rapid response to AngII infusion correlated with reduced AngII, where the infusion corresponded with decreased mortality [42]. This study identified rapid responders to AngII by measuring plasma renin activity (PRA), which was proposed as a potential biomarker.

Although this study evaluated the overall survival of patients with septic shock experience, these studies did not evaluate the overall impact of AngII infusion in relation to other components of shock. In the absence of comprehensive RAS levels and component data, it would be difficult to guide the development of these types of studies.

Soluble ACE2

Data supports the reduction of cell-associated ACE2 after SARS-CoV-2 infection can lead to reduced ACE2 enzymatic activity. A decline in ACE2 activity is accompanied by a reduction in the conversion of AngII to Ang(1-7), where the inability to produce adequate Ang(1-7) will allow AngII-mediated inflammation, vasoconstriction, pulmonary edema, and impaired lung function to go unopposed (2, 3, 9, 12). In addition, high levels of Ang II have been corresponded with exaggerated inflammatory response in severe cases of SARS-CoV-2 infection. Other biological activity attributed to ACE include the ability to metabolize des-Arg bradykinin and neurotensin.

Recombinant human soluble ACE2 (rHusACE2) is an engineered variant of ACE2 without the transmembrane domain. This protein retains its ability to bind onto the SARS-CoV-2 S protein and facilitate its neutralization and potential clearance [43, 44]. With these biological attributes, sACE2 may be a potential therapeutic agent for the treatment of SARS-CoV-2 [45]. In this context, soluble ACE2 is a target decoy capable of binding of SARS-CoV-2 and thereby competing with cell-associated ACE2, decreasing viral infection of susceptible cells [45]. rHusACE2 has been studied in healthy individuals in pilot clinical trials and was found to be safe at the dosages that were evaluated [46]. A subsequent clinical trial evaluated the safety of rHusACE2 in patients with ARDS which confirmed its safety [47].

To understand what impact if any rHusACE2 will have, focused RAS peptide evaluation alone may not be adequate. This may explain some of the controversies associated with the effectiveness of rHusACE2 in patients with SARS-CoV-2 infection. One unanswered question of ACE2 in SARS-CoV-2 is the presence of autoantibodies targeting the enzyme, although this is thought to be a host elimination pathway. ACE2 specific antibodies are present in individuals with SARS-CoV-2 infection and soluble ACE2 activity in plasma is reduced. The SARS-CoV-2/ACE2 complex leads to reduced ACE2 enzymatic activity [48]. Correspondingly, some have attributed this reduction in the ACE2 corresponds to increased AngII. Others have pointed to the role of SARS-CoV-2/ACE2 complex in driving inflammation [49]. Thus, symptoms of PASC (post-acute sequelae SARS-CoV-2 infection) are revealed [50].

In the absence of comprehensive data, it is obvious that the overall benefits associated with rHusACE2 for SARS-CoV2 will be difficult to identify. Evolving data regarding the sACE2 autoantibody complex may further complicate this already complex system. Clarification of the interactions between various RAS peptides and their respective receptors will require comprehensive evaluation (49).

Conclusion

The role of RAS in response to tissue injuries and infections have been dramatically highlighted by the SARS-CoV-2 pandemic. Our continued understanding of this complex system and its relationship with collateral mechanisms will provide significant insights as to the role of RAS in diseases. Additionally, changes in the bioactive peptides and its activation of GPCRs will define the timing and role in the overall responses. Understanding the various components and their interactions will require both metabolomics and transcriptomics analyses. What has not been clearly delineated is how samples from patients are collected and analyzed to give insightful information. In addition, this dynamic set of data must be evaluated in context of the receptor binding and biological mechanism that they activate. These methods will allow us to better understand the various components in the activation and resolution of diseases, like that caused by SARS-CoV-2.

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Part II
Lung, Liver and Kidney Diseases

Chapter 7
A Comprehensive Review of the Impact of the Renin Angiotensin System in the Liver, Lung, Infectious Diseases and Cancers

Renuka Munshi and Miteshkumar Maurya

Abstract Renin angiotensin aldosterone system has long been known for its role in the regulation of blood pressure and hydro-electrolyte balance in human body. There has been advances in the knowledge of local or paracrine renin angiotensin pathways in the various tissues of the human body. Derangements in these pathways has been implicated in the causation and complications of liver, lung, infectious diseases and cancers. Angiotensin II, being pro-inflammatory and pro-fibrogenic, becomes a causative factor in the pathogenesis of various diseases. Moreover, pharmacological agents modulating the expression of different components of the renin angiotensin system or its pathways have become areas of interest for many researchers as these may be emerging therapeutic targets that can influence the prognosis of different diseases.

Keywords Renin angiotensin system · Lung fibrosis · Hepatic fibrosis · Cancer · Angiotensin · RAS inhibitors · Infectious disease · Pharmacological agents · ACE inhibitors · ARBs

Introduction

The Renin Angiotensin System (RAS) is the hormonal system responsible mainly for regulation of the blood pressure and hydro-electrolyte balance in the human body [1]. In 1898, Tigerstedt and Bergman discovered the presence of the renin hormone from renal extracts after which studies exploring the renin angiotensin system accelerated [2, 3]. The Renin Angiotensin system is not just limited to the regulation of blood pressure and body fluid volumes but plays a vital role in different biological process. Each component of renin angiotensin system [RAS] has been explored in-depth adding to the human understanding about the complexity involved with the existence of local paracrine RAS system unique to each human body tissues

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and organs [4]. The cross talks between RAS related pathways involving receptors and enzymes are associated with several biological processes such as cancer stem cell proliferation, hematopoiesis, angiogenesis, tumorigenesis and metastasis [5, 6]. Thus RAS system has been linked with disease pathogenesis in lung, liver, infectious diseases and variety of cancers [7, 8]. However, whether RAS dysregulation is the cause or consequence of tumorigenesis still remains the subject of investigation and research.

Epidemiology of Liver, Lung, Infectious Diseases and Cancers Globally

Majority of the diseases that significantly contribute to mortality and morbidity are of hepatic, pulmonary, infectious and cancerous etiologies. Approximately 10 million deaths due to cancer were recorded in the year 2020 globally, of which the most common were breast, lung, colon, rectum and prostate cancers [9, 10]. In 2017, chronic respiratory diseases was the third most common cause of death followed by cardiovascular diseases and neoplasms while smoking was the leading cause of disability due to chronic respiratory disease among the men worldwide. According to the WHO annual global prevalence infectious disease data [2008], there are a million cases of malaria, sexually transmitted diseases, HIV/AIDS, tuberculosis, cholera and other diarrheal diseases that contribute annually to disease mortality and morbidity [11, 12]. Liver disease, especially viral hepatitis, hepatocellular carcinoma and cirrhotic complications account for 3.5% of all deaths worldwide and 10% of the global solid organ transplantation rates [13, 14]. The current epidemiology highlights the unmet need to explore new pharmacological targets for disease prevention and treatment.

Renin Angiotensin Aldosterone System [RAAS] Pathways

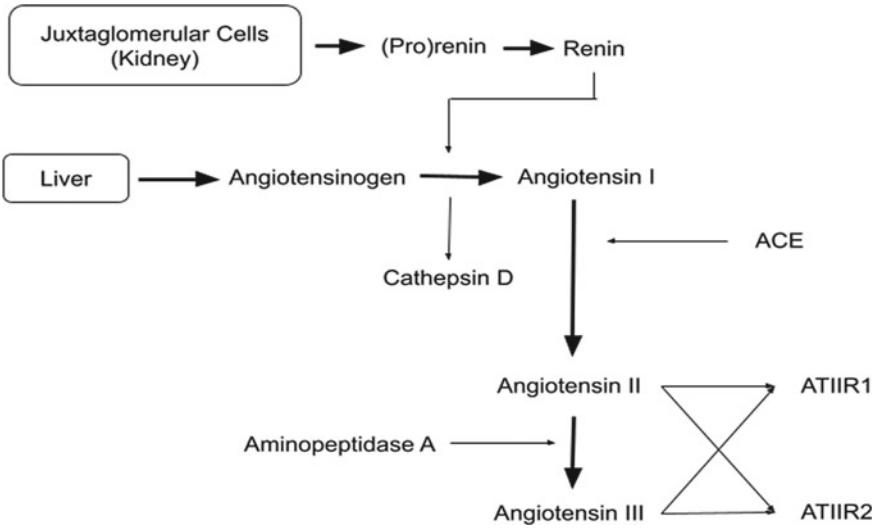
Figure 7.1 illustrates the normal physiology of the Renin Angiotensin Aldosterone System [RAAS] axis/pathways. The macula densa cells/pericytes also called as Juxta Glomerular Cells [JGC] near the afferent arteriole are sensitive to hypoxic stimuli due to a decrease in blood flow through the afferent arteriole of glomerular apparatus. In response to decrease in intraglomerular blood flow pressure and glomerular filtration, the juxtaglomerular cells of kidney secrete renin from storage granules. Renin was the first renal derived factors/hormones discovered and known to increase the blood pressure. It is the rate limiting hormone for the production of angiotensin II, known to influence the cardiovascular and renal function in synchrony to regulate the blood pressure and maintain salt and volume homeostasis in human body. Renin, also called as angiotensinogenase [340 amino acids and 37 Kda], is an aspartic protease

enzyme secreted by kidney to regulate the mean arterial blood pressure through its participation in renin angiotensin aldosterone axis [RAAS] [1]. Renin mediates its action through retention of volume of extracellular fluid (blood plasma, lymph and interstitial fluid) and arterial vasoconstriction. Though it targets the renin and prorenin receptor, it is not considered as hormone and is known to hydrolyse angiotensinogen to angiotensin I. Physiologically, f the human placenta is also known to secrete renin but its function remains unclear [15, 16].

There are only three stimuli known to affect the renin secretion viz.

1. Decrease in arterial blood pressure/decrease in blood volume sensed by pressure sensitive baroreceptors.
2. Decrease in sodium content in distal convoluted tubules sensed by macula densa of the Juxtaglomerular apparatus.
3. Stimulation of beta1 receptors of the sympathetic nervous system.

Once secreted in blood, circulating renin cleaves its substrate angiotensinogen to form the decapeptide angiotensin I (Ang I). This angiotensin I is then converted by angiotensin-converting enzyme (ACE) to angiotensin II (Ang II), the major active component of the RAS. Functionally, the major biological actions of Angiotensin II are mediated by angiotensin type 1 receptors [ATIIR1]. The excess activity of ACE-Angiotensin II-AT1 axis frequently leads to several pathophysiological changes which includes excessive renal sodium retention, excess vascular smooth muscle cell contraction and disproportionate increase in aldosterone secretion and disruption of



Abbreviations: ACE- Angiotensin Converting Enzyme, JGC-juxtaglomerular cells, ATIIR1-Angiotensin II receptor I, ATIIR2- Angiotensin II receptor 2

Fig. 7.1 Normal physiology of Renin Angiotensin System axis/pathways [RAS]

the cardiovascular responses. The pro-inflammatory, pro-thrombotic and pro-fibrotic pathways are stimulated by activation of AT1 receptor [17].

Liver Diseases

Renin Angiotensin System in Liver Disease

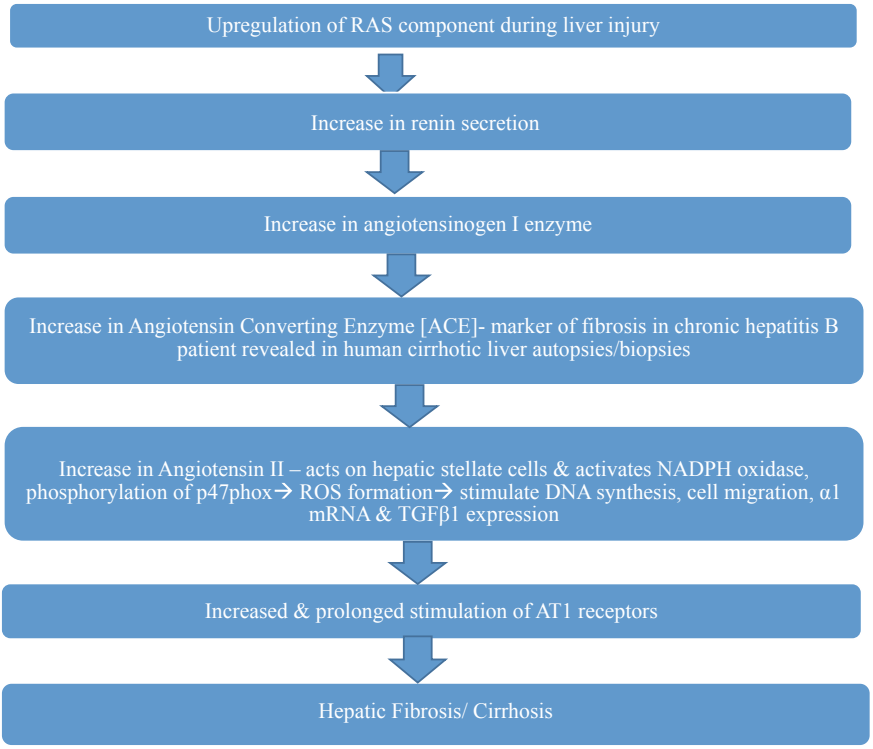
Liver diseases due to hepatitis B, hepatitis C virus infections, alcoholic liver disease and nonalcoholic fatty liver disease (NAFLD) contribute to the mortality and morbidity globally. If left untreated, all these chronic hepatitis cases may progress to the fatal end-stage liver diseases such as cirrhosis, liver failure and hepatocellular carcinoma. There has been upgradation in our knowledge about the renin angiotensin system (RAS) in the past few decades. The pro-fibrotic actions of Angiostensin II and the discovery of antagonistic Angiotensin (1-7) pathway with anti-pressor, anti-proliferative, anti-fibrotic, and pro-inflammatory effects has enlightened us about the existing two pathways in pathogenesis of various liver diseases. The concept of local RAS evolved with the findings that few components of RAS such as renin enzyme that are originally available in kidney is also present in brain and liver. Also it is hypothesized that tissue-based synthesis of Angiotensin II may be associated with independent function other than regulation of blood pressure. The involvement of local RAS has been implicated in many hepatic diseases mediated by angiotensin II which has inflammatory, oxidant, thrombotic properties with pro-fibrotic action. These fibrotic changes in liver is associated with increased hepatic vascularity followed by the portal hypertension clinically presenting as edema, ascites, hyper dynamic circulation and hepatorenal syndrome which may be restored to normal function based on the compensatory mechanism and hepatic reserve [18]. The local angiotensin-generating system is important for regulating tissue/organ functions with clinical implications via autocrine, paracrine or intracrine actions [19]. The 2-axes involved in the pathogenesis of liver diseases are:

A. The first axis consists of ACE enzyme regulating the pathway with angiotensin II as the end product that exerts its action through angiotensin type 1 (AT1) receptor.
B. The second axis consist of the ACE2-mediated hydrolysis of Angiotensin II resulting in production of Ang-(1-7) that exerts its actions on Mas receptor responsible for the vasodilatory, anti-proliferative, anti-fibrotic, and anti-inflammatory effects of Ang-(1-7).

The Role of Renin Angiotensin System in Pathophysiological Conditions Involving the Liver

Ang II effects are not limited to only vasoconstriction but it can activate AT1 receptors and induce proliferation of stellate cells in liver (Fig. 7.2). The up-regulation of transforming growth factor (TGF)-b1 in vitro contribute to the evolution of fibrotic changes in liver [20, 21].

Hepatic Fibrosis/Cirrhosis This can result from chronic hepatic injury. Liver fibrosis is a complex process triggered by several cell types and due to release of inflammatory mediators such as cytokines, chemokines and growth factors that cause disruption in normal hepatic environment. Even the RAS components are



Abbreviations: RAS-Renin angiotensin system, ACE- Angiotensin Converting Enzyme, NADPH- Reduced Nicotinamide adenine dinucleotide phosphate, p47phox-p47 phagocyte oxidase, ROS- Reactive Oxygen Species, mRNA-messenger Ribonucleic acid, DNA-Deoxyribonucleic acid, TGF β1-Transforming Growth Factor beta-1, AT1- Angiotensin receptor 1.

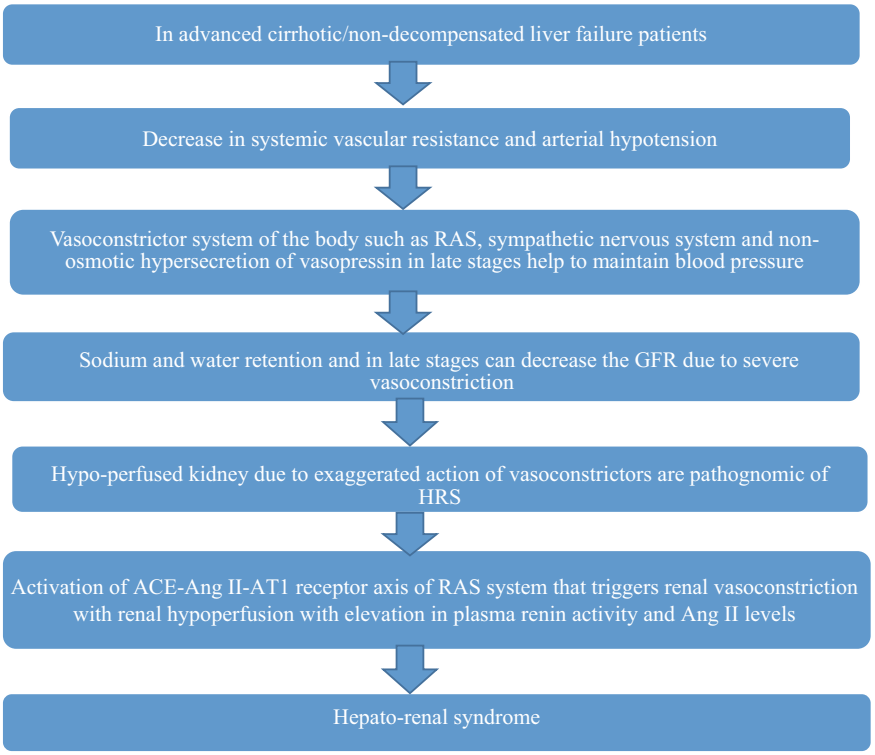
Fig. 7.2 Upregulation of RAS components in liver injury and subsequent liver cirrhosis

also involved in this process. It is well understood now that Angiotensin II is a pro-inflammatory and pro-fibrotic mediator, while Ang-(1-7) exerts the antagonistic effect in the liver tissue. AT1 receptor antagonist Candesartan and the ACE inhibitor Perindopril significantly attenuated the liver fibrosis in animal models of fibrosis. The fibrinogenic action of angiotensin II is mediated by Kupffer cells and special phagocytes present in the liver involved with the fibrotic process as these cells in liver express AT1 receptor, ATII receptor, renin, ACE receptors and other RAS components. Hepatic fibrosis was associated with RAS activation in experimental models demonstrating elevated levels of plasma renin activity, Angiotensin I, Angiotensin II and Angiotensin-(1-7). Moreover, the hepatic fibrosis condition deteriorated with use of Mas receptor antagonist, A-779 thus strengthening the protective role for endogenous Angiotensin-(1-7) [22–24].

Nonalcoholic Fatty Liver Diseases [NAFLD] It is one of the important risk factors for steatohepatitis [non-alcoholic], Type II diabetes mellitus and cardiovascular diseases. Angiotensin II is known to trigger insulin resistance, lipogenesis oxidative stress, pro-inflammatory cytokine production and fibrogenesis in the liver by activation of stellate cells. Experimental and clinical trials have demonstrated that either the inhibition of ACE-Ang II-AT1 axis or the activation of the ACE2-Ang-(1-7)-Mas axis may have a beneficial role in cases of non-alcoholic fatty liver disease [25, 26]. Pharmacotherapeutic agents such as ACE inhibitors block the ACE enzyme thereby halting angiotensin II synthesis while angiotensin receptor blockers inhibit the response of AT1 receptors to angiotensin II, both agents having demonstrated efficacy in treating chronic liver diseases [27, 28]. Thus, understanding the ACE2-Ang-(1-7)-Mas axis pathway is an emerging and promising pharmacological target for the treatment of liver diseases [29]. In the rodent model 2 weeks post bile duct ligation studies, liver injury in those rodents lacking the p47phox enzyme was minimal due to reduced synthesis of inflammatory cytokines such as TNF-α, IL-1β, IL-8, TGF-β1 and MCP-1 thereby delaying progression of liver injury to fibrosis [30]. Moreover, the ACE blocking agents such as lisinopril, losartan or NADPH oxidase inhibitors like N-acetylcysteine and diphenylene iodonium played a hepatoprotective role by preventing RAS induced fibrogenic process [31–33].

Hepatorenal syndrome [HRS] This is one of the main cause of progressive renal insufficiency in chronic liver disease and non-compensated hepatic failure patients. The pathophysiology of hepato-renal syndrome and involvement of RAS has been illustrated in Fig. 7.3. In the bile duct ligated rat models of liver injury, Pereira et al. and Paizis et al. demonstrated pro-fibrogenic activity due to increased circulating Ang II and Ang-(1-7) levels and upregulation of ACE2 enzymes respectively [34, 35]. Herath et al.*'s* experimental model on biliary fibrosis also added to the available evidence that upregulation of Mas receptor in the ACE2-Ang-(1-7)-Mas arm played a role in initiating the fibrogenic process after the onset of liver injury [36]. However, very little is known about how Ang-(1-7) affects renal hemodynamics in HRS. The

only treatment that has proven lifesaving is liver transplant as standard pharmacological interventions such as systemic use of vasoconstrictors like terlipressin and albumin use have hardly shown any survival benefit in patients. With the expanding armamentarium of RAS axis modulating agents, there is need to prove the safety and efficacy of these therapeutic agents in clinical trials.



Abbreviations: RAS-Renin Angiotensin System, GFR- Glomerular Filtration Rate, HRS-Hepato-renal Syndrome, ACE- Angiotensin Converting Enzyme, AngII- Angiotensin II, AT1-Angiotensin receptor 1.

Fig. 7.3 Role of Renin Angiotensin System in Hepatorenal syndrome [HRS]

Lung Diseases

Pulmonary Renin Angiotensin System

The circulatory RAS system is known to maintain circulatory homeostasis but the local RAS system has been investigated for its role in tissue injury and repair response. Pulmonary expression of several RAS components such as excess angiotensin converting enzyme [ACE] in lung has been associated with interstitial lung diseases. Increase ACE activity is associated with more conversion of Angiotensin I to Angiotensin II within lung circulation and parenchyma may play key role in pathogenesis of lung injury. This could possibly be due to the action of angiotensin II that increases vascular permeability, vascular tone and activation of fibroblast cells thereby reducing alveolar epithelial cell survival. This concept is supported by many researchers demonstrating the pulmonary protective role of ACE inhibitors and ARBs that attenuates the risk of lung injury in experimental lung injury models supporting its use in various parenchymal lung disease. However, further studies are needed to explore the RAS gene polymorphism and effector cell types actually affecting RAS component expression in lungs to develop treatment targets in future.

Renin Angiotensin System in Pulmonary fibrosis

Pulmonary diseases are complex with varying etiologies, countless trigger factors and each with limited disease modifying therapeutic options. Pulmonary fibrosis is a one such disease characterized by the excessive deposition of extracellular matrix (ECM) in the lung distorting the alveolar architecture in lung decreasing lung compliance, oxygen diffusing capacity ultimately affecting the normal lung function. In such cases, the triggers are allergens, chemicals, radiation and environmental particles with impaired wound healing response in the lung. Immune infiltration in lung end with release of inflammatory cytokines, chemokines and growth factors. Pro-fibrotic factors such as IL-13 and Transforming growth factor [TGF-b] in excess stimulates proliferation of interstitial fibroblast, myofibroblast and production of extracellular matrix causing lung fibrosis. In the RAS system, we know that angiotensin converting enzyme [ACE] present in the lung converts angiotensin I to angiotensin II. Angiotensin II is known to exert proliferative and pro-fibrotic actions on fibroblasts mediated through angiotensin I receptor. Pulmonary ACE enzyme and angiotensin II levels are found to be raised in broncho-alveolar fluid in fibrotic lung diseases associated with overexpression of angiotensinogen genes. Angiotensin triggers the release of two potent pro-fibrotic factors called TGF- beta and CTGF production which ultimately result in fibroblast/myofibroblast proliferation and ECM protein expression.

Table 7.1 Modulation of RAS expression by Pharmacological agents and its components

Pharmacological agents	Expression of RAS components	Effects of RAS modulations
ARBs [Angiotensin receptor blockers] or Angiotensin Converting Enzyme [ACE] inhibitors	Slight increase in the expression of both ACE and ACE-2	Increases the stimulation of AT-2r by Ang II, with vasodilating antifibrotic and antioxidant effects
Direct Renin Inhibitors [DRI]	Inhibiting the upstream RAS cascade, seems to inhibit expression and consequently decrease ACE and ACE-2 concentrations	Decrease in angiotensin II synthesis and subsequently less effect on AT1 receptor promoting antifibrotic action

Role of Pharmacological Agents: ACE Inhibitors or ATI Receptor Blockers

ACE inhibitors or ATI receptor blockers have shown anti-fibrotic action in many experimental lung fibrosis models and few in-vivo studies. In the transgenic mouse model, chronic exposure to the activated RAS system led to progressive lung fibrosis and marked reduction in pulmonary lung function independent of association with blood pressure supporting the pro-fibrotic role of RAS in the pathogenesis of pulmonary fibrosis. RAS also plays a key role in injury/repair response. Lung inflammation and injury lead to an increase in vascular permeability, vascular tone and fibroblast activity and reduces the survival of alveolar epithelial cells. Overexpression of RAS components such as angiotensin II and ACE levels contributes to the pathogenesis of interstitial lung disease as a consequence to lung injury. There is still a need to investigate key effector molecules, polymorphism of RAS genes and its association with physiological phenotypes that will suggest novel targets for future therapies. Anti-hypertensive drugs that target Ang II (angiotensin converting enzyme inhibitors [ACEIs]) or block its action on its pathological receptor, AT1 (angiotensin receptor blockers [ARBs]) are known to reduce oxidative stress and inflammation hence finding their therapeutic use in different diseases such as pulmonary fibrosis (Table 7.1), hypertension, heart failure, diabetes and coronary heart diseases [37–39].

Infectious Diseases

Renin Angiotensin System in Infectious Disease

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2 or COVID-19) infection has led global pandemic outbreak since November 2019. Many aspects of its

pathophysiology has been understood by the researchers and few aspects need further evaluation. One such association between the renin angiotensin system and SARS COV-2 infection has been explored to plan the treatment strategies. The SARS-CoV-2 virus docks at the angiotensin-converting enzyme 2 (ACE-2) receptor, a component of RAS to gain entry and infect the pulmonary cell. There is some correlation between the role of RAS modifying agents, angiotensin-converting enzyme inhibitors (ACEIs), angiotensin II receptor blockers (ARBs) and direct renin inhibitors (DRIs), with SARS-CoV-2 infection. ACE-2 enzyme is most commonly expressed in renal, cardiovascular, pulmonary and gastrointestinal tissues while the ACE activity is highly expressed in the lungs, brain and kidneys [40]. As we know the SARS-CoV-2 infection is categorized into three phases: the first being the asymptomatic or slightly symptomatic, the second is the moderate and the third is the more severe phase, characterized by hyperinflammatory state. Maximum damage is observed in the second and third phases characterized by generalized inflammatory state associated with "cytokine storm" ending up with severe lung injury and multi organ dysfunction [41]. Several studies have established the association between RAS and all stages of SARS COV-2 infection. Since ACE-2 is the membrane receptor that allows the SARS-CoV-2 virus to permeate through the epithelial lung cells, it was hypothesized that there is a higher risk of SARS-CoV-2 infection with increased expression of ACE-2 enzymes. In addition, there were variations in component of RAS enzymes during the course of different stages of SARS-CoV-2 infection with increased early stage activation followed by a decrease in the subsequent stages though the data on this is still unclear [42, 43]. The activation of RAS system is known to affect the patient condition or disease severity in COPD, asthma, viral infections and in smokers. In the SARS-CoV-2 viral infection, the physiological balance of the RAS system especially ACE/ACE-2 is disturbed. The lung lesion leads to activation of inflammatory cytokines that can further worsen the lung function. ACE/ACE-2 modulating pharmacological agents could pave the path to treatment of infectious lung disease. However, it may be possible that ACE-2 has a protective effect and when it decreases, there is a worsening of the inflammatory state of the lungs. It should also be noted that Ang II, Ang 1–7 and Ang 1–9 have different biological effects. Angiotensin II are vasoconstrictor and known to stimulate aldosterone release that remains the underlying cause of the myocardial hypertrophy, interstitial fibrosis, endothelial dysfunction, increase of inflammation state, oxidative stress and increased of coagulation that may complicate the survival outcomes in SARS-CoV-2 infection [44] The pathogenic effects of Angiotensin II is mediated by AT-1 receptors (AT-1r) that trigger excessive release of IL-6, TNF (Tumor Necrosis Factor) and other inflammatory cytokines [45–47]. On the contrary, Ang1-7 has antagonistic effects to angiotensin II mediating its agonistic action through Mas receptor (MASr) located on surface of bronchial muscle and alveolar epithelium of lungs and Type II receptors of Ang II (AT-2r). Ang 1–7 also acts on the MASr as an agonist present on platelets and exerts its anti-aggregating effect through the release of excess prostacyclin [48, 49]. A pharmacological approach modulating RAS could be of clinical benefit to fight SARS-CoV-2 infection. Pharmacological agents such as angiotensin converting enzyme inhibitors [ACEIs], angiotensin receptor blockers [ARBs] and

direct renin inhibitors [DRIs] have variable effects on the RAS components. The controversy still ongoing regarding whether increased ACE-2 using ACEIs or ARBs could be a risk factor for COVID-19 infection but the epidemiological data do support any association between the use of ACEIs or ARBs and increased likelihood of infection or complications of SARS-CoV-2. However, the positive aspect of increase in ACE-2 in more severe SARS-COV-2 infection may have survival benefit. This is because increase in ACE-2 lead to increase in synthesis of Ang 1–7 and Ang-1–9 that are linked to vasodilating, antifibrotic and anti-inflammatory properties. At present, there is no evidence that ACEIs or ARBs should be discontinued, because they are not the risk that can increase SARS-CoV-2 infection, but activation of the Ang II-AT-1r axis promotes lung damage, while increasing ACE-2 and activating the Ang 1–7/MASr axis may protect against lung damage. RAS modulating agents may have beneficial effect if administered at the right time or early stage of SARS-COV-2 Infection. More clinical studies are necessary to confirm these hypotheses.

Cancers

Cancer Stem Cells and Role in Pathogenesis of Cancer

The renin angiotensin system dysregulation has become subject of research interest to explore its carcinogenic potential. The concept proposed for the tumorigenic spread of cancer cells is based on the presence of Cancer Stem Cells [CSCs]. Cancer stem cells are produced due to either mutation in embryonic stem cells [ESCs]/progenitor cells or due to epigenetic/environmental factors. The complexity behind is the ability of these cancer stem cells within tumor to replicate and constitute the tumor bulk. Cancer stem cells have been characterized in different types of cancer such as pancreatic cancer, myelogeneous leukemia, breast cancer, oral and buccal mucosa cancer, squamous cell cancers and CNS tumors like glioblastoma multiforme. The loss of control/ disturbance of localized paracrine action of RAS system has been implicated for the abrupt start of cancer cell production. There is reduced risk of cancer in patients on Angiotensin Converting Enzyme [ACE] inhibitors supporting the hypothesis of preventive role of RAS antagonism in development of cancers. Moreover, these CSC cells are identified in these cancers confirming the role of paracrine RAS system present in these Cancer stem cells. This could be either due to normal cells turning

pluripotent aberrantly or differentiated cells get converted to de-differentiated stem cell like phenotype due to one or mutated genes [50–52].

Renin-Angiotensin System in Cancers

The renin angiotensin system plays a crucial role that may affect tumor growth and tumor microenvironment. RAS is expressed in many tissues including various human cancers and cell lines where it acts locally and mediates the cellular proliferation, growth and metabolism irrespective of the functional systemic RAS system. RAS components are also expressed in many cell types of the tumor microenvironment, such as endothelial cells, monocytes, macrophages, neutrophils, fibroblasts, dendritic cells, and T cells. RAS signaling in any of these cells can either facilitate or hinder growth and/or dissemination and has been shown to affect cell proliferation, cell migration, invasion, metastasis, apoptosis, angiogenesis, cancer-associated inflammation, immunomodulation, and tumor fibrosis. RAS inhibitors [RASi] used in the treatment of cardiovascular diseases activate the immunostimulatory pathways in solid metastatic cancers. The role of RASi in various cancer pathogenesis has been investigated in many retrospective and prospective studies. RASi has shown improved survival outcomes in patients of pancreatic ductal adenocarcinoma [PDAC] [53, 54]. The presumed mechanism hypothesized is via stimulation of the tumor's immune microenvironment, extracellular matrix changes and decreasing the risk of cells turning malignant. The AngII/AT1R axis conditions the tumor microenvironment and it has been found that AT1 receptor overexpression is associated with tumor growth progression with poor prognostic features (increase in tumor size, higher grade, and more vascular density) with worst outcomes. The AngII/AT2R and Ang(1-7)/MAS signaling works exactly the opposite interfering with tumor cell growth and proliferation by modulating desmoplasia, vasculature, inflammation, and immune cells [55]. Thus RASi drugs given as adjunct with immunotherapy may affect either cancer pathway restricting cancer growth and progression [56, 57]. The variable of expression of RAS components and its carcinogenic potential is elaborated in Table 7.2 [58–61].

Table 7.2 Overview of role of RAS components as hallmarks for carcinogenesis

RAS factors affecting the cancer risk	Enzyme expression in tissue	Physiological function	Enzyme or receptor activity	Carcinogenesis risk	Clinical implications
Prorenin [PRR] and renin receptor	Juxtaglomerular cells [JGCs] of kidney secrets both PRR is abundant in heart, brain, placenta, visceral and subcutaneous adipose tissue, liver CSCs in oral cavity cancers and GBM	Regulates blood pressure by stimulating release of angiotensinogen from liver Brain development and embryogenesis Wnt/b catenin signaling pathway	Alteration in Wnt/b catenin pathway [increased signaling]	Increases risk	Congenital birth defects, neoplastic and degenerative diseases, proliferation of cancer stem cells Increased Wnt signaling associated with risk of pancreatic ductal carcinoma due loss of control over cell proliferation
Angiotensin converting enzyme [ACE] or CD143	Cellular marker for primitive hemopoetic cells called hemangioblasts Endothelium of microvessels in cancerous stromal tissue Lung capillaries and epithelial cells of kidney Infantile hemangioma	Regulation of blood pressure by inhibiting conversion from Angiotensin I to Angiotensin II Angiogenesis Inflammation	Overactive ACE enzyme	Increase cancer growth and progression	ACE enzyme DD and TT allele [homozygous] associated with highly active ACE and increase cancer risk
			Inhibited or low ACE enzyme activity	Anti-cancer effects	ACE enzyme allele II and AA [homozygous] associated with low activity of ACE and low cancer risk Patient on ACE inhibitors [ACEi] have low cancer risk eg. Captopril, Perindopril

(continued)

Table 7.2 (continued)

RAS factors affecting the cancer risk	Enzyme expression in tissue	Physiological function	Enzyme or receptor activity	Carcinogenesis risk	Clinical implications
Angiotensin II receptor I [ATIIR1] [G protein coupled receptor]	RAS dysregulation role in VEGF expression and angiogenesis in cancer tissues Connective stromal tissue and tissue-associated macrophages [TAM] surrounding tumor Downstream signaling lead to vasodilation, hypertrophy and NF-kB activation with TNF-A and PAI-1 expression	Binds both angiotensin II and angiotensin III [more functions] Regulation of blood pressure, cellular growth and fibrosis, migration, angiogenesis, metastasis, inflammation, glucose release from liver, increased triglycerides synthesis and reduced gluconeogenesis	Overexpression	Estrogen receptor positive Breast cancer tumors [20%] [No risk in those with Erbb2 overexpression] Squamous cell cancers [oral cavity] Tumor invasiveness in ovarian and cervical cancers GFAP + GBM cancer stem cells	Increase VEGF levels within cancer tissue triggers angiogenesis and prevent immune cell infiltration Poor overall patient survival and progression to high grade cancers
			Under expression or low activity	ARB blockers users eg. Candesartan, Irbesartan prevented metastasis from colon cancer to lung	reduce the tumor size, vascularization, metastasis and low VEGF levels
Angiotensin II receptor II [ATIIR2]	Stem cells in brain Cancer stem cells within tumor nest cells and SOX2 + CSCs of GBM And micro vessels of endothelium	binds with both Angiotensin II and III Associated with Vasodilation, growth inhibition, natriuresis, nervous tissue growth Normal fetal development Counteracts with the ATIIR1 effects in cancer and cardiovascular health Apoptosis of cancer cells via extrinsic pathway with use of caspase 3	Overexpression	Decreased risk of cancer	Gene coding for ATIIR2 interacting proteins [ATIPs] inhibit EGFR-mediated proliferation and identified as putative tumor suppressor gene
			Low expression or activity	Increase in cancer risk or no beneficial effect	ATIIR2 knock-out mice reported faster growth of tumor vasculature and pancreatic ductal carcinoma size implanted subcutaneously

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Abbreviations

RAS	Renin Angiotensin System
HPV	Human Papilloma Virus
WHO	World Health Organization
COVID	Coronavirus Disease
HIV	Human Immunodeficiency Virus
AIDS	Acquired Immunodeficiency Syndrome
RAAS	Renin Angiotensin Aldosterone System
JGC	Juxta Glomerular Cells
ACE	Angiotensin Converting Enzyme
Ang I	Angiotensin I
Ang II	Angiotensin II
Ang-[1-7]	Angiotensin-[1-7]
ATI	Angiotensin type I receptor
ATII	Angiotensin type II receptor
ACE	Angiotensin Converting Enzyme
NAFLD	Non Alcoholic Fatty Liver Disease
TGF	Transforming Growth Factor
MCP	Monocyte Chemoattractant Protein
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
ADH	Anti-Diuretic Hormone
HRS	Hepatorenal Syndrome
ECM	Extracellular Matrix
IL	Interleukin
ACEIs	Angiotensin Converting Enzyme Inhibitors
ARBs	Angiotensin Receptor Blockers
SARS-CoV-2	Severe Acute Respiratory Syndrome- Coronavirus-2
DRIs	Direct Renin Inhibitors
RNA	Ribonucleic acid
COPD	Chronic Obstructive Pulmonary Disease
TNF	Tumor Necrosis Factor
MASr	Mas receptor
CSCs	Cancer Stem Cells
ESCs	Embryonic Stem Cells
CNS	Central Nervous System
RASi	Renin Angiotensin System Inhibitors

PDAC	Pancreatic Ductal AdenoCarcinoma
MAS	MAS1 oncogene
CTGF	Connective Tissue Growth Factor
ROS	Reactive Oxygen Species
GFR	Glomerular Filtration Rate

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Chapter 8
Changes in Renin Angiotensin System (RAS) in Cancers and Lung Diseases: Application of Biosensors for Monitoring These Changes

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Abstract In humans, almost every organ has varying degrees of the functional renin-angiotensin system (RAS). Increased RAS activity has been shown in various tumors, including kidney, prostate, bladder, stomach, cervix, brain, pancreas, colon, lung, liver, skin, and hematopoietic cancers. Although there are controversial data, most

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experimental studies show that angiotensin receptor blockers (ARBs) have anti-proliferative effects in breast cancer, induce cell death in pancreatic cancer, and ameliorate liver metastases in colon cancer. Improve in survival rate in non-small cell lung cancer patients is another finding in this regard. In addition, angiotensin-converting enzyme (ACE) inhibitors (ACEI) may decrease the risk of developing esophageal cancer. In recent decade, biosensors have been widely used in biomarkers detection worldwide as the most reliable, fast, and precise analytical method. Many approaches have been developed, each with its distinct advantages and limitations.

Keywords Cancer · Genetics · Epigenetics · Biosensor · RAS · Renin · Angiotensin · Lung

Abbreviations

ACE	Angiotensin converting enzyme
ACEI	Angiotensin converting enzyme inhibitor
ADAM17	ADAM Metallopeptidase Domain 17
AGT	Angiotensinogen
AGTR1	Angiotensin II receptor type 1
AngII	Angiotensin II
ARB	Angiotensin receptor blocker
ARDS	Acute respiratory distress syndrome
ATR	Angiotensin receptor
CYP11B2	Aldosterone synthase gene
DALY	Disability-adjusted life year
EZH2	Enhancer of zeste homolog 2
HAT1	Histone Acetyltransferase 1
HDAC2	Histone deacetylase 2
HTN	Hypertension
I/D	Insertion/Deletion Polymorphism
I/I	Insertion/Insertion
KDM	Lysine demethylase
miRNA	MicroRNA
ORF1ab	Two open reading frames a and b
PRR	Pattern recognition receptor
RAAS	Renin-angiotensin-aldosterone system
RAB1A	Ras-related protein Rab-1A
RAS	Renin-angiotensin system
REN	RASS Gene Encoding Renin
rS	Reference single nucleotide polymorphism identification
SNP	Single Nucleotide Polymorphism
SPR	Surface Plasmon Resonance
VEGF	Vascular endothelial growth factor

Introduction

Cancer has the highest burden in years of disability-adjusted life years (DALYs) among all human diseases. The overall risk of cancer between 0 and 74 is 20.2%. A total of 18 million new cases of cancer were diagnosed in 2018, the most common of which are lung (2.09 million), breast (2.09 million), and prostate (1.28 million) cancers. Every year about 8.97 million deaths happen due to cancer worldwide. This number makes cancer the second cause of death. Moving on with the same situation will probably make cancer the first cause of death in the world in 2060. Lung, liver, and gastric cancers are the three most fatal cancers. Prostate and thyroid cancers have the best prognosis, with a 5-year survival of 100%, while esophageal, liver, and especially pancreatic cancers have the worst prognosis, typically < 20% at five years [1].

Cancer in Canada

Cancer has a significant impact on Canada's population and health care system. About half of Canadians are expected to get cancer in their lifetime. Although standardized cancer deaths have dropped significantly since reaching their peak in 1988, cancer remains Canadians' leading cause of death [2, 3].

In 2020, about 225,800 new cases of cancer were diagnosed in Canada. Lung and bronchial (lung) cancers are the most commonly diagnosed, with about 29,800 cases, followed by breast cancer (27,400), colorectal cancer (26,900), and prostate cancer (23,300). These four cancers are about half (48%) of all cancers diagnosed in 2020 [4].

Cancer is a costly disease. Cancer care costs in Canada increased from $ 2.9 billion in 2005 to $ 7.5 billion in 2012. Due to the increasing number of cancer diagnoses, the costs of treating cancer patients, supporting their families, and the health care system, in general, are increasing [5].

The health and economic burden of cancer is increasing worldwide. It can be considered that there is a 'cancer epidemic' [5].

RAS and Cancer

Renin-angiotensin system (RAS), especially angiotensin (AT), plays the leading role in regulating blood pressure and aldosterone secretion. Angiotensin regulates cell turnover by accelerating cell proliferation and planned cell death in many tissues, including the cardiovascular system, adrenal cortex, kidney, liver, muscles, and connective tissue [6].

It has recently been suggested that in epidemiological and experimental studies, RAS activation and occlusion are associated with different types of cancer. Various studies have also shown that RAS blockade is protective in some cancers. However, with cancer, conflicting data also showed that RAS obstruction was either unrelated to cancer or related to cancer. Although the reasons for these findings are unclear, different types of receptors and actuators in RAS may explain these findings [7].

In humans, almost every organ has a functional RAS of varying degrees [8]. Different types of cancers currently express angiotensin receptors, especially AT1 and AT2 receptors expressed in breast cancer [9]. AT1 receptor is increased in some kinds of hyperplastic tissues and cancer [10]. Angiotensin polymorphism, AT1 receptors, and angiotensin-converting enzyme (ACE) are associated with the risk of breast cancer [11]. ACE polymorphism may increase benign prostate hyperplasia risk and prostate cancer, while A1166c substitution in the AT1 receptor only increases the benign prostate hyperplasia risk [12].

On the other hand, C allele carriers decrease breast cancer [13]. Three AT1 substitutions (T825A andA168G, C535T) receptors reduce breast cancer risk [11]. Patients with breast cancer with low ACE expression have worse outcomes than those with high ACE expression [14]. A low dose of losartan and the other RAS inhibitors effectively improve outcomes in patients with advanced pancreatic cancer [15].

Contrary to patients' data, ACE inhibitions and ACE receptor antagonists in vitro conditions inhibit the growth of many tumoral cells, including breast cancer [16].

Overexpression of the AT1 receptor is associated with advanced tumors (large, high-grade, more vascularization tumors) and poor outcomes [16–20]. RAS elements are expressed in many tumoral cells, such as endothelial cells, fibroblasts, monocytes, macrophages, neutrophils, dendritic cells, and T cells [21–26].

Some studies showed that RAS could have a role in liver fibrosis; for example, angiotensin II is overexpressed in the liver fibrosis process [27]. Hepatic stellate cell activity and angiotensin secretion are essential factors in exacerbating the process of liver fibrosis, and liver fibrosis underlies hepatocellular carcinoma. Inhibiting hepatic stellate cell activity and angiotensin II secretion considerably reduces the liver fibrosis process. For example, short-term treatment with losartan reduces liver fibrosis [28, 29].

A cohort study reported that ACE inhibitors (ACEIs) were associated with reducing cancer prevalence [30]. Local RAS causes angiogenesis and proliferation by expressing vascular endothelial growth factor (VEGF) or endothelial growth factor receptor [31]. RAS inhibition causes apoptosis in pancreatic cancer cells [32, 33]; therefore, using ACEI and angiotensin II receptor blockers (ARB) inhibits tumor growth in patients with pancreatic cancer. In a study that was done on patients with pancreatic cancer from 2001 to 2009, the patients were divided into three groups; ACEI/ARB group (the patients who used ACEI or ARB for high blood pressure), Non—ACEI/ARB group with high blood pressure (the patients who used blood pressure medications except for ACEI/ARB), Non- HTN (the patients who did not use any medication for blood pressure). This study showed that ACEI/ARB was associated with more prolonged progression-free survival and overall survival in patients with advanced cancer who were under monotherapy with gemcitabine. These data suggest

that RAS inhibition in patients with pancreatic cancer may inhibit tumor growth and improve survival. Besides, there was no meaningful difference in survival between the patients using ACEI and ARB [15].

A pilot study reported that ARBs had cytostatic activity in hormone-refractory prostatic cancer by decreasing PSA levels [34]. In another study, adding ACEI/ARB to chemotherapy based on platinum was associated with more prolonged survival in non-small cell lung cancer patients [35]. In a study, ACEI, in combination with vitamin K inhibited the relapse of hepatocellular carcinoma [36].

The expression of AT1R is strongly associated with ovarian tumors and histological classification; AT1R is highly expressed in invasive ovarian adenocarcinoma and borderline tumors, but it is expressed in a few benign cystadenomas [37].

RAS inhibitors, especially ACEI and AT1R blockers, considerably improved the general survival of patients with renal clear cell carcinoma (RCCC) and increased the effectiveness of treatments with VEGF [38–40]. In a study, five elements of RAS in RCCC were seen (renin, Pro Renin Receptor, ACE2, AT1R, and AT2R); considering immunohistochemistry, AT2R was positive in all 15 RCCC patients. Detection of positive immunohistochemistry for pattern recognition receptor (PRR) and ACE2 with western blot and RT-q PCR was proved in the sample of six patients with RCCC. AT2R expression was confirmed in the sample of 4 patients with western blot. Renin expression with RT-q PCR was confirmed [41]. A summary of RAS's contribution to cancers is illustrated in Table 8.1.

Genetic and Epigenetic Factors Regulating RAS in Lung Disease

Genetic Factors

Angiotensin I-converting enzyme

Increasing D allele frequency significantly reduced the prevalence of covid -19 infection but increased mortality rates in many parts of the world [70–72]. The European population has a higher incidence of SARS-CoV-2 infection and higher mortality rates [73]. As ACE DD genotype is higher in frequency in the European population than in Asians, higher mortality rates in the European population might likely be explained by ethnic differences in allele frequency of ACE I/D polymorphism [74].

Increased I/I genotype frequency was inversely correlated with Covid-19 morbidity and mortality in different populations [73, 75].

Table 8.1 Renin-angiotensin system (RAS) involvement in various cancers

Cancer type	Renin-angiotensin system	Change	Citations
Colorectal cancer	AT1R, AT2R	Upregulated	Mehranfard et al. [42]
hepatocellular carcinoma *(Liver)*	AT1R, ACE2	Enhanced migration and invasion, downregulation	Ziaja et al. [43] Huang et al. [44]
Breast cancer	AT1R, AT2R	Upregulated	Mehranfard et al. [42]
Uterine corpus endometrial carcinoma	ACE, ACE2, AGT	Downregulation	Cu et al. [45]
Pancreatic cancer	AT2R, ATII	Upregulated	Mehranfard et al. [42]
Endometrial cancer	AGTR1, ACE1, ACE2, ATP6AP2	Increase	Delforce et al. [46]
Lung cancer	AT2R	Upregulated	Danial Mehranfard et al. [42]
Cervical carcinoma	AGTR1, ATIIR1,ATIIR2	Increase, localized	Zhang et al. [47] Shivapathasundram et al. [48]
Ovarian cancer	AGTR1, ANGII	Increase	Zhang et al. [47]
Skin cancer	AGTR1	Increase	Zhang et al. [47]
Prostate cancer	ARBs, ACE	Increase	Uemura et al. [49]
Gastric cancer	ACE, AT1R	Inhibitors	Hashemzehi et al. [50]
Pituitary gland	AngII, ATRs, AT1R, AT2R, ACEIs	Downregulation	Ziaja et al. [43]
Adrenal gland	AT1R, AT2R	Localized	Ziaja et al. [43]
Adrenocortical	AGTR2	Downregulation	Cui et al. [45]
Kidney cancer	AT1-R, AT2-R	Decrease	Sobczuk et al. [51]
Renal cell carcinoma	ACE, PRR, ACE2, AT1R, and AT2R	Localized, downregulation	Siljee et al. [52]
Brain and glioblastoma	PRR, ACE, ATIIR1 and ATIIR2 AGTR1, AGTR2, ACE, ACE2	Localized Downregulation	Wickremesekera et al. [53] Cui et al. [45]
Head and neck metastatic malignant melanoma	AT1R, AT2R, PRR and ACE	Localized	Siljee et al. [54]
Testicular tumor	ACE	Localized	Cui et al. [45]
Epididymis	ACE, AT1 receptors	Localized	Leung et al. [55]
Lymphoma	ACE, AT1R l	Increase	Haznedaroglu [56] Zhang [57]
Leukemia	ACE, Ang-II	Development	Turk et al. [58]
Oropharynx cancer	ACEIs, ARBs	Improved	Magnuson et al. [59]

(continued)

Table 8.1 (continued)

Cancer type	Renin-angiotensin system	Change	Citations
Laryngeal cancer	ACE	Upregulation	Sun et al. [60]
Esophageal cancer	ACEIs, ARBs	Decrease	Chen et al. [61]
Thyroid cancer	ACE2	Increase	Sadoughi et al. [62]
Urothelial cancer	ACEI, ARBs	Regression	Skelton et al. [63]
Osteosarcoma	AGTR1	Decrease	Zhao et al. [64]
Retina	ANGII	Upregulation	Forrester et al. [65]
Gallbladder	ACE2	Increase	Walther et al. [66]
Thymus	ACE2	Increased	Zemlin et al. [67]
Spleen	AGTR1	Increased	Renziehausen [68]
Oral tongue squamous cell carcinoma	ATIIR1 and ATIIR2, ACE	Localized	Itinteang [69]

ACE2 genetic variants

Genetic variants of ACE2 are associated with hypertension, coronary heart disease, and other cardiovascular events in different races and ethnicities [76].

The allele frequencies of expression quantitative trait loci are significantly associated with ACE2 expression suggesting that susceptibility or response to SARS-CoV-2 could vary in populations [77].

Darbani et al. in 2020 reported that 13 ACE variants could boost the interaction between ACE2 and S1 protein. Among them, rs73635825 (S19P), rs1244687367 (I21T), rs4646116 (K26R), rs781255386 (T27A), rs1199100713 (N64K), and rs142984500 (H378R) variants significantly differed between the Europeans and Africans [78].

Consequently, N720D carriers have an increased S protein binding and viral entry caused by enhanced interaction between ACE2 and transmembrane serine protease 2 (TMPRSS2) [79].

rs143936283 (E329G) and rs73635825 (S19P) alleles changes may confer resistance against the SARS-CoV-2 attachment with the human ACE2 receptor [80]. K26R and I468V variants may affect the binding characteristics of the S protein of the virus and the hACE2 receptor [81].

p.(Asn720Asp), p.(Lys26Arg), and p.(Gly211Arg) missense variants were common and predicted to interfere with ACE2 protein structure and stabilization, whereas p.(Pro389His) and p.(Leu351Val) variants were rare and predicted to interfere with SARS-CoV-2 spike protein binding [82].

Contrary to these results, Novelli et al. reported there was not a significant association between ACE2 variants such as c.1888G > C p.(Asp630His) and Covid-19 severity in Italian populations [83]. Also, there are conflicting results for ACE2-S1 protein-binding dynamics [78, 82, 84]. A summary of RAS components' contribution in COVID-19 is illustrated in Table 8.2.

Table 8.2 Renin-angiotensin system (RAS) component involvement in COVID-19

RAS component	Genetic changes COVID -19		
	Variation, minor allele frequency (%)	Main finding	Citations
ACE2	S19P I21V, I21T, E23K, A25T, K26R, T27A, E35D, N64K, E75G, T92I, Q102P, H378R	• Interaction booster between ACE2 and S1 protein, H378R, and S19P were identified as specific variants of Europeans and Africans	Darbani [85]
	E35K, E37K, Y50F, N51D, N51S, M62V, K68E, F72V, M82I, G326E, E329G, G352V, D355N, Q388L, P389H, H505R, R514G/*, Y515C	• Interaction-inhibitor variants, • Q388L and M82I were identified as specific variants of Americans and Africans	Darbani [85]
	R219C, R219H, M383T, P389H, D427Y, R514G, R708W, R710C, R710H, R716C, L731F, R768W	• The distribution of deleterious variants varies among different populations • 39% and 54% of deleterious variants occur in Europeans and Africans	Hou et al. [86]
	N720D	• N720D variant impacted the stability and the flexibility of ACE2, led to more favorable site for binding and cleavage of TMPRSS2	Mohammad et al. [79]
	S19P, E329G	• S19P, K26E, and M82I might unfavorably have impacts on the stability of the encoded protein • S19P and E329G illustrated evident dissimilarities in interactions with the S protein	Hussain et al. [80]

(continued)

Table 8.2 (continued)

RAS component	Genetic changes COVID -19		
	Variation, minor allele frequency (%)	Main finding	Citations
	K26R	• Mutated more often in Non-Finnish Europeans, • Increased the binding free energy, and to some extent decreased the binding affinity	Li et al. [87]
	I468V	• Mutated more often in East Asians • Increased the binding free energy, and to some extent decreased the binding affinity	Li et al. [87]
	N720D, K26R, G211R	• Predicted to affect protein structure and stabilization	Benetti et al. [88]
	L351V, P389H	• Predicted to affect SARS-CoV-2 spike protein binding	Benetti et al. [88]
	K26R, I21V, E23K, T27A, N64K, T92I, Q102P, H378R, S19P	• Predicted to rise susceptibility	Stawiski et al. [89]
	K31R, N33I, H34R, E35K, E37K, D38V, Y50F, N51S, M62V, K68E, F72V, Y83H, G326E, G352V, D355N, Q388L, D509Y	• Putative protective variants projected to indicate reduced binding to S-protein	Stawiski et al. [89]
	c.439 + 4G > A intronic, N630H, N720D	• Three germ line variants recognized in Covid-19 patients • No association of ACE2 variants with Covid-19 severity	Novelli et al. [90]

(continued)

Table 8.2 (continued)

RAS component	Genetic changes COVID -19		
	Variation, minor allele frequency (%)	Main finding	Citations
	K26R	• Most frequent in the Ashkenazi Jewish population, and lowest frequent in the Asian populations • Decreased the binding energy	Ali et al. [91]
	I468V, R219C, K341R, D206G, G211R	• Increased the electrostatic attraction • Most frequent in East and South Asians, and European populations	Ali et al. [91]
	H378R	• Direct weakening of the catalytic metal binding atom to diminish activity of ACE2	Guo et al. [92]
	S19P	• Alters the vital helix of the S-protein	Guo et al. [92]
	G211R, N206G, R219C, R219H, K341R, I468V, S547C	• Might interfere with secondary structures	Guo et al. [92]
	G405E, W461R, F588S	• Destabilized the structure of the bound complex	Khalid and Naveed [93]

Epigenetic Factors

Epigenetic mechanisms regulate gene expression and protein levels through several pathways, including DNA methylation, histone modifications, and noncoding RNA-mediated mechanisms [94].

Epigenetic Regulation of ACE2

Histone modifications

Many potential regulators of ACE2 in the human lung, including genes related to histone modifications, such as *KDM5B* (lysine demethylase 5), specific histone acetylation (H3K27ac), and histone methylation (H3K4me1 and H3K4me3) dynamics were regulated ACE2 expression. ACE2 was highly expressed in critical Covid-19

cases [95]. Thus, ACE2 might be epigenetically upregulated in the lungs of severe Covid-19 patients and during lung cancer and COPD [95].

KDM5 demethylases might be considered potential targets for COVID-19 prevention [95]. H3K27me3 acts as a repressive mark and is regulated by the action of histone methyltransferase EZH2. This enzyme's loss of function enhances ACE2 expression by downregulating the H3K27me3 repressive mark in mouse germline cells [96].

The transcriptomic and system biology approach revealed a close association between induced ACE2 expression with RAB1A, HAT1, HDAC2, and KDM5B in COVID-19 patients with comorbidities such as hypertension, diabetes, and chronic obstructive lung disease [95].

Several histone deacetylases have also been reported regulating ACE2 function. For example, silent information regulator T1 (SIRT1), a histone deacetylase (HDAC) class III, affects ACE2 expression after interacting with its promoter. Hence, SIRT1 impacts viral entry over the host cells [97].

DNA methylation

DNA methylation is an epigenetic modification associated with various clinical conditions, such as cancer and asthma. ACE2 overexpression is regulated by DNA hypomethylation mediated through several CpG sites in the ACE2 promoter region proximal to the transcription start site, the 5'-UTR, and the 3'-UTR epigenetic dysregulation increases the risk and severity of SARS-CoV-2 infection in lupus patients [98]. There are three CpGs (cg04013915, cg08559914, cg03536816) at the ACE2 promoter region with a lower expression in lung epithelial cells. Data revealed a correlation between smoking habits and gender-specific ACE2 methylation status. Female smokers likely to be infected by SARS-CoV-2 have hypomethylation of two CpG sites (cg23232263, cg16734967) for ACE2 in lung tissue compared to male smokers [99]. Nasal ACE2 DNA methylation reflects differences in sex, race/ethnicity, and biological aging [100].

The microRNA regulation of ACE2

The avian influenza virus, SARS-CoV, and the respiratory syncytial virus can downregulate the ACE2 expression associated with acute lung injury or acute respiratory distress syndrome (ARDS) [101]. This downregulation of ACE2 protein expression might be mediated by miRNAs [101]. Also, the avian influenza virus can induce the upregulation of miR-200c-3p, which downregulates ACE2 protein expression. In severe pneumonia patients, increased plasma concentrations of miR-200c-3p, thus blocking the function of this miRNA, might be a potential therapeutic target [101]. Another mechanism for progression of ARDS is enhancing miR-200c-3p, that leads to the downregulation of a lncRNA (lncGAS5) and reduction of ACE2 expression [102]. Also, the levels of circulating miR-421 has an inverse association with ACE2 expression in patients with chronic kidney disease [103].

Another microRNA named miR-1246, mediates pulmonary endothelial dysfunction and apoptosis in vitro and lung inflammation and permeability in vivo by targeting ACE2; therefore, this miRNA is a potential therapeutic target for ARDS [104].

let-7c-5p targeted the *ORF1ab* gene in SARS-CoV-2 contributing to H1N1 influenza A suppression by targeting its M1 protein [105].

Several microRNAs contributing to ACE2 modulation might be considered a potential clinical target in pulmonary disorders, including miR-1246 [104], miR-200c-3p [101], miR-421 [106], and let-7b [107].

ADAM17 downregulates by miR-145, significantly expressed in the lungs and heart, is contributed to promoting the shedding of ACE2, thereby may provide a strategy to interfere with SARS-CoV-2 entry into the cells [108].

For example, hsa-miR-125a-5p is involved in ACE2 regulation in various tissues like the lung, kidney, and esophagus. Some factors also regulate the transcription of these microRNAs. This report explained the involvement of lysine-specific demethylase 5B (JARID1B) in transcription repression machinery of hsa-let-7e/hsa-mir-125a microRNAs, and miR-200 family (including miR-141, miR-200a, miR-200b, miR-200c, and miR-429) by inducing H3K4me3 histone repressive mark in the regulatory regions of microRNAs and hence downregulates their transcription. This finally triggered an induced ACE2 expression as microRNAs target 3′ UTR of ACE2 mRNA [109]. Table 8.3 demonstrates epigenetic changes in RAS in lung diseases.

Biosensors

A biosensor could be a device that can measure biological or chemical reactions. The device generates signals based on the concentration of the target analyte in the reaction. Biosensors might be used in various ways, such as monitoring diseases, detecting disease indicator markers, delivery of drugs. A typical biosensor entails an analyte, bioreceptor, transducer, electronics, and display. The analyte is the substance of interest. For a biosensor designed to detect glucose, glucose would be the analyte. Bioreceptor is a molecule that recognizes the analyte, and this recognition is precise. When bioreceptor and analyte interact, a signal is generated in various forms. Heat, charge, PH, light, and mass change are some examples of signals. The transducer is responsible for converting one form of energy into another. The goal is to convert the recognition signal into a measurable one. The energy conversion process is named signalization. Electronics process the measurable signal and make it ready for display. It converts signals from analog form into digital form. The display is a user interpretation system that helps in data visualization and makes the data understandable. The output signal on display can be numeric or graphic. Biosensors research is attracting enormous interest from its application in clinical treatment, pharmacy, biomedical, and healthcare sectors. Biosensors are successfully implemented for disease identification, prevention, rehabilitation, patient health surveillance, and human health

Table 8.3 Epigenetic changes in RAS in lung diseases

RAS element	Epigenetics changes	Main finding	Citations
ACE2	DNA methylation	• Variability of ACE2 DNA methylation profiles of human lung tissues in men and women • Significant hypomethylation in females compared to males	Corley and Ndhlovu [99]
		• Significant hypomethylation in the ACE2 gene • Higher ACE2 messenger RNA expression in lupus patients	Sawalha et al. [110]
		• Expression of ACE2 expression being regulated by DNA methylation dynamics	Sawalha et al. [110]
		• Sex variances in the methylation of specific CpG sites in ChrX: 15,621,573–15,622,147 in healthy cases	Mahrooz et al. [109]
		• ACE2 hypomethylation in nasal epithelium varies in different genders, ethnicities, and during biological aging • ACE2 hypomethylation in nasal epithelium in black males might lead to SARS-CoV-2 infectivity and severity	Cardenas et al. [111]
		• Decreased DNA methylation of CpG (cg085599149) near the ACE2 transcription start site (TSS200 region) then Increased ACE2 expression	Corley and Ndhlovu[99]
		• Hypomethylation of ACE2 gene then increased ACE2 expression	Pruimboom [112]
		• Hypermethylation of ACE2 gene and then decreased ACE2	Zoghbi and Beaudet [113]

(continued)

Table 8.3 (continued)

RAS element	Epigenetics changes	Main finding	Citations
		• Hypomethylation of ACE2 promoter and then increased ACE2	Corrado and Fontana [114]
	Histone modification	• Inhibition of ACE2 expression in human embryonic stem cells by histone methyl transferase enzyme EZH2 via H3K27 trimethylation	Li et al. [87]
		• KDM5B, specific histone acetylation (H3K27ac), and histone methylation (H3K4me1 and H3K4me3) dynamics regulate many genes associated with ACE2	Pinto et al. [95]
		• Increased KDM5B histone demethylase then increased ACE2 expression	Crackower et al. [115]
		JAK–STAT pathway and ACE2 gene regulation	Wrapp et al. [116]
	microRNAs	• miR-421and miR-143 as regulators of ACE2 expression	Wan et al. [117]
		• miR-200b, hsa-miR-200c and hsa-miR-429 as predictors of the miR-binding site	McCray et al. [118]
		• miR-200c-3p upregulated by avian influenza virus H5N1	Yang et al. [119]
		• miR-1246: lower expression of miR-1246 in smoker cases as compared with nonsmokers • Upregulation of ACE2 mRNA in smoker cases	Gurusaravanan Kutti Sridharan et al. [120]
		• Upregulation of miR-let-7b by hypoxia then decrease in ACE2 expression in cultured cells	Zhang et al. [107]

(continued)

Table 8.3 (continued)

RAS element	Epigenetics changes	Main finding	Citations
		• Repression of miR-200b, hsa-miR-200c and hsa-miR-429, miR-141 by KDM5B and then induced H3K4Me3 methylation mark and then Induced ACE2 expression	Yehualashet and Belachew [121]

management. Biosensors can also detect bacteria, pathogens, and virus microorganisms. Trackers of physical exercise and steps allow people to build and improve healthier behaviors. These instruments also unlock doors for those involved in clinical science. These sensors detect chemicals without drawing blood spontaneously from the human body. They search for many variables at once continuously. In order to track body chemistry continually, people usually need to remain in a hospital. This device allows people to access information about the chemicals in our bodies from anywhere. They will track different body functions by altering chemicals. People can collect all this information on a mobile phone app and share it with a doctor, caretaker, or anyone they want [122, 123].

Cancer has caused millions of deaths [124], and most cancer burden is in developing countries. Cancer combines a group of diseases. Early stage detection of cancer will make it more curable. There are invasive conventional detection methods such as biopsy; however, innovative and more feasible strategies for cancer detection are needed [125, 126].

The definition of biomarker-based on the World Health Organization is "any substance, structure or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease." Some biological markers, named cancer biomarkers, illustrate the specific genetic characteristics of the cancerous cells [125, 126].

Cancer detection biomarkers are detected by biosensors that use cancer cells, nucleic acid, enzymes, and antibodies, as probes [127]. Recently biosensing approaches for the detection of cancer biomarkers have been reported. Various types of cancer biosensors might be used based on the type of the transducer or the biological response expected. Electrochemical, mass-sensitive, and optical biosensors are three types of cancer biosensors with a specific transducer for cancer or specified for biological response in cancer. Biosensors can be designed based on the types of cancer biomarkers. For example, nucleic acid probes are designed to detect a nucleic acid. Specific ligands are designed for receptor detection, and specific antibodies detect secretary protein biomarkers [125, 128, 129].

However, The importance of biosensors to detect diseases and the limitations of previous methods have composedly fueled an ongoing attentiveness to improving

methodologies for designing and evaluating biosensors using different and sometimes combined matrices. Many approaches have been developed, each with its distinct advantages and limitations.

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Chapter 9
Bioinformatics Study on Renin Angiotensin in Lung, and Liver Cancer Using Plant-Based Extracts

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Abstract Cancer has been one of the leading causes of increasing mortality rate globally. It is the most prominent cause of death worldwide and in most cases incurable if there is delay in its diagnosis. The etiology of cancer is mostly dependent on its exposure to carcinogens consistently. The Renin-Angiotensin system (RAS) plays a significant role in the field of cancer biology that affects the growth of the tumor, and its dissemination either directly or indirectly. Targeting the RAS and by activating the immunostimulatory pathways, the RAS inhibitors (RASi) can enhance cancer immunotherapy by improving cancer treatment. Currently, researchers are more interested in using bioactive compounds from medicinal plants in anticancer therapy since it has no side effects. Plant-derived bioactive compounds having anticancer properties are generally non-toxic or are less toxic. Several phytochemicals have potential anticancer properties with effects on signaling pathways and cellular processes. This review focuses on the RAS in lung and liver cancer. It also highlights the extraction of bioactive compounds having anticancer effects from medicinal plants by using bioinformatic databases to treat liver and lung cancers.

Keywords Renin-angiotensin system · Bioactive compounds · Liver cancer · Lung cancer · Bioinformatics · Databases

Introduction

Angiotensin-converting enzyme (ACE) is the central unit of the Renin-Angiotensin system (RAS). It controls the blood pressure and regulates the volume of body fluids. ACE enhances the blood pressure and thereby, causes the constricting of blood vessels. ACE helps in the hydrolyzation of peptides by removing a dipeptide from C-terminus end.

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The RAS has been viewed as 'Endocrine RAS' due to its association with the endocrine system. It plays a crucial role in regulation of the blood pressure. In the endocrine system of RAS, an enzyme produced by the kidney, renin acts on circulation of the Angiotensinogen (AGT) protein [1]. Renin helps in cleaving the AGT protein by producing a particular fragment of 10 amino acids, Angiotensin I (Ang I). The inactive decapeptide form of Ang I later gets converted to the active octapeptide, Angiotensin II (Ang II) by removal of the His-Leu dipeptide. Ang II binds to its specific cell surface receptors, AT_1 and AT_2, belonging to the seven transmembrane, G-protein receptor superfamily [1]. The AT_1 receptor plays a significant role in mediating the classical actions like sodium retention, cell proliferation and growth, and vasodilation of Ang II. Simultaneously, the AT_2 receptor helps in promoting the apoptosis, inhibiting cell growth, cell differentiation, and vasodilation that may counterbalance the effect of Ang II on the AT_1 receptor [2].

Angiotensin-converting enzyme 2 (ACE2) plays a pivotal role in protecting the inflammation and fibrogenesis of lung and liver [3, 4]. Cirrhosis and advanced level liver fibrosis are the major risk factors resulting in hepatocellular carcinoma (HCC) [5]. In addition, Idiopathic pulmonary fibrosis (IPF) has been reported to be associated with increased risk of lung cancers [6]. The occurrence of dysplastic or atypical changes in the fibrosis may progress to malignancy, thereby, resulting in the development of cancer in the major fibrosis region [7]. ACE2 is one of the most important parts of the RAS.

Both ACE2 and Ang 1-7 provides protection in liver fibrosis against liver injury and progress of cirrhosis [8]. Since RAS participates in regulation of hepatic fibrosis, tissue modelling, and hepatic inflammation post liver injury, the role of ACE2 in liver disease has been of special interest in comparison to other organs. RAS helps in inducing transforming growth factor β1 (TGF-β1) and activating the hepatic stellate cells [9]. The treatment of the liver disease with angiotensin receptor blockers and Angiotensin-converting enzyme inhibitors (ACEIs) can attenuate the progression of fibrosis in human studies [10]. In addition, ACE2 supplementation is reported to prevent bile duct liver fibrosis [4, 10].

Similarly, ACE2 mediated by the effects of Mas oncogene, peptide product of ACE2 known as the ANG1-7 receptor plays a major protective role in diseases related to lungs [3, 11–13]. ACE2 has been reported to be downregulated in fibrotic condition of neonatal and adult human lungs [3, 12, 13]. They also reported a significant reduction of ACE2 and identification of their protective effects in the IPF human lung [3]. They also demonstrated the regulation of ACE2 in the alveolar epithelial survival cells by balancing both proapoptotic Ang II and its antiapoptotic product, Ang 1-7 [14].

HCC being one of the most frequently occurring cancers has a high mortality rate. Several treatment methods have been reported for treating HCC but most of them also have side effects. Alternatively, the use of plant products not only helps in the prevention but also deals with the co-treatment of HCC. Abdel-Hamid et al. [15] reported the results of a study on the management of HCC by using herbal medicinal products. They demonstrated the mechanism of action and pathways involved in the herbal products and also their bioactive molecules involved in the co-treatment as

well as prevention of the HCC. They also reported that these herbal products and their bioactive molecules inhibit the development and progression of liver cancer in various ways including enhancing the effects of several chemotherapeutic drugs, suppressing the chronic inflammation and oxidative stress, protection against the liver carcinogens, and inhibition of metastasis and cancerous cell growth. Another study by Chen et al. [16] reported the anticancer mechanism and the mode of action of the formulations of some Chinese herbal medicines. They reported the efficacy of these herbal medicines as an alternative therapeutic therapy for liver cancers.

A study by Li et al. [17] reported the effect of Traditional Chinese medicine (TCM) in lung cancers, especially non-small cell lung cancer (NSCLC). They demonstrated that TCM has a profound effect compared to other standard treatments in the treatment of lung cancer. Similarly, another study was reported by Chota et al. [18] on the potential treatment of lung cancer by using an African herbal medicinal plant, *Dicoma anomala.*

Yuan et al. [19] reported the role of several bioactive compounds extracted from plant products in the treatment of cancer. They also demonstrated the mechanism and mode of action of their anticancer effect. A recent study by Chavda et al. [20] reported the use of several natural bioactive components in anticancer therapy. They also reported the encapsulation of natural bioactive molecules in several drug delivery methods to improve their anticancer effect. A new drug delivery system that helps in entrapping the natural bioactive compounds was also developed by them.

This present review mainly focuses on the Renin-Angiotensin system (RAS) and also on the enzymes and inhibitors associated with RAS present in both lung and liver cancers. The treatment of lung and liver cancer using the medicinal plant products is also highlighted. This study also reviews the phytochemicals and bioactive compounds extracted from various medicinal plants by the use of bioinformatic databases and tools and their use in the treatment of liver and lung cancers.

Liver Cancer—Hepatocellular Carcinoma

Zhang et al. [21] reported that liver cancers are reported to be ranked sixth worldwide for most incident cases in the year 2020. It is considered to be the third main leading cause of cancer-associated death with 830,180 mortality per year and 905,677 new reported cases approximately. Based on the estimation of the World Health Organisation (WHO) approximately more than 1.3 million individuals are predicted to die from liver cancers by the year 2040 [21].

Among all the primary liver cancers occurring, hepatocellular carcinoma (HCC) is the one which causes tumor in 75–85% cases [22]. HCC is known to be one of the most malignant lethal tumors commonly occurring over the globe. Molecular studies have revealed that the source of HCC is the mature hepatocytes [23]. They are often diagnosed at a late or advanced stage of cancer and hence, there are very limited options available for their effective treatment at that stage [24].

During the past decade, only one systemic drug, Sorafenib has been reported to be clinically to be effective against advanced HCC. It has been approved by the Food and Drug Administration (FDA) and is the standard drug for treating advanced HCC [21].

In recent years, there has been an advancement in the treatment of advanced HCC. Several molecular targeted drugs as well as immune checkpoint inhibitors (ICIs) have been approved as therapeutic drugs for treating advanced HCC. Moreover, Lee et al. [25] reported that a combination of two drugs, bevacizumab and atezolizumab, brought about a significant change in the treatment of advanced HCC. They demonstrated that the incorporation of ICIs into advanced HCC therapies and also their combination with the molecular targeted therapy is emerging as a novel tool in the enhancement of immune responses.

Liver cirrhosis is the most significant risk factor for liver cancers. Although there has been progress in both diagnosis as well as treatment, surgical resection or orthotopic liver transplantation (OLT) is the only cure [23]. Once the degree of differentiation in the liver worsens, the prognosis for liver cancer patients becomes a worse. Hence, restoring the differentiation state may bring about an improvement in the prognosis. The treatment of differentiation of liver cancer is done for reversing the dedifferentiation method of hepatocyte cells to liver cancerous cells by using drugs. Hence, this can be used as a novel treatment tool for HCC in improving the prognosis, reducing the reoccurrence, and restoring the normal characteristics of the liver [23].

Lung Cancer

Lung cancer is considered as the leading cause of the death of cancer patients [26]. Mortality data has been collected and published by the National Center for Health Statistics (NCHS). In the year 2022, approximately 609,360 deaths from cancer and 1,918,030 new cases are projected in the US. Among them, lung cancer has been projected to cause most of the deaths, accounting for 350 deaths each day [27]. Immunotherapy is now a line of treatment for early stages of lung cancer which has drastically changed the quality of care for the patients with metastatic non-small-cell lung cancer (NSCLC) [26].

Slebe et al. [28] reported that currently immune oncology (IO) therapy has become a significant treatment method for patients living with a NSCLC. However, they revealed that only a limited number of patients benefit from this IO therapy. Hence, biomarkers and predictive biomarkers providing insights into the functional biological pathways at tumor microenvironment (TME) level have the capability of elevating the impact of IO therapy. This may lead to the development of improved and novel drug strategies. They also reported that by using several highly-specific radiolabeled tracers, immune positron emission tomography (immunoPET) could provide these biomarkers, to investigate the main targets in TME with immunoPET imaging. In addition, immunoPET has the ability to provide the pharmacodynamic

biomarkers to help in the development of novel drugs and predictive biomarkers to guide in the decision making of innovative and clinical treatment strategies in lung cancer [28].

Angiotensin Converting Enzyme Inhibitors (ACEI)

Angiotensin-converting enzyme inhibitors (ACEI) are drugs that are prescribed mainly for treating heart problems like stroke, heart failure, coronary artery disease (CAD), heart attacks, and others involving high blood pressure (hypertension), hardening of connective tissues and skin (Scleroderma), diabetes, migraines, chronic kidney disease etc. [29, 30]. These drugs help to relax the arteries and veins and lower the blood pressure. The ACE inhibitors block the ACE from converting to Angiotensin II, thus preventing their production in the body. They can narrow the blood vessels and thereby, results in increasing the blood pressure and forcing the heart to work harder.

ACEI includes Captopril, Benazepril (Lotensin), Fosinopril, Enalapril (Vasotec), Lisinopril (Zestril, Prinvil), Perindopril, Ramipril (Altace), Moexipril, Quinapril (Accupril) and Trandolapril. The exact mechanism of action of ACEIs is still not known completely. Although, they interfere with the Renin-Angiotensin-Aldosterone system, the efficacy of ACE inhibitors is indirectly related to renin level in blood.

Hypertension

ACE inhibitors lower both the diastolic and systolic blood pressure mean arterial blood pressure effectively in normotensive and hypertensive subjects [30–32]. These drugs have been reported to have antihypertensive effects in several randomized clinical [33]. The Eighth Joint National Commission (JNC8) in the year 2014 reported evidence-based guidelines to treat hypertension in adults. They recommended ACE inhibitors as one of the four classes of drugs for initial therapy in adults with hypertension [34]. Currently, the guidelines published by the European Society of Cardiology (ESC) and the American Heart Association/American College of Cardiology (AHA/ACC) recommend the ACEI as first-line antihypertensive therapeutic drugs. They are mainly recommended for patients with cardiovascular diseases and diabetes mellitus [35, 36]. The ACE inhibitors are proven to be very effective drugs in White race people and less effective in case of Black race people in clinical practice [30].

Post Myocardial Infarction (MI)

Over the past few decades, there have been multiple randomized trials conducted on mortality due to ACE inhibitors post MI [30, 37, 38]. A majority of the trials conducted have reported a remarkable decrease in the mortality rate as well as slow progression of congestive heart failure after the MI patients were treated with ACEI [30, 39]. The clinical practice for patients with heart failure or dysfunctional left ventricle is treatment with ACEI without delay post MI. It is suggested that all the patients be treated with ACEI initially based on the functional assessment of the left ventricle with a review before proceeding further [40].

Heart Failure

ACE inhibitors improve heart failure by lowering the preload, afterload, and systolic wall stress, resulting in increase of cardiac output without much increase in the heart rate [30, 41, 42]. ACEIs play a significant role in excreting salts by reducing the production of antidiuretic and aldosterone hormones, and augmentation of renal blood flow. The ACE inhibitors also reduce cardiac myocyte hypertrophy. Several large, randomized, prospective placebo-controlled trials have been reported since the 1980s. They revealed that patients with heart failure when treated with ACEIs reduces the overall mortality rate with reduction in ejection fraction [30, 43]. They proved that ACEIs also reduce mortality in asymptomatic patients with dysfunction in their left ventricles [30, 44]. Hence, these trials proved that ACE inhibitors can be recommended strongly as first-line therapeutic drugs for patients suffering from heart failure [45].

Diabetes

Due to diabetes mellitus associated neuropathy, the elevation of glomerular capillary pressure and the Renin-Angiotensin-Aldosterone system have been reported to increase the progression of renal dysfunction [30, 46]. A large, randomized, prospective placebo-controlled trial has proved that ACEIs reduce the neuropathy progression in diabetes mellitus insulin-dependent patients. They also reduce the combined endpoints of transplantation, dialysis, and death significantly [30, 47]. Recent recommendations prescribe ACEIs as first-choice therapy in patients with high blood pressure having no history of CAD, which improves the heart function and decreases the incidence of MI [48].

Chronic Kidney Disease (CKD)

ACE inhibitors are the first-line therapeutic drugs used in CKD patients. The effect of ACEI has proved to be more superior compared to placebo-controlled treatment on slowing the CKD progression and reducing proteinuria [49].

Proteinuria or Nephrotic Syndrome

ACEIs have been reported to reduce the glomerular capillary pressure by dilating the efferent arterioles selectively as well as lowering the arterial pressure [30, 50]. The use of ACEIs has been proven to prevent microalbuminuria progression to proteinuria [30, 51]. The ACE inhibitors provide protection for longer duration against proteinuria progression and development. Hence, they maintain stability in renal function in untreated patients with renal function impairment [30, 51].

Post-transplant Glomerulonephritis

The use of ACEIs is the mainstay and first-line drug in patients suffering from glomerular diseases. It gradually slows down the decreasing proteinuria and glomerular filtration rate (GFR) [30, 52]. Moreover, the Renin-Angiotensin-Aldosterone inhibitors can prolong the survival of graft in patients suffering from post-transplant glomerulonephritis [30, 53].

Bioinformatics Study on Angiotensin Enzymes in Liver and Lung Cancer

Bioinformatic Tools Used in Lung Cancer

Patients with lung cancer are reportedly more susceptible to severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2) infection that began spreading during the Coronavirus disease 2019 (COVID-19) pandemic. The SARS-CoV-2 virus enters the host's body through the Angiotensin-converting enzyme 2 (ACE2) receptor. Samad et al. [54] reported the identification of ACE2 protein as a potential biomarker in the SARS-CoV2 infection and its association with lung cancer by using bioinformatic tools and computational analyses.

ACE2 Expression Analysis Using the Cancer Genome Atlas (TCGA) Datasets

ACE2 transcriptional expression analysis was explored in multiple cancers by applying an online server tool, Gene expression profiling interactive analysis 2 (GEPIA2) that is available publicly using the datasets, "The cancer genome atlas (TCGA)" [55]. They studied the data of mRNA expression of ACE2 gene in lung cancer using TCGA dataset through UALCAN website [56, 57]. Further, they analyzed the mRNA expression of the ACE2 gene using another tool, Oncomine and the TCGA datasets [58, 59]. Both web-platforms, UALCAN and Oncomine are user friendly and are used for revealing the gene expression of the TCGA data and also relative expression of a particular query gene set.

Mutational and Survival Analyses of AEC2 Gene in Lung Cancer

The Genotype 2 outcome server was used to perform the survival analysis of the AEC2 gene in lung cancer. This server provides Kaplan–Meier plots using TCGA data. The position of mutational analysis was performed using cBioPortal in lung cancer through TCGA data [60].

COVID-19 Gene Identification and Co-expression Profiling of AEC2 Gene Using the Comparative Toxicogenomics Database (CTD)

The co-related genes of lung squamous carcinoma (LUSC) and lung adenocarcinoma (LUAD) in Visualization platform and R2 genomics analysis were collected using the TCGA dataset [61]. Then using the comparative toxicogenomics database (CTD) all the COVID -19 genes were downloaded [62] in text format and visualized by FunRich software.

Protein–Protein Interaction Using the GeneMANIA Database of Co-expressed Genes

An extensive database, GeneMANIA was used that provided a flexible interface for the gene function, proteomic, and genomic data query. This database has functional associated data that includes co-expression, gene and protein interactions, and pathways [63, 64].

Gene Ontology Analysis and Functional Pathway of Common Co-related Genes with COVID-19 Associated Lung Cancer

Kyoto encyclopedia of genomes and gene (KEGG) database is used for high-level function and biological system utilities through high-throughput technologies and information related to genome sequencing at molecular level [65]. Gene ontology analysis was predicted for computation of biological system. Gene ontology was performed by using web portal, Enrichr. In addition, the analysis of commonly co-related genes of the COVID-19 pandemic and lung cancer was done for their enrichment functional pathway [66].

Bioinformatic Tools Used in Liver Cancer

ACE2 is the SARS-CoV-2 receptor causing COVID-19 pandemic and Transmembrane serine protease 2 (TMPRSS2) is the coreceptor. The abnormality in the hepatic function due to COVID-19 infection suggested bystander or specific liver disease. The liver cells are widely used as SARS-CoV-2 infection models in vitro since they can express the viral ACE2 receptor. Desquilles et al. [67] demonstrated the analysis of TMPRSS2 and ACE2 expressions and their localization in non-tumorous livers and human cancerous livers. They reported that the differentiated liver cancers displayed the potential relation between metabolic breakdown and dysfunction of AEC2 gene using transcriptomic datasets.

Datasets Used in HCC Liver Cancers

The Désert's microarray meta dataset was used. This is composed of many human HCCs. In this particular dataset, the HCCs that express an activated β-catenin transcriptomic program was identified with 5 genes including LGR5, VNN1, GHUL, HAL and ODAM, predicting activated Catenin-β1 (CTNNB1) mutations with specificity as well as high sensitivity [68]. The TCGA dataset was composed of many HCCs and non-tumorous samples. The methylation, RNA sequencing and mutation data were extracted using TCGA Abiolinks R package [68].

Plant Extracts for Treating ACE in Liver and Lung Cancer

Plant Extracts for Treating ACE in HCC

Bioactive compounds obtained from plant products have been successfully tested for treating the HCC as an alternative to chemotherapeutic drugs. The bioactive molecules from plants reported for treating liver cancer are listed in Table 9.1.

Plant Extracts for Treating ACE in Lung Cancer

Plant extracts have been reported to give significant results when used for treatment of several disorders either acute or chronic, and even cancer of the lungs. Bioactive compounds reported from plants for treating lung cancer are listed in Table 9.2.

Bioinformatics Work Using Plant Extracts for Treating Lung and Liver Cancer

A meticulous and detailed search has been performed on several electronic databases including PubMed, Scopus, Google Scholar, and Web of Science to retrieve relevant literature. A proper and detailed query set was designed on the databases. During the search, all the phrases, keywords, and relevant terms were used including "Natural medicinal plants," "Lung cancer," "Liver cancer," "carcinoma," "cancerous," "hepatocellular carcinoma," "Anticancer," "Anticancer plants;" "Anticancer herbs," "Renin-Angiotensin," "Angiotensin-converting enzymes," "in vivo activity," "in vitro activity," and "Animal models" [69].

The number of articles found to be relevant to our search were finalized. The data obtained after the curation, analysis, and extraction through the designing of phrases/keywords combination with their inclusion criteria were noted [69].

The inclusion criteria were based on two sets. The first set of criteria is the 'general criteria'. In this criterion, the articles chosen for the manuscript had reported both the anticancer activity of traditional plants and the anticancer role of pure compounds or their extracts from the plants. The second criteria included the selection of specific plants having anticancer properties with a detailed list of phytochemicals present in them. Articles in current journals on the anticancer activity of relevant plants were selected. The in vivo and in vitro anticancer activities of the medicinal plants and the antitumor/anticancer activities of the bioactive compounds extracted from them was reported [69].

An in-silico study by Putra et al. [70] reported on the anticancer potential of several Indonesian herbal plants. They first used databases like Pubchem, Protein data bank (PDB), Marvin Sketch software, and Ramachandran plot to retrieve the

Table 9.1 Bioactive compounds from plant extracts for treating liver cancer (HCC)

S. No.	Plants	Bioactive compounds	References
1	*Zingiber officinale* (ginger)	Zingerone, 6-gingerol, 6-shogaol, zerumbone	[71]
2	*Curcuma longa* (turmeric)	Curcumin	[72]
3	*Allium sativum* (garlic)	Allicin, diallyl sulfide, diallyl disulfide, S-allylcysteine	[73, 74]
4	*Ferrula asafoetida* (asafoetida)	Ferulic acid, Farnesiferol A, Umbelliferone	[75, 76]
5	*Cinnamomum verum* (cinnamon)	Cuminaldehyde, 2-methoxy-cinnamaldehyde	[77, 78]
6	*Crocus sativus* (saffron)	Crocin, α-carotene, β-carotene, lycopene	[79–81]
7	Cruciferous vegetables like *Brassica oleracea* var. *capitata* (cabbage), *Brassica oleracea* var. *italica* (broccoli), *Raphanus sativus* (radish), *Nasturtium officinale* (watercress), *Brassica rapa* (turnip), *Lepidium sativum* (garden cress), *Brassica oleracea* var. *sabellica* (kale)	Benzyl isothiocyanate, phenethyl isothiocyanate, allyl isothiocyanate, DL-Sulforaphane, indole-3-carbinol	[82–85]
8	*Coffea* sp. (coffee)	Caffeine	[86–90]
9	*Camellia sinensis* (tea)	Catechins, flavins, epigallocatechin gallate	[91–95]
10	*Taraxacum officinale* (dandelion)	α-amyrin, β-amyrin, taraxasterol, lupeol	[96]
11	*Solanum lycopersicum* (tomato)	Lycopene, α-tomatine	[97]
12	*Allium cepa* (onion)	Quercetin	[98–100]
13	*Punica granatum* (pomegranate)	Cuanidin-3,5-O-diglucoside, pelargonidin, delphinidin, gallic acid, ellagic acid, urolithin A	[102–104]
14	*Malus domestica* (apple)	Chlorogenic acid, Phloretin, Proanthocyanidin B2, epicatechin, catechin, rutin, maleic-acid styrene	[105, 106]
15	*Fragaria ananassa* (strawberry), *Vitis vinifera* L. (grapes)	Fisetin	[107]
16	*Vaccinium* sect. Cyanococcus (blueberries, tree wood, grapes	Pterostilbene	[108, 109]
17	*Arachis hypogaea* (peanuts), grapes, red wine, *Pinus* sp. (pines)	Resveratrol, tanshinone I, Tanshinone IIA, β-elemene, Emodin, Aloe-emodin	[110–112]
18	*Panax Ginseng* (ginseng)	Ginsenosides, Protopanaxadiol (PPD)	[113–116]

(continued)

Table 9.1 (continued)

S. No.	Plants	Bioactive compounds	References
19	*Citrus reticulata* (tangerine)	Tangeretin	[113–115]
20	*Amaranthus spinosus* (spiny amaranth)	(14E, 18E, 22E, 26E)-methylnonacosa-14, 18, 22, 26 tetraenoate	[117]
21	*Ziziphus jujuba* (jujube)	Ursolic acid, Oleanolic acid	[118–120]
22	*Diospyros kaki* (persimmon)	Flavonoids and non-specified terpenoids	[121]
23	*Digitalis ferruginea* (rusty foxglove)	Lanatoside C	[122]
24	*Astraeus hygrometricus* (hygroscopic earthstar)	Astrakurkurone	[123]
25	*Artemisia annua* (sweet sagewort)	Artemisinin	[124, 125]
26	*Scutellariae radix* (Scutrllaria)	Oroxylin A	[126, 127]
27	*Fagopyrum tataricum* (tartary buckwheat)	Tatariside F	[128]
28	*Garcinia mangostana* (mangosteen)	Mangostanaxanthone V and VI, α-mangostin	[129]
29	*Nelumbo nucifera* (sacred lotus)	Neferine	[130]
30	*Rhizoma coptidis* (Huang Lian)	Berberine	[131]
31	*Alpinia galanga* (galangal)	Acetoxychavicol acetate	[132]
32	*Glycyrrhiza glabra* (licorice)	Glabridin	[133, 134]

Hassan [115], Rodriguez et al. [116], Rawat et al. [135]

protein and ligand. They later performed molecular docking by using the AutoDock tools software, and visualized the docking analysis using the Discovery Studio 4.0 for evaluating the binding value of Gibbs free energy and their amino acid residues. They reported three bioactive compounds, physcionin, berberine, and pinostrobin that showed potential anticancer properties against the lung cancer.

Conclusion

Cancer, being a public health problem has a profound impact on the health of people in both developing and developed countries across the globe. Clinical studies provided enough compelling evidences that Ang II helps in regulating all the hallmarks of several cancers, including liver and lung cancer. To overcome the problems involved in the use of currently available drugs for the treatment of cancer, there is a need to look for certain effective and novel anticancer agents that can improve the efficacy with minimum side effects. In the present review, bioinformatic databases were used to obtain the potential bioactive compounds from medicinal plants to treat lung and liver cancers. Later, anti-cancerous as well as bioactive medicinal compound

Table 9.2 Bioactive compounds from plant extracts for treating lung cancer

S. No.	Plants	Bioactive compounds	References
1	*Angelica keiskei* Koidz	Xanthoangelol E	[136]
2	*Erythrophelum succirubrum*	Pyrogallol; Resorcinol; β-glyceryl monostearate; ethyl linoleate; 2,6-Diphenylimidazol[1,2-b]-[1,2,4]-triazine; ethyl hexadecanoate; ethyl gallate	[137]
3	*Psoralea corylifolia* (babchi)	4′-O-methylbavachalcone	[138]
4	*Nigella sativa* (black cumin)	α-hederin	[139, 140]
5	*Alnus japonica* (Japanese alder)	Hirsutenone	[141]
6	Chili	Capsaicin	[142]
7	*Polygonum* sp. *Rheum* sp.	Emodin	[143]
8	*Bridelia ovata*	Palmitic acid; Friedelan-3-one; Citrostadienol; Fucosterol; Epifriedelanol; 1-Tricosene; Elemicin; Petroselinic acid; Hebesterol; Stigmast-5-en-3-ol; α-Tocopherol	[137]
9	*Rheum officinale* (Chinese rhubarb)	Emodin	[144]
10	*Salvia rosmarinus* (rosemary)	Carnosic acid	[142]
11	*Petroselinum crispum* (parsley), *Citrus paradasi* (grapefruit), *matricaria chamomilla* (chamomile), *Citrus sinensis* (orange), tea, onion, wheat sprouts	Apigenin	[145]
12	*Paulownia tomentosa*	Tomentin E	[146]
13	*Salvia miltiorrhiza* (danshen)	Cryptotashinone, Dihydrotanshinone I	[147]
14	*Dendrobium pulchellum*	Chrysotoxine	[148–150]
15	*Dracocephalum rupestre*	Eriodictyol	[151]
16	*Ecklonia cava* (marine brown algae)	Dieckol	[152]
17	*Piper nigrum* (pepper), *Apium graveolens* (celery), *Mentha piperita* (peppermint), *Thymus vulgaris* (thyme), *Lonicera caprifolium* (honeysuckle)	Luteolin	[153]

(continued)

Table 9.2 (continued)

S. No.	Plants	Bioactive compounds	References
18	*Croton oblongifolius*	Benzoic acid; Kaur-16-en-18-oic-acid; Stigmast-5-en-3-ol; Aflatoxin G1	[137]
19	*Uncaria tomentosa* (cat's claw)	Mitraphylline	[154]
20	*Tanacetum parthenium* (feverfew)	Parthenolide	[142]
21	*Azadirachta indica* (neem)	Nimbolide	[142]
22	*Tribulus terrestris* L. (gokshur)	Terrestrimine, Lianhuaqingwen, Herbacetin, Rhoifolin, Pectolinarin	[155–157]
23	*Vanilla planifolia* (flat-leaved vanilla)	Vanillin	[158, 159]
24	*Aglaia perviridis*	Myricetin	[160]
25	*Triterygium regelii* (Regel's threewingnut)	Tingenone	[161]
26	*Torreya nucifera* L. (Japanese nutmeg-yew)	Amentoflavone	[161]
27	*Scutellaria baicalensis* (Chinese skullcap)	Scutellarein	[160]
28	*Sambucus javanica* (Chinese elder)	Gallic acid, chlorogenic acid, caffeic acid	[162]

Poofery et al. [137], Benarba and Pandiella [163], Rahman et al. [164]

libraries from several endemic plants from different regions can be developed by using high-throughput techniques.

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Chapter 10
Role of Renin-Angiotensin System in the Pathogenesis and Progression of Non-alcoholic Fatty Liver

Amira M. Badr, Iman O. Sherif, Yasmen F. Mahran, and Hala A. Attia

Abstract Non-alcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver diseases and is increasing simultaneously with obesity and Type 2 Diabetes Mellitus, which are on the rise. Currently, no FDA-approved drug exists to treat NAFLD, and pharmacological treatments are directed at managing its associated comorbidities. Therefore, a better understanding of NAFLD pathogenesis and converging pathways helps in improving prognosis and preventing progression to non-alcoholic steatohepatitis (NASH). Renin-angiotensin system (RAS) plays an important role not only in regulating blood pressure but was also found to contribute to obesity, insulin resistance, lipotoxicity, and inflammation, which are considered the key players in NAFLD pathogenesis. Moreover, a prominent role of RAS has been identified in hepatic fibrosis; activated hepatic stellate cells (HSC) express renin, angiotensin-converting enzyme (ACE), and angiotensin II (AngII), which cause HSC to proliferate and produce reactive oxygen species and inflammatory mediators. On the other hand, the inhibition of RAS improved insulin resistance and inhibited hepatic fibrogenesis. The maintenance of the balance between the two arms of RAS showed to play a meaningful role. One of the two arms is known as the classical arm; ACE-AngII-Angiotensin I receptor arm, and the other is the new one; ACE2/Ang1-7/Mas/MasII. Ang1-7 was found to increase insulin sensitivity and counteract the effects of AngII. Therefore, RAS blockers have emerged as a promising therapeutic modality for NAFLD. Accordingly, the present review will discuss the role of RAS in NAFLD and the potential therapeutic value of RAS modulators.

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Introduction

As a major contributor to chronic liver diseases, non-alcoholic fatty liver disease (NAFLD) incidence has recently increased along with obesity and Type 2 Diabetes epidemics. It is universally recognized as the hepatic manifestation of metabolic syndrome, a global public health concern associated with the involvement of multiple systems and resulting in high mortality rates. A sedentary lifestyle, increased dietary fructose consumption, and obesity are the root causes of NAFLD. NAFLD includes a wide range of liver conditions, from simple steatosis to the more serious non-alcoholic steatohepatitis (NASH). Simple steatosis is known as NAFL and is considered benign. However, NASH is characterized by liver injury, hepatocellular ballooning, inflammation, and various degrees of fibrosis [1].

The renin-angiotensin-system (RAS) is a body system known for decades ago to consist mainly of a single hormonal cascade responsible for controlling cardiovascular and renal functions [2]. Evidence that RAS contributes to NAFLD pathogenesis do exist through both experimental and clinical studies. A discussion of the role of RAS in the development and prevention of NAFLD will be presented in this context.

RAS

RAS is one of the body's hormonal systems with multiple functions. Members of this system include mainly renin and Angiotensin II (Ang II). Through the circulating Ang II, RAS functions in the homeostatic control of blood volume, systemic vascular resistance, hormone secretion, and renal function in a prolonged manner [2, 3]. It has been reported that RAS is present in the systemic circulation and is also produced in local tissue [4]. There is evidence that some cell types express several RAS components intracellularly, such as adrenal medullary chromaffin cells and pituitary glandular cells [5], adipocytes [6], and renal cortical cells [7]. Both local and systemic RAS operate in an integrated way and have diverse physiological roles through autocrine and paracrine actions [8, 9]. Cell proliferation, differentiation, apoptosis, the generation of reactive oxygen species (ROS), tissue inflammation, and fibrogenesis are some of the physiological pathways they can modulate [8, 10, 11].

The renal juxtaglomerular cells secrete renin in response to the stimulation of β-adrenergic receptors located on the cell walls [12]. Renin, the rate-limiting step in the RAS cascade, interacts with a plasma protein called angiotensinogen to create a decapeptide prohormone; Ang I, Fig. 10.1 [13]. Ang I is then rapidly hydrolyzed through the angiotensin-converting enzyme (ACE) action to form the major biologically active peptide generated by RAS, Ang II, Fig. 10.1 [14]. ACE is located on the

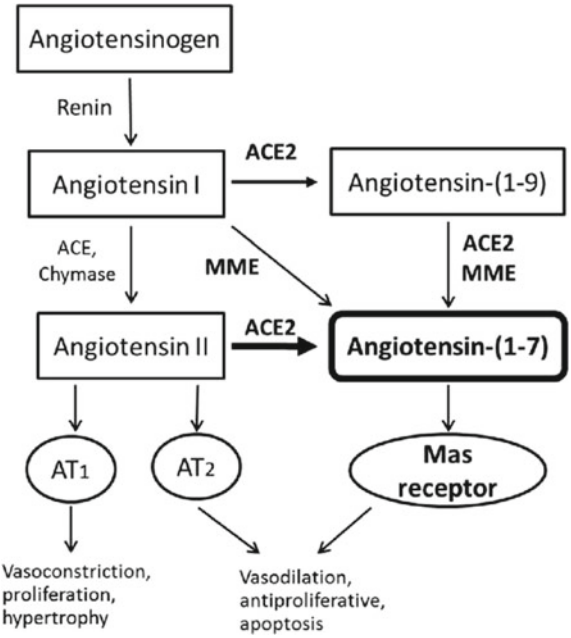

Fig. 10.1 Members of the RAS system; Ang II, Ang-(1-7), their receptors and producing enzymes. ACE: Angiotensin 1-converting enzyme; ACE2, Angiotensin 1-converting enzyme2; AT1, Angiotensin II type 1 receptor; AT2, Angiotensin II type 2 receptor; MME, Membrane metallo-endopeptidase; RAS: Renin-Angiotensin System. The figure is adopted from Ghadhanfar et al. [18]

luminal side of the vascular endothelium, predominantly in the lung and the kidney, besides other organs [15, 16]. In 2000, a new gene in the ACE gene family was discovered, ACE2 [16]. ACE 2 is also expressed on the endothelial cell surface with a limited expression pattern compared to ACE [17].

Ang II

The most crucial active peptide of the RAS is Ang II. Two main receptors, angiotensin type-1 and type-2 receptors (AT1R and AT2R) mediate the Ang II functions in the body. AT1R mediates most of the significant physiological effects of Ang II, such as regulating blood pressure, salt and water retention, hormone secretion, kidney function, as well as the modulation of cell proliferating and migrating [8, 19]. Also, the AT1R axis mediates much of the disease change associated with chronic RAS activation, such as fibrosis, inflammation, angiogenesis, vascular aging, and atherosclerosis [20]. While the AT1 receptor is abundant in adult tissues, AT2R is mainly expressed in fetal tissues and is upregulated in pathological conditions. AT2R is generally reported to mediate antagonistic effects to those pathophysiological conditions induced by AT1R with harmful consequences [16, 19].

Other Angiotensins

In addition to Ang II's primary function in the RAS, Ang I and Ang II's other metabolites may also possess significance in the body. There are three bioactive angiotensin fragments, Ang III (Ang 2-8), Ang IV (Ang 3-8), and Ang (1-7) derived from Ang II by the action of various enzymes. Angiotensin III and IV are generated after the aminopeptidases removed amino acids from Ang II's N terminus. Similar activities are shared between Ang III and Ang II via AT1R and AT2R [21, 22]. Furthermore, Ang IV through acting on Angiotensin receptor 4 is also involved in regulating cardiac hypertrophy, angiogenesis, and plasminogen activator inhibitor 1 (PAI-1) expression, among other physiological functions and pathological conditions [23, 24].

A1-7 is produced either by ACE2 or by endopeptidases from Ang I. ACE2 can also convert Ang I to Ang-1-9, which is further metabolized to A1-7 by ACE, Fig. 10.1. The MAS1 oncogene (Mas) receptor appears to be primarily in charge of mediating the angiotensin (1-7) effects, which mostly counteract Ang II. The ACE2-angiotensin-(1-7)-Mas axis appears to favor vasodilation, to exert antiproliferative, anti-inflammatory, antifibrotic, and antithrombotic activities [8, 25, 26, 18].

The Two Arms of the RAS

Based on previous information, RAS can be described as having two arms, the classical ACE-AngII-AT1R arm and the new one consisting of ACE2/Ang1-7/Mas/Mas II and is known to antagonize the classical pathway, Fig. 10.1. A combination of the two biologically active peptides, Ang II stimulates inflammation, oxidation, fibrosis, and vasoconstriction by activating AT1R, whereas Ang1-7 inhibits and reduces many of Ang II's detrimental effects [27, 28]. The role of the new arm has been evolving recently [29, 30]. According to research, ACE2 is involved in the pathophysiology of cardiovascular diseases, and a reduction of ACE2 directly correlates with hyperactivation of the ACE/Ang II/AT1R axis [31].

The Pathogenesis of NAFLD

NAFLD is a heterogeneous group of liver diseases in which excess triglycerides (TG) are accumulated in the liver. The liver is the main organ responsible for fatty acids (FA) metabolism. FA acquirement is attained by hepatocellular uptake from the plasma and de novo lipogenesis. However, elimination of FA occurs via β-oxidation in the cell or through exportation into plasma in the form of TG-rich very-low-density lipoproteins (VLDL). Under normal conditions, only small TG are stored in the liver

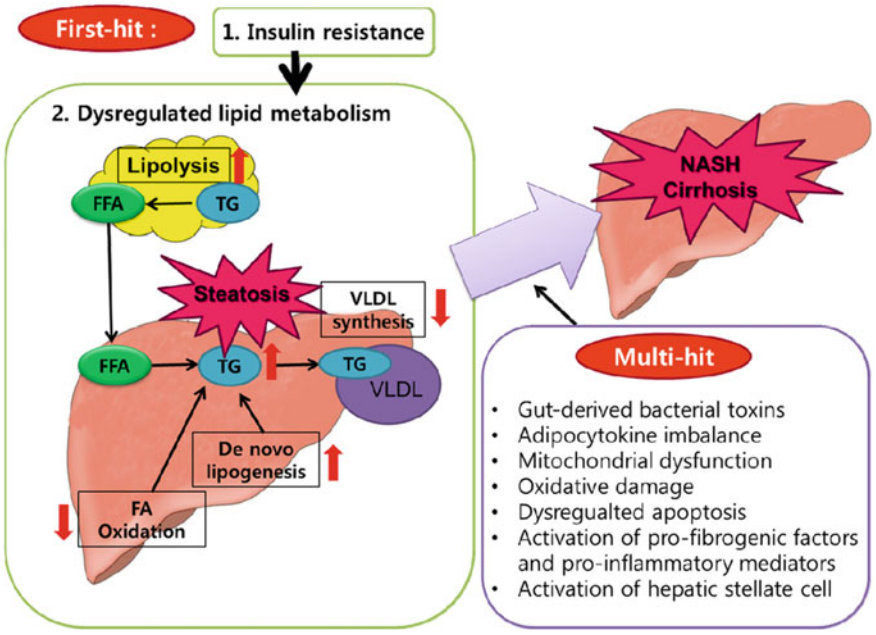

Fig. 10.2 IR and dysregulated lipid metabolism is associated with increased release of FFA from the adipose tissue to the liver, increased glucose production, and TG accumulation. Simple steatosis renders the hepatocytes susceptible to "multiple hits" which include gut-derived bacterial toxins, adipocytokine imbalance, mitochondrial dysfunction, oxidative damage, activation of pro-fibrogenic factors, and pro-inflammatory mediators ultimately leading to NASH and cirrhosis. FFA: Free fatty acids, IR: Insulin Resistance, TG: Triglycerides. The figure is adapted from [35]

in cytoplasmic lipid droplets. Under certain conditions, if an imbalance between lipid acquirement and lipid elimination is settled, a clinical state of NAFLD occurs [32].

The pathogenesis of NAFLD can be described as a "multiple-hits hypothesis". Hepatic steatosis development through TG accumulation in the liver and insulin resistance (IR) is considered the first hit. The first hit subsequently prepares the liver to be vulnerable to the other hits; such as lipotoxicity, oxidative stress, mitochondrial dysfunction, gut-derived bacteria, and inflammatory cytokines/adipokines, that promote inflammation and fibrosis, Figs. 10.2 and 10.3 [33, 34].

Lipotoxicity

As a part of lipid metabolism, the liver imports free FA (FFA), synthesizes, stores, and exports lipids. Disturbances in any of these processes may result in NAFLD. Adipose tissue lipolysis produces FFA that plays a critical role in developing lipotoxicity; which occurs when they accumulate in non-adipose tissues, and lead to hepatic

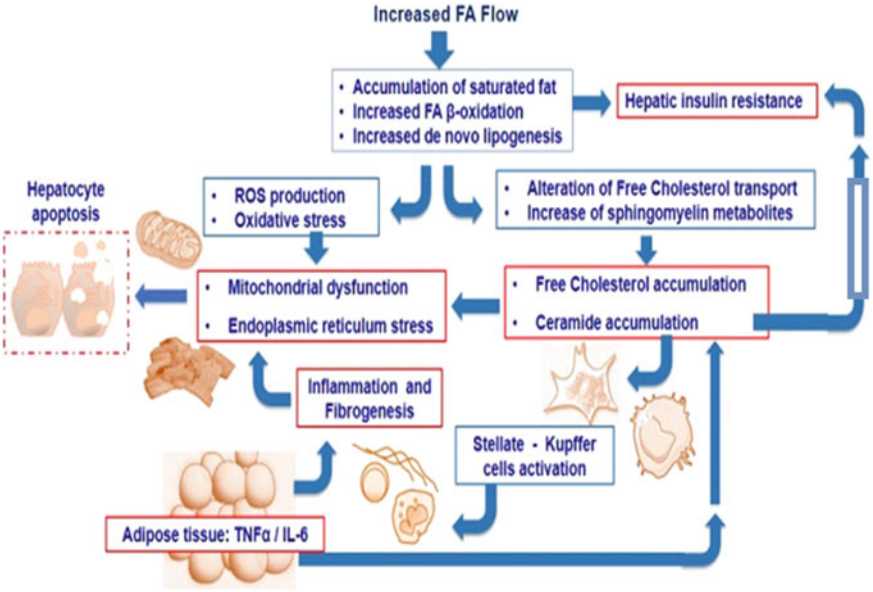

Fig. 10.3 The major pathogenic pathways involved in NAFLD: IR is associated with increased FFA flux that contributes to increased TG production. In parallel, the accumulation of free cholesterol and ceramides enhances the activation of inflammatory pathways; TNF-α and IL-6 from adipose tissue enhance the inflammatory process. Fat accumulation in the liver culminates in oxidative stress, lipotoxicity and increased inflammatory markers. ROS generation induces oxidative mitochondrial damage and ER stress, ER: Endoplasmic Reticulum, FFA: Free fatty acids, IL-6: Inteleukin-6, IR: insulin resistance, NAFLD: Non-alcoholic fatty liver, ROS: reactive oxygen species, TG: Triglycerides, TNF-α: Tumor necrosis factor-α. Figure is adapted from Gaggini et al. [36]

dysfunction and death. When the liver is in a physiological state, FFA are either transported to the mitochondria for β-oxidation, esterified to be excreted in VLDL, or stored as lipid droplets. However, when the liver is in a pathological state, FFA and phospholipids accumulate in the hepatocytes. Accumulated long-chain saturated FA such as palmitate and stearate can stimulate pattern recognition receptor, toll-like receptor4 (TLR4), which activates the pro-inflammatory cytokines. Additionally, uncontrollable lipotoxicity leads to increased production of ROS, which results in hepatic endoplasmic reticulum (ER) stress and mitochondrial dysfunction, which are critical in the pathogenesis of NAFLD as will be discussed, Fig. 10.2 [37].

IR

A growing body of evidence from experimental and clinical studies consider IR the key pathophysiological hallmark of NAFLD and its progression to NASH. Insulin plays a crucial role in lipid metabolism; it suppresses adipose tissue lipolysis, thus

decreasing plasma FA levels and promoting TG storage in adipose tissue [38]. Thus, hyperinsulinemia promotes hepatic FA and TG uptake, de-novo lipid synthesis and impaired β-oxidation of FA by negative feedback, leading to TG accumulation [38, 39]. IR is necessary for lipotoxicity establishment, oxidative stress, and the activation of inflammatory cascades, Fig. 10.2 [40].

Mitochondrial Dysfunction and Oxidative Stress

Subsequently to increased FFA in the hepatocytes, the process of FA β-oxidation will increase as an adaptive mechanism, which will be associated with ROS production. Fat oxidation in the liver is critical for preventing fat accumulation, but too much FA oxidation is detrimental to the liver through oxidative stress, which reduces antioxidant defences [37]. The oxidative stress further increases the hepatocyte's susceptibility to toxic stimuli. Mitochondria are the first organelle to be impaired due to aggressive lipids like FFA and cholesterol accumulating there, resulting in decreased mitochondrial FA oxidation. At the same time, the minor pathways of ER α- and ω-oxidations will be activated leading to overproduction of ROS, which can lead to lipid peroxidation producing malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) that can enhance inflammation, activate cell necrosis and apoptosis, and be implicated in fibrosis [33, 37].

Adipocytokine Imbalance and Inflammation

The cross talk between lipotoxicity, IR, and other factors can activate the inflammatory cascades and stimulate the release of pro-inflammatory cytokines such as nuclear factor-κB (NF-κB), tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) [41]. In addition, excessive FFA and oxidative stress encountered in NAFLD can activate pattern recognition receptors like TLRs and NOD-like receptors (NLRs). Researchers have reported that NLRP-3 inflammasomes may contribute to fibrosis progression in NAFLD [40, 41], and they observed higher levels of NLRP3, pro-IL-1b, and pro-IL-18 protein expression in NASH patients compared to those with simple steatosis [42].

Moreover, adipose tissue macrophages are the primary source of local and systemic inflammatory mediators. TNFα and IL-6 are two important pro-inflammatory adipocytokines, and their expression is markedly increased in the adipocytes of obese subjects and patients with IR. Thus, excess caloric intake, obesity, and adipose tissue expansion lead to a chronic mild inflammatory state that plays a pivotal role in the onset of IR [43]. On the other hand, adiponectin is a prototypic anti-inflammatory and anti-diabetic adipocytokine which inhibits the phagocytic activity in macrophages and stimulates the release of the anti-inflammatory IL-10. Thus, high TNFα and low adiponectin lead to IR and NAFLD [44].

Endoplasmic Reticulum Stress

In hepatocytes, the main site of lipid synthesis is the ER [45]. Many insults including physiological, pathological, and environmental can cause accumulation of unfolded/misfolded protein, leading to disruption of ER homeostasis, a condition that is known as ER stress [46]. It has been reported that there is a crosstalk between ER stress and progression of NAFLD, as increased ER stress was found to induce IR in both human and rat and impair Glucose Transporter Protein-4 (GLUT4) production and insulin-induced glucose uptake [47]. Furthermore, an increasing body of evidence demonstrates that ER stress plays an important role in the activation of hepatic Sterol regulatory element-binding proteins (SREBP-1); the most important transcription factor that regulates the expression of the enzymes for FA synthesis; and thus the development of NAFLD [48]. Moreover, a close association between ER stress and the inflammatory response was reported [49]. ER stress is well documented to activate JNK and NF-κB as a consequence of unfolded protein response (UPR) signalling, resulting in the impairment of hepatic metabolism [50]. The major pathogenic mechanisms involved in the pathogenesis of NAFLD were illustrated in Figs. 10.2 and 10.3.

Genetic Factors

Finally, though hepatic steatosis is prevalent in patients with obesity and IR, only a small percentage of these patients proceed to NASH and cirrhosis, implying a complex interaction between genetics and environmental factors. One of the evolving treatments of NAFLD is the inhibitors of RAS, emphasizing the role of RAS in the pathogenesis and development of NAFLD. Moreover, it has been documented that single nucleotide polymorphisms (SNP) in angiotensin II type I receptor (ART1) are linked with increased risk of NAFLD and its related fibrosis [51]. Therefore, the RAS and its contribution to NAFLD will be further discussed in this chapter.

The Role of RAS in NAFLD

In recent years, RAS has become more widely acknowledged as a modulator of body metabolism. Many studies demonstrated that the classical RAS axis is upregulated in the liver during NAFLD [8, 27, 51, 52]. Ang II causes many deleterious effects that contribute to a wide range of histological changes observed in NAFLD and NASH. Among these effects are the stimulation of IR, de novo lipogenesis, mitochondrial toxicity, endothelial dysfunction, ROS generation, and the release of pro-inflammatory cytokines like TGF-β and IL-6, Fig. 10.4 [8, 42, 53, 54]. Thus, NAFLD pathogenesis and progression may be influenced by RAS.

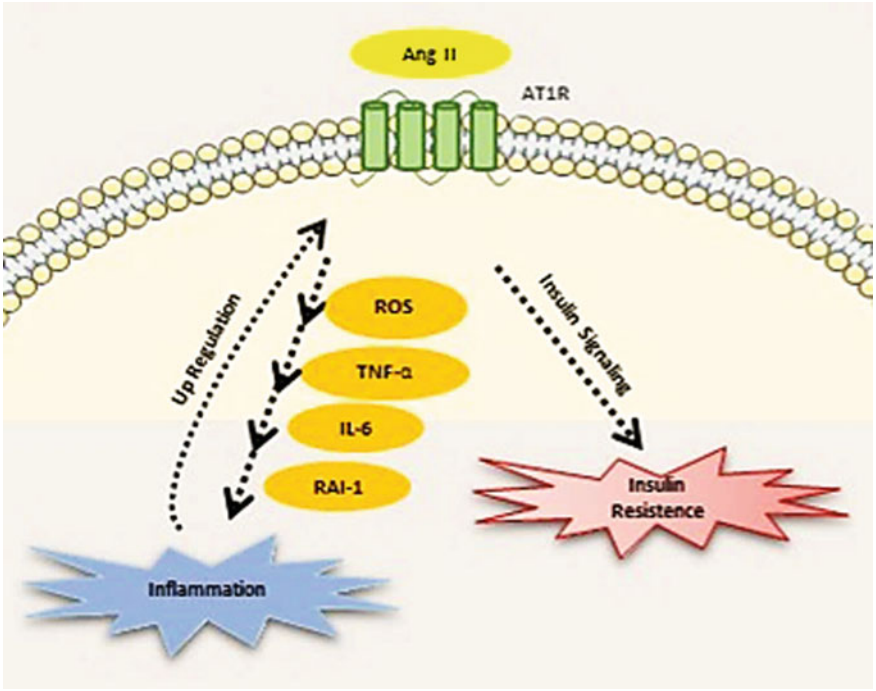

Fig. 10.4 Ang II is a major player in NAFLD. It impairs intracellular insulin signaling contributing to IR. It also induces the generation of ROS, initiating and propagating the production of pro-inflammatory mediators, including TNF-α, IL-6, and PAI-1. These results in inflammation, additional impairment of insulin signaling, and upregulation of the AT1R genes, contributing to the vicious cycle of steatosis–necroinflammation–fibrosis. Ang II: Angiotensin II, AT1R: Angiotensin type 1 receptor, IL-6: Inteleukin-6, IR: insulin resistance, NAFLD: Non-alcoholic fatty liver, PAI-1: plasminogen activator inhibitor-1, ROS: reactive oxygen species, TNF-α: Tumor necrosis factor-α [55]

Evidence that the two axes of RAS contribute to NAFLD pathogenesis do exist. In experimental models of NAFLD, the two axis of hepatic RAS were imbalanced with the upregulation of the ratios ACE/ACE2, AngII/Ang (1-7), and Mas/AT1R, which ultimately lead to liver steatosis [27, 56]. Accordingly, it was demonstrated that the maintenance of local balances of the two axis is important for the prevention of liver metabolic diseases [57], in which the activation of ACE2/Ang (1-7)/Mas inhibits liver injury. This was further supported by the findings that the use of AT1R blockers [52, 58], inhibition of ACE/Ang II/AT1R by gene knockout animal models [59], or/and the activation of the alternative ACE2/Ang1-7/Mas axis [60, 61] can ameliorate NAFLD. In humans with liver disease, increased expression of both ACE2 gene and plasma Ang-(1-7) was reported, confirming that the alternative axis of the RAS is upregulated in response to hepatic injury as a protective remedy [62]. Taken together, the above studies demonstrate the essential participation of the RAS in NAFLD. In harmony with these findings, recent research have explored the therapeutic efficacy

of RAS inhibitors (RASi) for the treatment of NAFLD and they are now considered as a promising evolving treatment of NAFLD, and thus emphasizing the role of RAS in its pathogenesis and progression [63].

The Role of RAS in the Development of IR and Lipogenesis

Results from experimental animals and humans suggest that obesity activates the ACE/Ang II/AT1R arm. In fact, NAFLD's altered hepatic lipid metabolism and IR has been linked to an alteration in Ang II, and rodent models that lack renin, ACE, and liver-specific deletion of ATR1 showed reduced hepatic steatosis and improved IR [64]. Further evidence of the contribution of Ang II to IR is provided by increasing number of experimental and clinical studies showing improved insulin sensitivity following the use of RASi; ACE inhibitors and AT1R Blockers (ARBs) [65, 66]. Recent studies have shown that the signaling pathways of insulin and Ang II cross talk at several levels and share downstream effectors. Insulin activates the phosphoinositide-3-kinase (PI3K)/Akt pathway, which stimulates the translocation of GLUT-4 in insulin-dependent tissues. Ang II inhibits insulin-mediated PI3K pathway activation, which results in impaired GLUT-4 translocation, resulting in IR [67]. Moreover, Ang II can induce the generation of ROS and regulate the production of pro-inflammatory mediators, resulting in the impairment of insulin signalling, Fig. 10.5 [68, 69].

Moreover, Ang II induces IR by its effect on the adipose tissue. The adipocytes express components of RAS, including angiotensinogen, ACE, AT1R, and AT2R. Adipocyte differentiation is inhibited by Ang II via the AT1R, resulting in increased secretion of inflammatory cytokines or diabetogenic adipokines, which inhibit insulin signalling and lead to increased hyperglycemia and weight gain [71]. These effects were also ameliorated by ARBs [66, 72]. Additionally, elevated plasma Ang II has been associated with increased FFAs, resulting in augmented hepatic FFA uptake and promoting the hepatic lipogenesis [73].

On the other hand, there is increasing evidence that the Ang-1-7/Mas axis has beneficial effects by reducing obesity, via improving insulin sensitivity and glucose tolerance, decreasing body fat, increasing adiponectin production, and reverting IR [74, 55]. Cao et al. proposed that Ang-(1-7)/ACE2/Mas pathway can ameliorate fatty liver through regulating lipid-metabolizing genes and via its ability to improve Akt signalling and, thus improving insulin sensitivity [61]. Recently, Song et al. found that Mas deletion in mice has contributed to the severe glucose intolerance, IR, and hepatic steatosis. Meanwhile, the upregulation of Ang (1-7)/Mas arm or Ang-(1-7) administration attenuated NAFLD by downregulating the expression of hepatic lipogenic enzymes, and improving the Akt-induced insulin signaling [75].

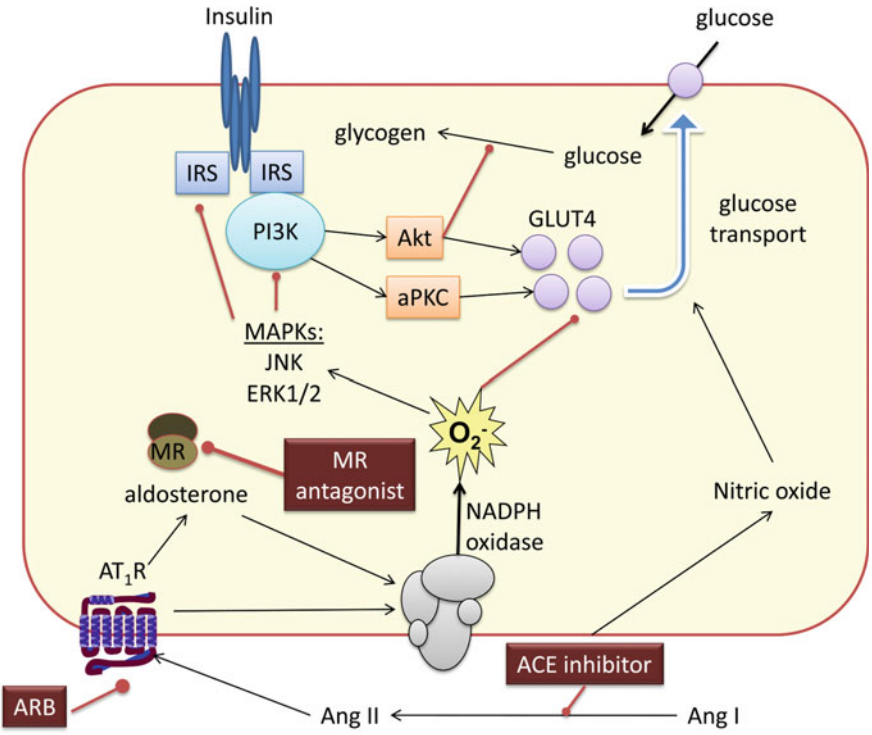

Fig. 10.5 Ang II activates NADPH oxidase to generate ROS (O_2^-). Activation of redox-sensitive serine kinases such as JNK and ERK-1 induced the phosphorylation of IRS-1 and reduced binding with PI3K, with decreased translocation of GLUT4 and reduced glucose transport. The use of ARBs prevents these effects of Ang II. Direct renin inhibitors and ACE inhibitors improve IR by decreasing the formation of Ang II and Aldosterone. ACE inhibitors also increase glucose transport via a nitric oxide-dependent mechanism. ACE: Angiotensin converting enzyme, AKt: protein kinase A, Ang II: Angiotensin II, ARBs: Angiotensin receptor blockers, ERK: extracellular signal-regulated kinase, GLUT4: Glucose transporter protein 4, IR: Insulin Resistance, IRS-1: insulin receptor substrate-1, JNK: c-jun N-terminal kinase, NADPH: nicotinamide adenine dinucleotide phosphate hydrogen, PI3K: phosphatidylinositol 3 Kinase, PKC: protein kinase C. ROS: Reactive oxygen species [70]

The Role of RAS in Promoting Hepatic Oxidative Stress, Inflammation, and Fibrosis

RAS system is also a contributor to hepatic oxidative stress and inflammation. Ang II can induces the generation of ROS mainly by activating the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase [69]. Moreover, it regulates the production of pro-inflammatory mediators, including TNF-α, IL-6, and Plasminogen Activation Inhibitior (PAI-1) [76]. Previous studies in transgenic rats with elevated plasma AngII levels indicated that AngII-induced NAFLD is primarily caused by oxidative stress-mediated mitochondrial dysfunction and impaired mitochondria-mediated

FA β-oxidation via AT1R [54]. The Ang II-mediated ROS formation induces mitochondrial damage with the depletion of mitochondrial genes and proteins leading to reduced FA oxidation, thereby contributing to the development of NAFLD. Both increased activity of NADPH oxidase and decreased activity of cytosolic Cu–Zn superoxide dismutase (SOD) contribute to mitochondrial oxidative stress in these transgenic rat livers. Excessive ROS formation causes lipid peroxidation and release of reactive aldehydes such as 4-HNE. Lipid peroxidation has been shown to increase mitochondrial permeability and reduce gene transcription [77].

Ang II-induced ROS production also mediates inflammation and further impairment of insulin signalling [68, 69]. Furthermore, a high Ang II expression level was proven to trigger inflammatory cell recruitment into the liver and, as a result, induces NAFLD [54, 78]. In accordance, Ang II-infused rats expressed higher levels of IL-6 in the liver, as well as higher levels of monocyte recruitment and inflammation [57, 61]. Moreover, It has also been reported that RAS is linked to fibrosis in NAFLD, and RASi have been shown to prevent stellate cell activation. Activated hepatic stellate cell transforms into hepatic myofibroblasts (HMs), which possess a localized RAS that continuously produces Ang II and stimulates fibrogenesis, in addition to the production of pro-inflammatory cytokines and tissue growth factor (TGF)-β1. HMs thus aggravates hepatic inflammation and fibrogenesis in a vicious manner. Accordingly RASi has the potential to slow down the vicious cycle originating from steatosis to necroinflammation and fibrosis [79].

On the other hand, Cao et al. documented that the ROS levels were increased in the liver of *ACE2* knockout mice, whereas in HepG2 cells, Ang-(1-7) could protect against oxidative stress by inhibiting NADPH oxidase expression. In parallel, ACE2 downregulation led to increased activation of JNK and NF-κB, that is associated with increased induction of pro-inflammatory cytokines; TNFα and IL-6, and both ACE2 overexpression and Ang-(1-7) ameliorated that effect [80, 81]. Thus, ACE2 can protect against inflammatory stress by inhibiting pro-inflammatory cytokines expression [61].

Role of RAS in ER Stress

A recent work revealed that ameliorating ER stress contributes to the role of ACE2 in maintaining hepatic metabolic homeostasis. In ACE2 knockout mice, several ER-related genes were activated while, and importantly, ACE2 upregulation alleviates ER stress and restores hepatic metabolic homeostasis in the HepG2 cells. Interestingly, the protein levels of SREBP-1c protein, as well as FA synthas and acetyl coA carboxylase, which are SREBP-1c targeting enzymes for de novo FA synthesis, were downregulated in ACE2- overexpressed HepG2 cells. This provides an avenue to treat fatty liver disease and other ER stress-associated pathologies [80]. It is well known that Akt is associated with ER stress [82], and an evidence showed that the suppression of Akt signalling can induce ER stress subsequent apoptosis [83, 84]. Previous data revealed that ACE2 downregulation decreased the activity of Akt [81], however,

the activation of Akt increased markedly in ACE2-overexpressing HepG2 cells [80], indicating that the Akt signalling pathway may be involved in the regulation of the ER stress by ACE2 in hepatic cells.

The Potential Therapeutic Efficacy of RASi in the Treatment of NAFLD

According to previous illustration of the potential implication of RAS in NAFLD, growing interest in the therapeutic efficacy of RASi such as ACE inhibitors (ACE-I) and ARBs, in patients with NAFLD has emerged, supported by their widespread use and excellent safety profile [55]. Different clinical studies were conducted [85–89]. A recent one by Kim et al., showed that RASi significantly reduced NAFLD development and progression in obese patients, especially with increased cumulative dose [63]. They also reported that ACE-I use was superior compared to other RASi, in terms of reducing the risk of NAFLD progression, and suggested that additional hepatoprotective effects of ACE-I may be mediated through increasing bradykinin. However, others propose ARBs to be more effective, as ACE-I blocks both ATR1 and ATR2, with the loss of the counterbalancing effects of AT2R on the actions of AT1R [63, 90].

A meta analysis conducted by Li et al., of clinical studies using ARBs, concluded that an efficient lowering of low density lipoprotein and cholesterol was observed, however the data is still insufficient to support the efficacy of ARBs in preventing the progression of NAFLD to fibrosis [65]. The limitation is that most studies in the RAS field have focused only on the progression to liver fibrosis, not NAFLD itself, and were restricted to limited study populations or rodent models. Thus, further well-designed randomized controlled trials of RASi in NAFLD patients should be encouraged [91]. Several investigations have demonstrated that RASi improve IR, hepatic steatosis and inflammation in NASH models [92, 93]. ACE-I and ARBs not only inhibit the ACE/Ang II/AT1 arm, but they also stimulate the activity of the ACE2/Ang-(1-7)/Mas axis and increase Ang-(1-7) level by several times [94]. Both showed significant effects in improving lipid and glucose metabolism [55, 80, 92, 93]. The effect of ARBs on hepatic fibrosis in different animal models is also well-established, e.g. ARBs inhibit stellate cell activity in obese mice, leading to a decrease in hepatic fibrosis [63, 95]. Evidence in humans show that NAFLD patients using ARBs commonly have decreased fibrotic markers [85]. However, ARBs are not the same, they differ in their receptor selectivity and binding mode. Telmisartan ranks higher among the ARBs from the standpoint of safety and efficacy in the treatment of NAFLD. It ls also the only ARB to have partial Perixesome Proliferator Activated Receptor (PPAR)-γ agonist activity, Fig. 10.6 [96]. Recently, the use of RASi in managing NAFLD was recommended in Japan, when hypertension is a comorbidity [97].

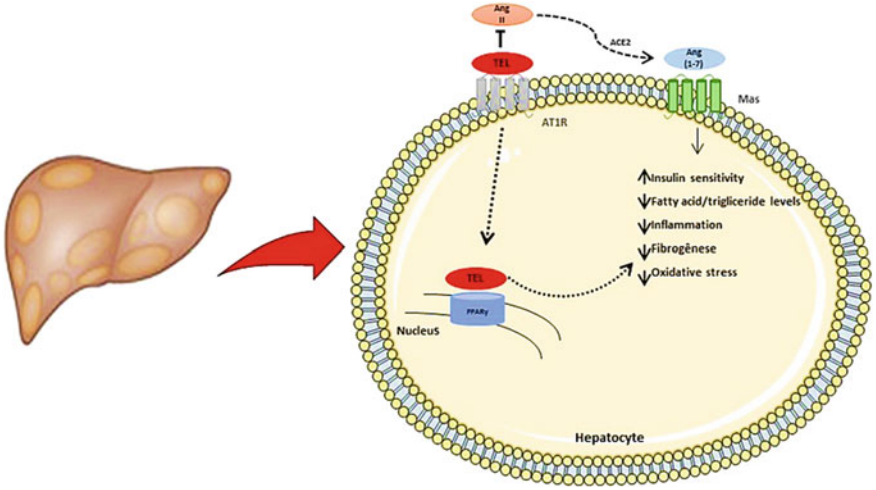

Fig. 10.6 The liver-protecting actions of telmisartan, an AT1R in NAFLD are shown. It indirectly stimulates the Ang-(1-7)/Mas axis, improves insulin sensitivity, lipid metabolism, and decreases the expression of proinflammatory cytokines, with the suppression of macrophage infiltration into the liver. The result is morphological improvement in hepatic steatosis and in fibrogenic markers. It also has anti-oxidative properties and works as a partial agonist of the nuclear receptor PPAR-γ. Ang: Angiotensin, AT1R: angiotensin type 1 receptor blocker, NAFLD: Non-alcoholic fatty liver disease, PPAR: Peroxisome Proliferator Activity Receptor. The figure is adopted from [55]

Conclusion

Collectively, RAS has been found to be highly associated with NAFLD pathogenic pathways. Thus, scientists can speculate that by inhibiting RAS, NAFLD progression can be halted. RASi can do so by improving insulin signaling, controlling adipokines and inflammatory cytokines production, reducing oxidative stress, and inhibiting fibrosis. Although not yet approved as a treatment for NAFLD, future clinical studies are expected to provide better understanding of RASi value and their therapeutic potential.

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Chapter 11
The Classical and Nonclassical Renin-Angiotensin-Aldosterone System in Liver Cirrhosis

Giovanni Sansoè and Florence Wong

Abstract In chronic liver disease, excess hepatic deposition of extracellular matrix, the ensuing development of cirrhosis, the associated renal dysfunction, which ranges from pre-ascitic sodium retention to hepatorenal syndrome, are all dependent, to large extent, on altered function of the renin-angiotensin-aldosterone system (RAAS). The RAAS, once believed to be a hormonal system for blood pressure control and extracellular fluid volume regulation, is now considered a flexible and branching network of enzymes, peptides and receptors that regulates, in addition to arterial circulation, local and systemic inflammation, development of fibrosis in several organs, tumorigenesis, and even bodily reactions to common viruses. In patients with liver disease, besides production of angiotensin II by angiotensin converting enzyme in the vessel wall, there are adaptable synthesis and degradation of bioactive peptides within several tissues by means of enzymes that may be different from those located in arterial endothelium and smooth muscle cells. These 'nonclassical' RAAS metabolic pathways that lead to arterial vasodilatation, increased natriuresis and blunting of inflammation (e.g. through angiotensin 1-7 or 3-8) have been identified and can be manipulated pharmacologically, with foreseeable advantages in the treatment of circulatory and renal complications of liver cirrhosis and portal hypertension. Therefore, a comprehensive understanding of the classical and nonclassical RAAS pathways together with the enzymes and peptides involved, especially those that operate inside the liver and the kidney, will provide insights into disease pathogenesis and help to devise treatment strategies for the various disease processes.

Keywords Renin angiotensin system · Liver cirrhosis · Sodium retention · Liver fibrosis · Aldosterone · Chymase · ACE · ACE2 · Neprilysin

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Introduction

Ever since Tigerstedt and Bergman observed in 1898 that an extract of canine kidney, which they called renin, led to increased arterial blood pressure when injected into another animal, a challenge has been presented to scientists and renewed many times over [1]. The dilemma facing the investigators was not elucidated until 1940, when Prinzmetal showed the presence of renin in ischemic kidneys. Concepts were made clearer when Braun-Menendez identified the octapeptide, angiotensin II (Ang II), as the actual vasoactive compound leading to increased arterial pressure through its binding to a single cell membrane receptor, later called angiotensin type 1 receptor (AT_1R) [1]. Soon the 'classical' renin-angiotensin-aldosterone system (RAAS) was characterized (Fig. 11.1), only to become, over the following decades, less important as many other 'nonclassical' pathways of the RAAS were identified (Fig. 11.2) through the discovery of many more critical enzymes capable of producing a further series of angiotensin peptides. These peptides of different lengths, most of which endowed with specific functions, interact with at least four, and maybe five, different cell membrane receptors.

The challenge in this review is to explain concisely the role of the new and expanded RAAS in relation to the development of chronic liver disease up until the end stage of liver insufficiency, along with the related progressive derangement

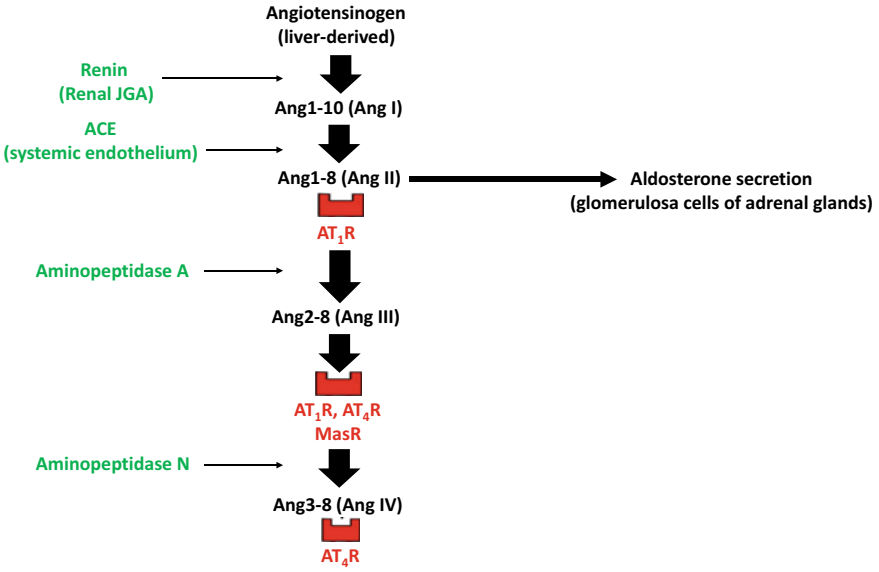

Fig. 11.1 Schematic representation of 'classical' renin-angiotensin-aldosterone system, with peptide receptors. Ang: angiotensin; AT_1Rs: angiotensin type1 receptors; AT_4Rs: angiotensin type4 receptor; MasR: Mas receptor, ACE: angiotensin converting enzyme; Ang: angiotensin; AT_1Rs: angiotensin type1 receptors; AT_4Rs: angiotensin type4 receptor; JGA: juxtaglomerular apparatus

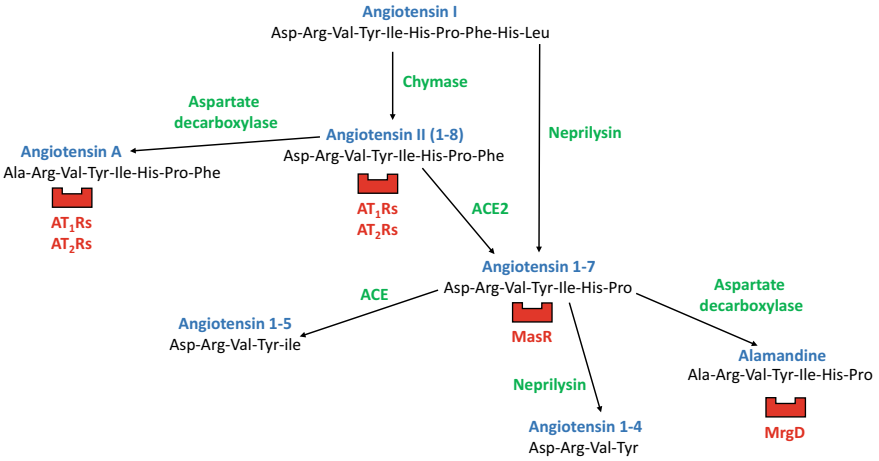

Fig. 11.2 Schematic representation of the 'non-classical' renin-angiotensin system, with peptide receptors. ACE: angiotensin converting enzyme; AT_1Rs: angiotensin type1 receptors; AT_2Rs: angiotensin type2 receptor; MasR; Mas receptor; MrgD: Mas-related G protein-coupled receptor member D

of renal and circulatory function that ensues. Several aspects of this system are worthy of note: (a) This complicated endocrine, paracrine, and even intracrine (i.e. intracellular) system of bioactive peptides of RAAS is now known for being deeply involved not only in the control of arterial blood pressure, as once was exclusively believed. It is also involved in the development of local and systemic inflammation, extracellular matrix deposition (fibrogenesis) in several organs, tumorigenesis, and even bodily reactions to common, sometimes lethal, viruses such as hRSV, SARS-CoV and SARS-CoV-2. (b) Many enzymes we will discuss (Figs. 11.1 and 11.2) can adapt their synthetic function when manipulated pharmacologically. Examples of such variable functional capability are shown in several alternative metabolic pathways: when angiotensin converting enzyme type 2 (ACE2) is blocked by metallopeptidase inhibitors, neprilysin starts converting Angiotensin I (Ang I) into angiotensin 1-7 (Ang1-7), the usual peptide product of ACE2 itself [2, 3]; when angiotensin converting enzyme (ACE) is blocked by ACE inhibitors (ACEis), chymase and cathepsin G start producing Ang II, with the result of a paradoxical increase in aldosterone plasma levels ('the aldosterone escape' phenomenon) during prolonged ACEi administration [3]; when renin is blocked by specific non-peptide inhibitors like aliskiren, Ang I and II are cleaved by chymase from the newly described angiotensins 1-25 (Ang1-25) and 1-12 (Ang1-12), which are polypeptides generated through non-renin pathways [4]. (c) These various enzymatic reactions sometimes occur in the systemic circulation (i.e. in the arterial endothelium and smooth muscle cells), sometimes in the local circulation (e.g. in heart, liver and kidney), sometimes in single cells that both produce and react to a peptide, sometimes inside the cytoplasm of definite cells (e.g. in cardiomyocytes). (d) Finally, these complex systems of 'classical' and 'nonclassical' systems (all cleaved from liver-derived α_2 globulin angiotensinogen)

can interfere with other endocrine or paracrine systems such as those of endothelins, kinins, plasmins, and with the secretion and function of catecholamines themselves [1].

In this review, we shall try to summarize all of this in relation to the development of chronic liver disease, mostly liver cirrhosis, and the cirrhosis-associated derangement of kidney function. Introduction to the basic physiology of the many protagonists of RAAS is essential to understand their perturbation in the setting of liver cirrhosis.

Physiology Considerations

(a) *Prorenin and its receptor*

The triggers to renin release are hypoperfusion of afferent glomerular arterioles, low chloride content in the macula densa segment of the nephron and stimulation of β_1 adrenergic receptors [5]. Losing a 43-amino acid N-terminal segment, a variable amount of the precursor prorenin is cleaved into the protease renin in the juxtaglomerular (JG) cells of the kidney, which are the only cells secreting active renin into blood, but a remarkable share of integral prorenin is also secreted into blood by the same JG cells and by adrenal glands [6], so much so that, normally, circulating prorenin levels are ten times higher than plasma renin concentrations. This ratio increases further in patients with diabetes and arterial hypertension [7]. One quarter of the circulating prorenin is proteolytically converted into renin in plasma by cathepsin B and proconvertase I or undergoes nonproteolytic activation into mature renin by binding to cell surface prorenin/renin receptors [(P)RRs]. Finally, half of circulating prorenin binds to nonspecific clearance receptors [8, 9] (Fig. 11.3).

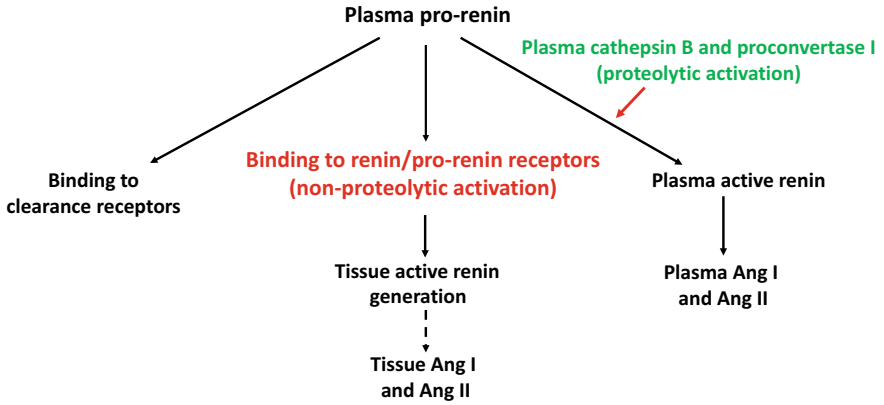

Fig. 11.3 Proteolytic and non-proteolytic activation of plasma prorenin into active renin. AngI: angiotensin I; AngII: angiotensin II

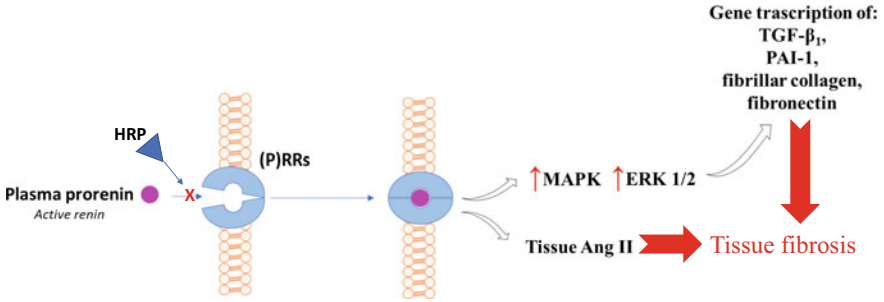

Fig. 11.4 Mechanisms of prorenin-induced tissue fibrogenesis. Agn II: angiotensin II; ERK 1 and 2: extracellular signal-regulated kinase 1 and 2; HRP: handle region of prorenin [(P)RR blocker]; MAPK: mitogen-activated protein kinase; PAI-1: plasminogen activator inhibitor-1; (P)RRs: tissue prorenin receptors; TGF-β_1: Transforming growth factor β_1

(P)RR is a 350-aminoacid protein that shows higher affinity for prorenin rather than active renin (Fig. 11.4). It is expressed in vascular smooth muscle cells and in mesangial, distal convoluted tubule and collecting duct cells of the kidney. In the distal nephron, (P)RRs are functionally associated with H^+-ATPases: these proton pumps transport protons across plasma membranes in the intercalated cells of collecting ducts and acidify urine in exchange with aldosterone-dependent Na^+ reabsorption [9].

Prorenin binding to (P)RRs promotes generation of Ang II in tissues. Independently of local Ang II, (P)RRs stimulation directly causes activation of stress related kinases such as mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase (ERK) 1 and 2, which upregulate transcription of pro-fibrogenic genes such as TGF-β_1, plasminogen activator inhibitor-1 (PAI-1), fibrillar collagen and fibronectin [10–12] (Fig. 11.4). Interestingly, estrogens increase ERK 1 and 2 phosphorylation and function through the same MAPK-dependent mechanism [13].

The peptide known as the 'handle region of prorenin' (HRP) prevents prorenin/renin binding to (P)RR (Fig. 11.4). Therefore, in experimental murine diabetes, HRP infusion reduces the glomerulosclerosis index and the renal content of TGF-β_1 and Ang II [14].

(b) *Role of calcium in renin secretion and renal sodium metabolism*

Pressor responses to sodium chloride loading in salt-sensitive essential hypertensive patients are preceded by a decrease in serum total and ionized calcium [15], and hypocalcemia with secondary hyperparathyroidism promotes arterial hypertension in chronic renal insufficiency [16]. Conversely, activation of the vitamin D receptor by 1,24-$(OH)_2$ vitamin D, which increases serum Ca^{++} concentrations through augmented intestinal absorption and decreased urine excretion of Ca^{++}, reduces renin secretion and indirectly sodium tubular retention [17]. Once again, the MAPK/ERK 1/2 pathway, notably stimulated by estrogens, was demonstrated to upregulate vitamin D receptors and therefore blunt renin secretion [13].

This means, as mentioned above, that fibrotic and sodium-retentive mechanisms might be regulated in opposite directions through the same sex hormone-dependent mechanism.

Plasma hypercalcemia stimulates the plasma membrane-associated receptors for extracellular calcium (calcium-sensing receptors or CaSR) in kidney JG cells, thus decreasing prorenin gene transcription and renin release through inhibition of adenylate cyclase, stimulation of phospholipase C, and production of diacylglycerol and inositol 1,4,5-triphosphate [18, 19]. CaSR stimulation by hypercalcemia also suppresses gene transcription and expression of vasopressin-dependent water channels in the kidney collecting duct [20] and reduces the content of sodium–potassium-chloride co-transporters in the thick ascending limb of the Henle's loop [21]. In other words, extracellular calcium acts as a natriuretic and diuretic agent.

(c) *Key endopeptidases and peptides of RAAS*

The 13-amino acid N-terminal sequence of angiotensinogen in humans is essential to understand the role of all RAAS peptidases [22]: N-Asp_1-Arg_2-Val_3-Tyr_4-Ile_5-His_6-Pro_7-Phe_8-His_9-Leu_{10}-Val_{11}-Ile_{12}-His_{13}…-C. Four different peptidases are the source of most bioactive peptides of the RAAS. The four peptidases are: ACE, a peptidyl-dicarboxypeptidase (EC 3.4.15.1 according to the EC system); ACE2, a peptidyl-monocarboxypeptidase (EC 3.4.17.23); chymase, a serine endopeptidase (EC 3.4.21.39); neprilysin, a Zn-metallo-endopeptidase (NEP, neutral endopeptidase, EC 3.4.24.11). ACE, ACE2 and NEP are classified as metallopeptidases. These four enzymes have membrane-anchoring domains that orient their active sites on the extracellular surface of the cell [23].

ACE. Once renin has cut the Leu_{10}-Val_{11} peptide bond of angiotensinogen to generate Ang I (Ang 1-10), dicarboxypeptidase ACE cleaves the Phe_8-His_9 bond of the decapeptide to make Ang II (Ang 1-8). ACE also cleaves the newly described angiotensin 1-12 (Ang1-12) into Ang I, angiotensin 1-9 (Ang1-9) into angiotensin 1-7 (Ang1-7) and, finally, Ang1-7 into inactive angiotensin 1-5 (Ang1-5) [24, 25]. Outside RAS, ACE degrades enkephalins, substance P and luteinizing hormone releasing hormone [26]. Kidney ACE is found in most tubular cells, vascular endothelial cells and glomerular mesangial cells. Outside the kidney, ACE is located in endothelial cells, especially in the lung [24].

ACE2. This monocarboxypeptidase is the main source of the vasodilator and natriuretic peptide Ang1-7 since it cleaves the Pro_7-Phe_8 bond of Ang II almost ubiquitously. Inside non-classical pathways of RAAS, ACE2 cleaves Ang I into Ang1-9 [24, 25]. ACE2 metabolizes also other peptide substrates (apelin, kinins and endorphins) and regulates the level of tryptophan in the blood [27].

NEP. This enzyme is a membrane-bound Zn-metallo-endopeptidase. It is also called atriopeptidase because it leads to the proteolytic clearance of urodilatin, atrial, brain-derived and C-type natriuretic peptides in the kidney, lung, brain, and heart [28]. NEP degrades opioid-peptides [29], bradykinin [30], bombesin-like peptide [31], substance P [32] and adrenomedullin [33]. Inside the RAAS, NEP cleaves Ang1-9, Ang1-12 and Ang I into Ang1-7 and, finally, the latter vasodilator and

natriuretic peptide into the inactive by-product angiotensin 1-4 (Ang1-4) [24, 25]. In other words, NEP generates Ang1-7, but continues to metabolize Ang1-7 at the Tyr_4-Ile_5 bond into Ang1-4 (Fig. 11.2), as shown in studies employing vasopeptidase inhibitors (i.e. combined ACE and NEP inhibitors) in patients or animal models with arterial hypertension [34, 35].

NEP also produces the vasoconstrictor, profibrogenic and anti-natriuretic polypeptide endothelin-1 (ET-1) from circulating precursors (big ET-1 and ET 1-31) [36, 37].

Chymase. Serine endopeptidase chymase, in heart, liver, renal tubules and mast cells, converts Ang I into Ang II; ACE catalyzes this same reaction in the arterial endothelium [38]. In tissues with chronic inflammation, chymase is over-expressed and converts big ET-1 into ET-1 [39] and activates TGF-β_1 through potentiation of Ang II action [40]. 80% of Ang II synthesized in kidney, heart and blood vessels is produced by chymase [41], but chymase inhibitors do not lower blood pressure and do not increase active renin [42] because chymase is confined to mast cells of the vascular adventitia of arterial vessels. Moreover, systemic plasma contains serine endopeptidase inhibitors [42]. Chymase also cleaves Ang1-12 into the Ang II. Upstream in this atypical metabolic pathway, Ang1-12 is not cleaved from angiotensinogen by renin, but through a hitherto unidentified protease that cuts the Ile_{12}-His_{13} bond of angiotensinogen in humans [43, 44]. Ang1-12 may be a precursor of Ang I through ACE [45, 46], of Ang1-7 and then Ang1-4 (in the tubular nephron) through NEP [46], and, above all, of Ang II through chymase in heart and kidney [43]. Recent studies underline that, alongside Ang1-12, another polypeptide derived from angiotensinogen through non-renin pathways (i.e. angiotensin 1-25) may be a suitable source of tissue Ang I and II by means of chymase action, at least in the heart [47, 48].

(d) *Angiotensin receptors so far described*

The cell surface receptors identified so far as binding sites of this host of angiotensins are five: $AT_{1\text{-}2\text{-}4}$Rs, Mas receptors (MasRs) and Mas-related G protein-coupled receptor member D (MrgD) (Figs. 11.1 and 11.2). The main endogenous ligand of AT_1R and AT_2R in vascular endothelium, kidney, adrenals, brain, heart, liver and testis is Ang II. Ang 1-9 is also a ligand of AT_2R (7). The main endogenous ligands of AT_4R and MasR are, respectively, angiotensin 3-8 (Ang3-8) and Ang1-7 [25]. Putative ligand of MrgD is newly described heptapeptide alamandine (Fig. 11.2).

AT_1 receptors. AT_1Rs greatly exceed the number of AT_2Rs after birth [25], leading to vasoconstriction, aldosterone secretion from the glomerulosa cells of adrenal glands, tubular sodium retention and increased arterial blood pressure, when stimulated by Ang II (classical RAAS).

AT_1R signaling is primarily mediated through G-proteins, leading to adenylyl cyclase activation and intracellular cAMP generation, activation of phospholipase C, production of inositol-1,4,5-triphosphate (IP3), Ca^{++} release from sarcoplasmic reticulum into the cytoplasm, and final Ca^{++}/calmodulin-dependent vasoconstriction. Further AT_1R signaling is mediated through small GTPase proteins, G-protein independent β-arrestin, reactive oxygen species (ROS) (through NADPH-oxidase [NOX]

activation, leading to tissue fibrogenesis) [49], non-receptor type tyrosine kinases, transactivation of receptor tyrosine kinases [50]. AT_1Rs also undergo homo and hetero oligomerization with other receptors, including AT_2Rs, bradykinin B_2 receptors, β_2 adrenergic receptors, and dopamine D_2 receptors [51]. Recently, it has been shown that AT_2Rs directly bind to AT_1Rs, inhibiting AT_1R functions. Bradykinin B_2 receptors potentiate AT_1R signaling, enhancing the vasoconstrictive effects of Ang II. Evidence also exists of direct interaction between the β_2-adrenergic receptors and AT_1Rs. β-blockers have been shown to interfere with Ang II signaling in heart failure patients and have become a mainstay of therapy in patients with chronic heart failure [52].

AT_2 receptors. These G protein-coupled receptors share only 34% amino acid sequence homology with AT_1Rs [53]. AT_2R signaling involves G protein, specific protein phosphatases (MKp-1, PP_2A, etc.) and scaffolding proteins, nitric oxide/cGMP ion channel protein, and constitutive activity (i.e. ligand independent activity of AT_2R) [50]. Stimulation of AT_2Rs in interlobular arterioles and the tubular nephron of the kidney leads to vasodilatation and natriuresis. The latter effect is mediated by stimulation of nitric oxide/cGMP/Sp 1 pathways that inhibit the proximal tubule Na^+/K^+-ATPase [25, 54]. Moreover, it appears that specific stimulation of AT_2R can down-regulate expression of AT_1Rs, resulting in the finding that, based on the AT_1R/AT_2R balance, Ang II itself can be hyper- or hypotensive and natriuretic or anti-natriuretic [54, 55].

AT4 receptors. AT_4Rs are mainly in brain, heart, kidney, adrenals and blood vessels. This receptor is the Angiotensin 3-8 (Ang3-8) binding site (Fig. 11.1). Ang3-8 binding protein was identified as insulin-regulated amino peptidase (IRAP, EC 3.4.11.3), which is a type 2 trans-membrane protein of the gluzincin amino peptidase family [56, 57].

Mas receptors. MasR is a G protein-coupled receptor and the binding site for Ang1-7. The action of Ang1-7 through MasR causes production of arachidonic acid and activation of nitric oxide synthase. MasRs exhibit the highest expression in brain and testis [50]. In common with AT_4R, stimulation of vasodilator and natriuretic MasR leads to nitric oxide production via enhanced phosphorylation of protein kinase B and increased cell levels of cyclic GMP [24, 25].

Mas-related G protein-coupled receptor member D (MrgD). MrgD expression is detected in arterial smooth muscle cells, endothelial nitric oxide synthase (eNOS)-positive endothelial cells, and in atherosclerotic plaques [58]. MrgD stimulation is thought to elicit phospholipase C activation and increased expression of nitric oxide synthase (NOS) enzymes [58]. MrgD is the putative binding site for alamandine, an heptapeptide derived from Ang1-7 through decarboxylation of the N-terminal aspartate residue (Fig. 11.2).

(e) *Intracrine RAAS*

Over the last decades, a large amount of literature has shown that not only do tissue renin-angiotensin systems exist, but so do intracellular (i.e. intracrine) renin-angiotensin systems. Various reports have identified intracellular location and actions

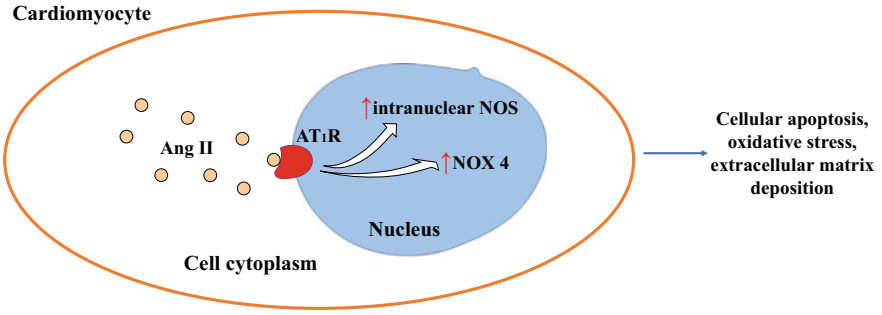

Fig. 11.5 Synthetic representation of intracrine RAAS inside cardiomyocytes. Ang II: angiotensin II; AT_1R: angiotensin type 1 receptor; NOS: nitric oxide synthase; NOX 4: NADPH oxidase 4

of such RAAS components as Ang II, Ang1-7, prorenin receptor, angiotensinogen, several isoforms of renin, AT_1Rs, AT_2Rs, MasRs, ACE, ACE2 and chymase [59]. Perhaps intracellular RAAS alone would warrant a separate review. What matters here may be summarized as follows. Ang II treatment produces a significant increase in nitric oxide (NO) and superoxide/H_2O_2 production in isolated nuclei (Fig. 11.5). These effects are inhibited by losartan (an AT_1R inhibitor) but not by an AT_2R blocker [60]. The likely sources of these intracellular NO and reactive oxygen species are intranuclear NOS and NADPH oxidase 4 (NOX 4). At least in diabetic rats, the intracellular Ang II content in the heart is correlated with cardiomyocyte apoptosis, oxidative stress and extracellular matrix deposition [61] (Fig. 11.5). There is strong experimental evidence to support the view that intracrine Ang II activity may function independent of the circulating RAAS [47]. Whether these findings can be transferred to the model of liver fibrosis is a matter of debate.

(f) *Aminopeptidases and Ang II clearance*

In the systemic circulation, degradation of Ang II may lead to Ang1-7 generation through ACE2, but, in wild-type mice and normal humans, low systemic levels of Ang1-7 and much higher levels of angiotensin 2-8 (Ang2-8 or Ang III) and angiotensin 3-8 (Ang3-8 or Ang IV) [62] emphasize that the actual clearance of Ang II is through the sequential actions of plasma aminopeptidases (Fig. 11.1). In plasma, aspartyl-aminopeptidase or aminopeptidase A (APA) cleaves the Asp_1-Arg_2 bond at the N-terminal end of Ang II to generate Ang2-8, which in turn is cleaved at the new N-terminal Arg-Val bond by arginyl-aminopeptidase or aminopeptidase N (APN), to form Ang3-8 [25]. The kidney synthesizes and secretes most APA and APN found in blood [62, 63].

Ang2-8 and Ang3-8, have important hormonal activities. Ang2-8 binds AT_1R, AT_2R and MasR, and Ang3-8 binds mostly AT_4R. AT_4R stimulation by Ang3-8 causes arterial vasodilatation and natriuretic responses [25].

In summary, all RAAS peptides that are generated downstream of Ang II are either vasodilating and natriuretic agents (Ang1-7, Ang3-8, alamandine and even Ang2-8 when stimulating AT_2Rs or MasR) or inactive by-products (Ang1-5, Ang1-4) (Figs. 11.1 and 11.2).

Liver Cirrhosis. Prorenin and Renin Regulation by Extracellular Calcium

By comparing normal and CCl_4 cirrhotic rats, it is found that (P)RR content in the liver is significantly lower, not higher, in the cirrhotic group (western blot analysis). Conversely, plasma concentrations of prorenin can be derived empirically from the ratio direct renin (DR)/plasma renin activity (PRA) [1], and DR/PRA ratios were 3.3 $\pm$ 0.8 and 7.9 $\pm$ 1.6 ($P < 0.03$) in healthy and cirrhotic rats respectively, showing more plasma prorenin in the latter group [64]. Significantly lower content of (P)RRs in the cirrhotic liver along with increased circulating prorenin may be the expression of physiological receptor downregulation after prolonged agonist stimulation. To summarize, it is clear that (P)RR is expressed also in the liver and, as such, its role as a pathogenic factor, among many others, of hepatic fibrogenesis cannot be excluded (Figs. 11.3 and 11.4).

Sansoè and Wong observed significant natriuretic and aquaretic responses to intravenous calcium loading in human compensated cirrhosis [65] and to intravenous administration of CaSR agonists (i.e. poly-L-arginine) in experimental pre-ascitic cirrhosis [66]. Of course, these calcium-driven diuretic responses were not accompanied by any increase in plasma renin activity, due to the already described downregulating effects of CaSR stimulation on renin gene transcription and secretion by JG cells [18, 19].

Liver Cirrhosis. Endopeptidases and Peptides of RAAS Are Protagonists in Chronic Liver Disease and Its Renal Complications

Within the liver, low levels of ACE activity are detected in sham/control animals, while significantly increased levels are shown in areas of active fibrogenesis in bile duct ligated or CCl_4-treated rats [67–69]. Inhibition of ACE reduces increased arterial blood pressure, and ACE inhibitors (ACEis) or AT_1R antagonists (ARBs) can attenuate experimental liver fibrosis [68, 70], but these two classes of drugs have severe hypotensive effects in patients with established cirrhosis [71, 72]. Concentrations of ACE2, Ang1-7 and MasR (Ang1-7 specific receptor) (Fig. 11.2) are increased in splanchnic vessels from cirrhotic patients and rats compared to healthy controls [73]. Therefore, MasR blockade reduces portal pressure, indicating that activation of this

receptor in splanchnic vasculature promotes mesenteric hyperdynamic circulation and increases portal inflow that contributes to portal hypertension [73]. However, non-peptidic MasR agonist AVE0991 reduces portal pressure without any change in arterial blood pressure [74]. At first sight, these data seem contradictory: it was apparently shown that both MasR blockers and MasR agonists reduce portal pressure. It is conceivable that MasR blockade reduces portal venous inflow, as stated, while MasR agonists reduce intrahepatic resistance to portal flow since ACE2 is upregulated in areas of active liver fibrogenesis [75]. As a matter of fact, recombinant ACE2 has anti-fibrogenic effects in bile duct ligated (BDL) and CCl_4-treated rats, both acutely and long-term [76, 77], and diminazene aceturate, commonly used to treat human trypanosomiasis, enhances hepatic ACE2 activity and inhibits tumor necrosis factor-α (TNF-α) synthesis and gene expression of NADPH oxidase (NOX), a key source of fibrogenic reactive oxygen species (ROS) [78]. So doing, this drug exerts strong hepatic anti-fibrotic properties. ACE2, indeed, is thought to be a negative regulator of the RAAS and, in the liver, this enzyme functions to limit fibrosis [79].

In the kidney of cirrhotic rats with ascites, there is a mean 170% increase in NEP protein content, and NEP localizes mainly in proximal convoluted tubule and macula densa [80]; the NEP inhibitor candoxatrilat promptly increases urinary volume, and urinary excretion of sodium, atrial natriuretic peptide (ANP) and cyclic GMP (ANP second messenger), without significant changes in plasma renin activity or mean arterial pressure [80]. These overall results depend on the key contribution of NEP to ANP, Ang1-7, bradykinin clearance and to tissue ET-1 generation [24, 25, 28]. Notably, in patients with cirrhosis and ascites, renal plasma flow (RPF) and glomerular filtration rate (GFR) inversely correlate with plasma levels of ET-1 [81], and intravenous infusion of ET-1 results in prompt anti-natriuretic responses [82]. In the cytosol fraction of the cirrhotic rat liver, there is a even greater increase in NEP content, 280% to be exact. This enzyme is in the desmin-positive myofibroblast-like cells of the fibrotic septa. NEP inhibitor candoxatrilat, administered to rats with CCl_4-dependent cirrhosis, acutely decreases portal pressure and increases liver plasma flow (evaluated through indocyanine green clearance) [83]. In the kidney of CCl_4 cirrhotic animals, chymase protein content and activity are significantly increased in cortical arterioles and the tubular nephron. In cirrhotic rats and hamsters, chronic dosing of SF2809E, a specific chymase inhibitor, decreases renal Ang II content and increases natriuresis and aquaresis [84, 85]. In the liver of CCl_4 cirrhotic rats, chymase is largely expressed in α-smooth muscle-positive myofibroblasts, while, in human cirrhosis, chymase is mainly found in hepatocytes of regenerative nodules. Moreover, chymase mRNA transcription is promptly upregulated by TGF-β_1 in human HepG2 cells and activated hepatic stellate cells in vitro. Finally, SF2809E, specific chymase inhibitor, reduces liver Ang II content, hepatic fibrogenesis and portal pressure in CCl_4-treated animals [84, 85].

To sum up, in the diseased liver, areas of active fibrogenesis express increased contents of ACE [67], chymase [85], and NEP [83], but also of ACE2 [75]. This leads to increased tissue levels of Ang II [85] and some five-fold increase in the Ang II/Ang1-7 ratio in the diseased liver [64]. Particularly critical is the role of desmin- and α-smooth muscle-positive liver myofibroblasts (HSC/MFs) of liver fibrotic septa:

these cells host over-expressed NEP [83], the enzyme that degrades the vasodilating and anti-fibrogenic Ang1-7 [24, 25], and are active sources of Ang II and ET-1 through cellular over-expression of ACE and chymase [69, 85].

Moreover, in patients with liver cirrhosis, renal RAAS is aberrantly activated: angiotensinogen is secreted into proximal tubular fluid [25], active renin is massively produced in advanced cirrhosis with ascites, and ACE, NEP and chymase are upregulated and hyperactive in the tubular nephron even before clinical decompensation [24, 25, 83, 85]. This leads to Ang II concentrations in the kidney interstitial and tubular fluids being much higher than normal well before ascites development and before secondary hyper-reninism (i.e. irrespective of systemic levels of Ang II) [25, 85], producing a net effect of sodium retention along all segments of the tubular nephron (see later for the specific mechanisms of Ang II-dependent renal sodium retention).

Liver Cirrhosis. Receptors of Angiotensins and Post-receptor Mechanisms of Disease

Ang II is a key contributor (through binding to AT_1Rs) to progression of liver fibrogenesis, cirrhosis development, and worsening of hepatic function in chronic liver disease. Liver fibrosis progression depends on interactions among injured hepatocytes, activated inflammatory cells, and hepatic myofibroblast (MFs)-like cells that originate mainly from activation of hepatic stellate cells (HSCs) or portal fibroblasts. Activated HSCs produce Ang II [67], Ang II binds to AT_1R expressed by most myofibroblasts, and transcription of genes encoding for extracellular matrix components, pro-fibrogenic cytokines (e.g. TGF-β_1) and collagenolysis inhibitors occurs [86–88].

The role of AT_1R signaling in HSC activation and collagen deposition in chronically diseased liver is predominant (Fig. 11.6).

- *RhoA/Rock-1 pathway*. Among members of the Rho small GTPase superfamily (AT_1R signaling mediators), Ras homolog gene family member A (RhoA) constitutes the RhoA/Rock-1 (Rho-associated coiled-coil-containing kinase protein-1) signaling pathway, with resultant activation of the small G protein Rac and reactive oxygen species (ROS) production, which plays a central role in the development of liver fibrosis [89]. Notably, one of the most important effects of ROS is the reduction of nitric oxide (NO) bioavailability: superoxide radical anion (O_2^-) reacts with NO, destroying it via its conversion to peroxynitrites [90] (Fig. 11.6). In BDL rats, liver collagen deposition can be blunted and portal pressure decreased through inhibition of the RhoA/Rock 1 signaling pathway, which is instead activated by Ang II through AT_1R, [91].
- *JAK2 pathway*. Through mechanisms that are not fully understood but probably involve Ca^{++} and PYK_2 or Src kinase, stimulation of AT_1Rs activates intracellular Janus kinase-2 (JAK2). JAK2 then phosphorylates Rho guanine nucleotide exchange factor 1 (ARHGEF1), which stimulates the RhoA-Rho kinase–myosin

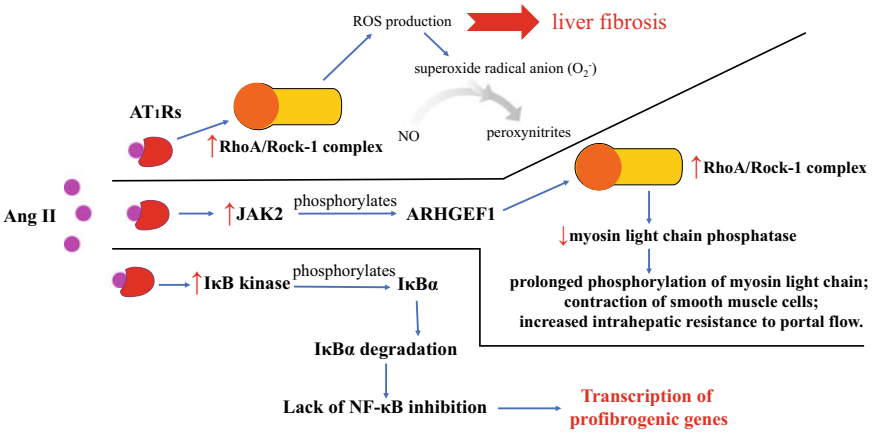

Fig. 11.6 Mechanisms of liver disease mediated through AT_1R stimulation by Ang II. Ang: angiotensin; AT_1R: angiotensin type 1 receptor; ARHGEF1: Rho guanine nucleotide exchange factor 1; IκB: inhibitor of NF-κB; JAK2: intracellular Janus kinase-2; NF-κB: Nuclear factor κB; NO: nitric oxide; RhoA: Ras homolog gene family member A; Rock-1: Rho-associated coiled-coil-containing kinase protein-1; ROS: reactive oxygen species

phosphatase target subunit cascade, and this inhibits myosin light chain phosphatase, leading to prolonged phosphorylation of myosin light chain and final contraction of vascular smooth muscle cells, the physiological effect of Ang II [92] (Fig. 11.6). Interestingly, JAK2 antagonists significantly attenuate HSC activation and collagen accumulation in experimental liver fibrosis models [93]. Notably, JAK2 phosphorylates and activates signal transducer and activator of transcription 3 (STAT3), and JAK2/STAT3 pathway is aberrantly expressed in tissues infected by SARS-CoV-2 during COVID-19 [2, 94], as well as in most malignancies: e.g., breast, pancreatic, bladder, colorectal, gastric cancers, lung adenocarcinoma, and natural killer/T-cell lymphoma [95].

- *NF-κB pathway*. Activated liver HSCs express constitutive nuclear factor-κB (NF-κB), which promotes HSC survival by stimulating the expression of anti-apoptotic proteins. Specific inhibition of NF-κB is sufficient to provoke apoptosis of mature human HSCs and blunting of liver collagen deposition. Human HSC activation is accompanied by a sustained transcriptional repression of IκBα, the natural inhibitor of NF-κB. Moreover, upon stimulation of AT_1Rs in activated HSCs, serine residues on IκBα are phosphorylated by the IκB kinase. This results in progressive degradation of IκBα, which releases NF-κB for nuclear transport and interaction with profibrogenic target genes, leading to their transcription [96] (Fig. 11.6).

In liver cirrhosis, intrarenal RAAS is activated earlier than its systemic counterpart, as confirmed in humans with pre-ascitic disease. In fact, lower-body negative pressure, which reduces central blood volume, enhances renal renin and Ang II secretion rates [97]. Moreover, despite baseline suppression of systemic RAAS, sodium

overload induced by high sodium diet is reversed by the AT_1R antagonist losartan administered at a dose not perturbing systemic hemodynamics, stressing exclusive intrarenal activation of renin-angiotensin system [98, 99].

In the kidney, Ang II constricts the efferent glomerular arteriole more than the afferent one, resulting in a tendency to preservation of GFR and filtration pressure. This occurs at the expense of reduction in renal plasma flow, increase in filtration fraction, and decrease in peritubular capillary hydrostatic pressure. The latter leads to retention of sodium and water in the tubular nephron [100].

In addition, Ang II causes direct sodium reabsorption in the proximal convoluted tubule through stimulation of tubular AT_1R and activation of renal cortical Na^+/H^+ exchanger 3, a process involving an increase in intracellular Ca^{++} and activation of JAK2 and calmodulin [100, 101]. Enhanced release of Ang II and increase in oxidative stress (through activation of NOX and RhoA/Rock 1 kinase pathways) are also the key to further renal sodium retention via increased activity of thiazide-sensitive sodium chloride cotransporter in the later segments of the distal convoluted tubule [90]. Finally, increased systemic levels of Ang II and secondary aldosteronism lead to aldosterone-dependent Na^+/K^+-ATPase and epithelial sodium channels (ENaCs) upregulation in the collecting duct and stimulation of arginine vasopressin (AVP) secretion. In turn, increased plasma AVP and increased reactive oxygen species (ROS) (due to stimulation of kidney AT_1Rs) enhance the activity of Na^+-K^+-$2Cl^-$ cotransporters in the thick ascending limb of the Henle's loop [90]. In other words, increased renal content of Ang II, firstly, and increased systemic levels of Ang II with secondary aldosteronism, secondly, completely control sodium retention along all segments of the tubular nephron in cirrhosis, both in pre-ascitic and in ascitic patients.

Unfortunately, in patients with cirrhosis and ascites or end stage liver disease, oral ACEis or ARBs, due to their arterial vasodilatory activity, do not improve natriuresis and may aggravate arterial hypotension and hyper-reninism, leading to final fall in both RPF and GFR [102–105]. This is due to the systemic activation of RAAS, which nonetheless tries to compensate for the peripheral arterial vasodilatation of advanced cirrhosis [102]. Perhaps compensated patients with early cirrhosis and no systemic RAAS activation might take advantage of ARBs administration, at least to reduce liver fibrogenesis [67, 103]. In any event, recent systematic reviews of available trials show that ARBs, in patients with ascitic cirrhosis, do not reduce portal pressure significantly and increase the risk of symptomatic hypotension and renal failure [1, 104]. Moreover, it has long been known that ACEis in liver cirrhosis do not reduce portal pressure in Child–Pugh A cirrhotic patients [105–107] and, of course, are detrimental in decompensated cirrhotic patients [108].

Finally, in clinical settings characterized by enhanced systemic production of Ang II (e.g. in decompensated cirrhosis), AT_1Rs-enriched exosomes transfer such receptors to peripheral target cells, in order to offset the physiological receptor downregulation after prolonged agonist stimulation. Exosomes are extracellular nanovescicles of 30–100 nm in size that are released into the extracellular space by cardiomyocytes through reverse budding of multivesicular intracellular bodies [109].

The discovery of non-peptidic AT_2R agonists offers hope for new therapeutic approaches to modify the AT_1R/AT_2R balance [110]. Among these AT_2R agonists, the most promising one, in relation to the management of cirrhosis complications, is Compound 21 (C21), which, in animal models of arterial hypertension, produces dose-dependent natriuretic and aquaretic effects but does not reduce blood pressure unless the AT_1Rs are also blocked [111].

Moreover, agonists of AT_2R do blunt fibrogenesis in chronic liver disease [55].

The putative MrgD ligand alamandine (Fig. 11.2) can attenuate arterial hypertension, alleviate cardiac hypertrophy in spontaneously hypertensive rat [58], and appears to attenuate hepatic fibrosis by regulating autophagy induced by NOX 4-derived reactive oxygen species [112]. Unfortunately, no human studies are available regarding this specific topic.

Liver Cirrhosis. Secondary Aldosteronism, Renal Sodium Retention and Progression of Liver Fibrosis

Patients with advanced liver cirrhosis and ascites display splanchnic and systemic hyperdynamic circulation, contraction of effective arterial blood volume, hyperreninism and secondary aldosteronism [113]. Beyond the expected worsening of sodium retention because of secondary hyperaldosteronism itself, aldosterone, whose secretion by glomerulosa cells of adrenal glands is under Ang II control through stimulation of AT_1Rs, has a definite role also in the initial development of cirrhotic ascites. In rats with CCl_4-induced cirrhosis, pre-ascitic renal sodium retention is temporally related with increasing renal aldosterone excretion and is prevented by the aldosterone antagonist spironolactone [114]. In upright pre-ascitic cirrhotic patients, renal sodium retention is associated with a borderline elevation in plasma aldosterone and increased tubular sodium reabsorption by the distal nephron [115].

This traditional view of aldosterone as a trigger of clinical decompensation of liver cirrhosis has been recently enriched after the observation that patients with arterial hypertension chronically treated with ACEis show paradoxically high levels of circulating aldosterone because of the so called 'aldosterone escape': when ACE is blocked by ACE inhibitors, chymase and cathepsin G start producing Ang II, with the result of increased aldosterone plasma levels during prolonged ACEi administration. This 'aldosterone escape phenomenon' is thought to be the cause of ACEi treatment failure in the prevention of progressive renal fibrosis that occurs in subgroups of patients with arterial hypertension. Indeed, sustained increased levels of plasma aldosterone, as occur also in patients with advanced liver cirrhosis, induce ubiquitous plasminogen activator inhibitor-1 (PAI-1) expression, and treatment with mineralocorticoid receptor antagonists reverses this phenomenon. PAI-1 is a member of the serine protease inhibitor (serpine) gene family and the main inhibitor of tissue-type and urokinase-type plasminogen activators (tPA and uPA), and therefore of fibrinolysis. Unfortunately, the same tissue PAI-1, as such induced by increased plasma levels

of aldosterone, is also a strong inhibitor of plasmin-dependent matrix metalloproteinases (MMPs) activation in the liver, where MMPs should provide the reabsorption of excess extracellular matrix deposition during chronic liver diseases [3, 116–118]. In brief, plasma aldosterone, through increased PAI-1 gene expression, is considered a relevant agent of progressive liver fibrosis in chronic liver disease [117, 118].

Liver Cirrhosis. Aminopeptidases and Chronic Liver Disease

Recently, it has been shown that plasma aminopeptidase A is significantly reduced in patients with liver cirrhosis [119]. In this clinical context, this means that lack of aminopeptidase A provides less Ang2-8 to aminopeptidase N, which in turn generates lesser amounts of the natriuretic Ang3-8 (Fig. 11.1). Furthermore, slowed degradation of Ang II itself means prolonged half-life of this key anti-natriuretic peptide, which perpetuates the vasoconstrictive and sodium retaining effects of Ang II.

Conclusions

With a more comprehensive understanding of the systemic and tissue RAAS, it is perhaps time to advance an updated theory of liver insufficiency and associated functional renal failure in cirrhosis. What was once thought of as secondary to mere hemodynamic abnormalities (i.e. the hyperdynamic circulation of liver cirrhosis with ensuing contraction of effective arterial blood volume) is now complementary to our understanding of the changes that occur both inside the diseased liver and inside the kidney: chymase, ACE, NEP are overexpressed and functioning in both organs, leading to a net imbalance towards too much Ang II and too little Ang1-7. The consequences in the organs are different: inflammation and progressive fibrosis inside the liver, vasoconstriction, tubular sodium retention and final GFR loss inside the kidney.

Conflicts of Interest None to declare.

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Chapter 12
Renin-Angiotensin-Aldosterone System Role in Organ Fibrosis

Maha Ghanem and Haidy Moustafa Abdelsalam Megahed

Abstract The normal healing process secondary to injury, disease or toxins is a mandatory process to human life. The healing process begins with hemostasis, then inflammation which is accompanied by stimulation of the immune system followed by macrophages and neutrophils that infiltrate and remove tissues and cell debris. The immune cells stimulate the formation of vasoactive, proinflammatory, and profibrotic effectors. Fibrosis pathogenesis involves several cellular and molecular signaling mechanisms. The renin-angiotensin-aldosterone system (RAAS) interacts with multiple pro-fibrotic pathways to mediate fibrosis in different cell types, including Transforming Growth Factor (TGF-β1), Tumor Necrosis Factor (TNF), Platelet-Derived Growth Factor (PDGF) and Interleukins as IL-6 and IL-13, to accelerate the proliferative phase of repair. Angiotensin converting enzyme-2 (ACE-2) has been shown to play a key role in the protection against lung disease and liver disease, via effects mediated by the MAS oncogene and the ACE-2 peptide product ANG1–7 receptor. Deviating healing by repeated tissue injury leads to increased proliferation and decreased myofibroblast apoptosis. Myofibroblasts have a cytoskeletal structure that includes actin and myosin, which link the actin filaments to the extra cellular matrix (ECM). Therefore, whenever myofibroblasts contract the surrounding matrix, there will be deformity and failure of organ function. Myofibroblasts produce ECM components including glycoproteins, proteoglycans, and collagen, which lead to the creation of fibrous scar tissue. Furthermore, interlinking of collagen in fibrous scar tissue results in tissue that is more resistant to protease degradation, leading to scarring that is irreversible, and tissue architecture destruction. Myofibroblasts activation occurs through signaling pathways. These signal transduction pathways [including TGF-β, Yes-associated protein 1 (YAP)/transcriptional coactivator with PDZ-binding motif (TAZ) signaling and Wingless/Int (WNT)], have been associated with the pathophysiology of fibrosis. Delaying the liver and lung fibrosis process can be achieved by the action of ACE inhibitors and angiotensin receptor blockers (ARB).

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Keywords Fibrosis · Lung · Liver · Signaling pathway · TGF-β1 · Int (WNT) · (YAP) · Metabolic disorder · α2-AR blockers

Introduction

Renin-Angiotensin-Aldosterone System (RAAS)

Renin-angiotensin-aldosterone system (RAAS) is an endocrine system that has been expressed in many tissues sites, so it is either circulating RAAS or local RAAS. The circulating RAAS has a well-known role in monitoring and controlling the blood pressure, systemic vascular resistance and fluid and electrolyte balance, through several mechanisms. Whenever there is a decrease in blood pressure, an immediate response occurs secondary to the stimulation of the baroreceptors which respond to the variations in the blood pressure. The delayed response in adjusting the blood pressure occurs through the RAAS. This system involves renin, angiotensin II, the kidneys, and aldosterone. In reaction to decreased renal blood pressure, decreased sodium transport to the distal convoluted tubule (DCT), and/or beta-agonism, the arterial pressure is raised. Its effect is through boosting salt and water reabsorption as well as vascular muscle tone [1].

Components of the RAAS System (Fig. 12.1)

1. **Renin**: juxtaglomerular (JG) cells contain prorenin. Low sodium load in the DCT will initiate activation of these cells and the transformation of prorenin to renin which will target angiotensinogen [2–4].
2. **Angiotensinogen**: the liver releases angiotensinogen into the plasma where it is changed to angiotensin I by the action of renin. Angiotensin I will be transformed to angiotensin II by the angiotensin converting enzyme (ACE) which is in the vascular endothelium of the lungs and kidneys [1].

   Angiotensin II degrades into angiotensin III and IV in the plasma. Angiotensin III has 100% of the aldosterone-stimulating impact of angiotensin II but only 40% of the pressor effects, whereas angiotensin IV has a lower systemic effect [1]. Angiotensin II leads to vasoconstriction of arterioles by binding to G protein-coupled receptors, resulting in a secondary messenger cascade. Also, in the brain, angiotensin II binds to the hypothalamus, stimulating thirst and increased water consumption [5]. It also stimulates the release of ADH which increase water reabsorption in the kidney through the aquaporin channels at the collecting duct. As well as it reduces the baroreceptor response to a rise in blood pressure [1].
3. **Kidney**: Angiotensin II acts by increasing sodium reabsorption in the kidneys which consequently leads to an increase in the blood osmolarity with shifting of fluids into the blood volume with an increase in the arterial pressure [6].

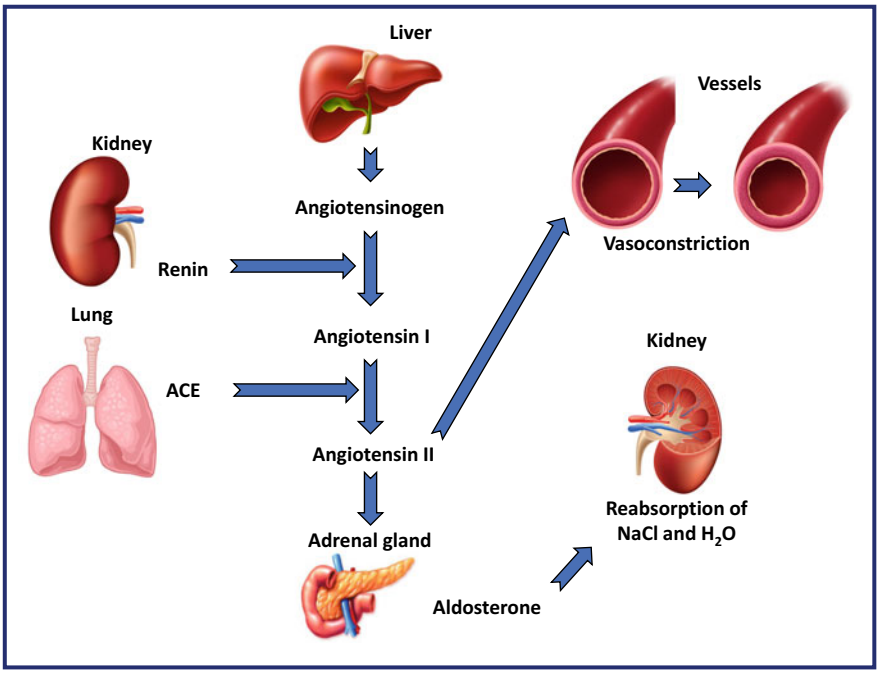


Fig. 12.1 Renin-Angiotensin-Aldosterone System (RAAS). It regulates the blood pressure through vasoconstriction of vessels with angiotensin II involvement, and hydro-electrolytic regulation with the intervention of aldosterone and its action in the kidney. ACE: angiotensin-converting enzyme; NaCl: sodium chloride; and H_2O: water [8]. Reproduced after journal and author permission

4. **Aldosterone**: It is released because of the action of angiotensin II on the adrenal cortex. Aldosterone increases sodium levels by reabsorption at the nephron's distal tubule and collecting duct, resulting in an increase in blood volume. It operates by triggering the insertion of luminal Na channels and basolateral Na–K ATPase proteins [7].

Renin-Angiotensin-Aldosterone System (RAAS) Role in Fibrosis

Fibrosis is a progressive buildup of ECM components produced by myofibroblasts. Whenever it is stimulated, fibrosis occurs in organs and ultimate failure takes place [9]. Fibrosis can affect any of the body's major tissues. Idiopathic pulmonary fibrosis (IPF) and liver cirrhosis are two of the most frequent fibrotic illnesses [10]. The incidence of IPF was estimated to be around 10/100,000/year, while incidence of

cirrhosis in adults, was estimated at 30.2/100,000 (95% CI 27.8–32.7), 38.8 for males (95% CI 35.0–42.9) and 21.8 for females (95% CI 19.0–24.9) [11, 12].

Causes of Liver and Lung Fibrosis

Oxidative stress and altered ECM deposition are frequent damage responses in the liver and lung (lung-liver axis). Local macrophages in both organs also play a key role in mediating the immune/inflammatory response. It was observed that IPF is an autoimmune response to HCV infection. Liver fibrosis is the result of chronic viral (such as chronic viral hepatitis), parasitic infections, chronic alcohol consumption, and nonalcoholic steatohepatitis (NASH). Furthermore, exposure to hepatotoxins (e.g., carbon tetrachloride [CCl4] and acetaminophen) or radiation and bile duct ligation results in pericentral or periportal liver fibrosis, respectively. Lung fibrosis is also associated with autoimmune diseases such as, scleroderma (systemic sclerosis) and sarcoidosis [13].

Local Renin-Angiotensin-Aldosterone System (RAAS)

Local RAAS and its precursor prorenin are highly expressed in tissues. They have been identified in tissues including the liver, lung, heart, kidney, etc. It participates in the injury process, inflammatory reaction, and fibrogenic diseases of many organs, as the liver and lung by another method which is not related to blood derived RAAS [13].

ACE-2 Protective Role in Liver and Lung Fibrosis

Angiotensin converting enzyme-2 (ACE-2) and its products Ang 1–7 protect against the development of liver and lung fibrosis. On inducing acute lung injury, ACE-2 has a clear protective effect in response to acid reflux. Its protective effect was associated with lower levels of Ang II following lung damage. According to an experimental investigation, Ang II downregulates ACE-2 mRNA via angiotensin receptor I in a positive feed-forward mechanism that amplifies Ang II-mediated responses [13]. The MAS oncogene and the ACE-2 peptide product ANG1–7 receptor has been demonstrated to have synergistic role in the protection against lung disease as well as to liver disease. Through MAS receptor pathways, ACE-2 is downregulated in fibrotic lung diseases in humans. It is decreased in the induced pulmonary fibrosis—IPF—and has a protective effect in that disease. Scientists found that ACE-2 affects alveolar epithelial cell survival by balancing proapoptotic Ang II and its antiapoptotic degradation product Ang 1–7 through MAS receptor. Histologically, humans have two

types of the pulmonary alveolar epithelium (AE). AE2 can synthesize and secrete of lung surfactant, and it differentiate into AE1 cells during repair process after injuries. Adult AE1 cells also are capable to proliferate and give rise to AE2 cells leading to post pneumonectomy regeneration [5]. Immunoreactive ACE-2 was missing in alveolar epithelia positive for proliferation markers in lung biopsy specimens from IPF patients, but abundant in alveolar epithelia devoid of proliferation indicators. This explained the decrease of ACE-2 in lung fibrosis and demonstrated that this protective enzyme is regulated by the cell cycle [5].

Hepatic inflammation, fibrosis, and tissue remodeling following injury are all regulated by ACE-2 and RAAS. By stimulating hepatic stellate cells and boosting the production of transforming growth factor, RAAS promotes hepatic fibrosis. Delaying the liver fibrosis process can be achieved by the action of ACE inhibitors and angiotensin receptor blockers (ARB). Furthermore, ACE-2 supplementation can prevent hepatic fibrosis in a bile duct ligation mouse model (acute form) through its receptor MAS [13–16].

Role of Angiotensin-Converting Enzyme 2 (ACE2) in COVID-19

Recently it was found that SARS-CoV infection can lead to multiple organ injury not only to the lungs but also the liver, heart, and kidney. Virus-induced inflammatory process and immune cell trafficking regulate and determine the nature of immune responses through excessive production of cytokines and chemokines, insufficient interferon response, and auto-antibodies that are responsible for the disease pathogenesis. Studies on SARS patients found that Monocyte Chemoattractant Protein-1 was highly expressed in SARS-CoV-infected ACE2+ cells, which means that the virus stimulates local immune response [17].

ACE2 that converts Ang-II to angiotensin-(1–7) and MAS, counteracts the negative effects of the RAAS and has anti-inflammatory effects, in addition to acting as a receptor for SARS-CoV and SARS-CoV-2 and this was evident by high expression of ACE2 in organs and tissues. Organ injury in SARS-CoV infection may be due to the imbalance between ACE/ACE2 and Ang-II/angiotensin-(1–7) due to downregulation of ACE2 expression [17] (Fig. 12.2).

Signal Transduction Pathway

Targeting the RAAS in Fibrosis

After tissues injury, the healing process starts with hemostasis, then inflammation which is accompanied by activation of the immune cells e.g., neutrophils and

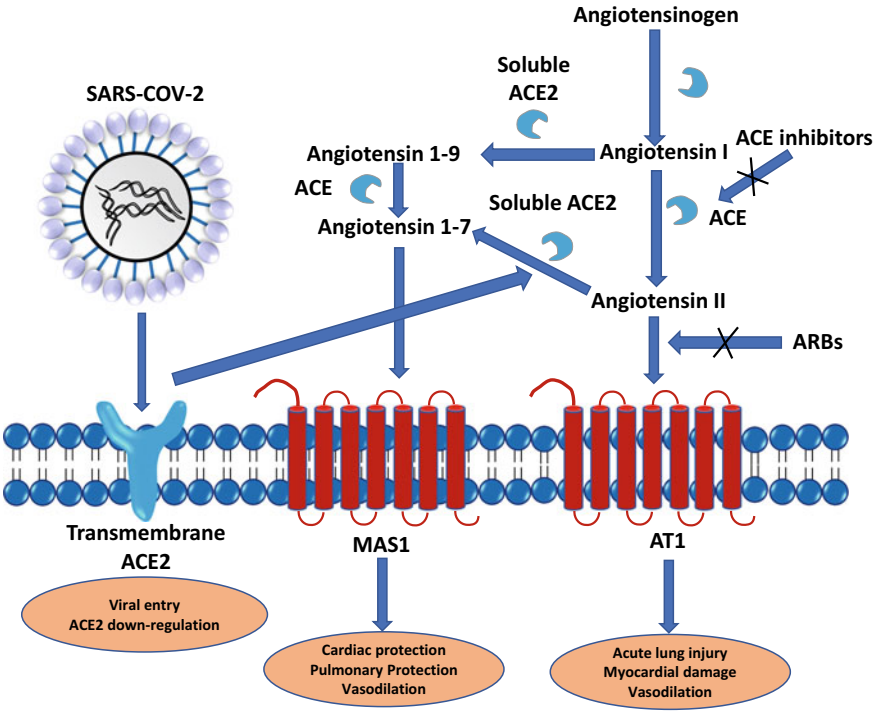


Fig. 12.2 Renin-angiotensin system (RAAS) and its interaction with SARS-COV-2 [17]. Reproduced after journal permission

macrophages which infiltrate and remove tissue and cell debris. Fibrosis pathogenesis involves several cellular and molecular signaling mechanisms. The RAAS interacts with multiple pro-fibrotic pathways to mediate fibrosis in different cell types, including Transforming Growth Factor (TGF-β), Tumor Necrosis Factor (TNF), Platelet-Derived Growth Factor (PDGF), IL-6 and IL-13, to accelerate the proliferative phase of repair [16].

Other than transforming growth factor (TGF-β), There are several signaling pathways involved in the process of fibrosis, including Wingless/Int (WNT) and Yes-associated protein 1 (YAP)/transcriptional coactivator with PDZ-binding motif (TAZ) signaling. Genes involved in myofibroblast cell increase the number of cellular component when the TGF-β-induced SMAD complex is engaged, and this mechanism is also regulated by long and short noncoding RNA molecules, as well as epigenetic alterations of DNA and histone proteins. The same thing happens when WNT-stabilized-catenin translocate to the nucleus and induces transcription of its target genes. YAP and TAZ are two transcriptional co-activators in the Hippo signaling pathway that rely on nuclear translocation to operate in addition to other signaling pathways [9, 17, 18].

Transforming Growth Factor (TGF-β)

TGF-β is the most effective element in causing precursor cells to differentiate into myofibroblasts, which release ECM to preserve the structure of damaged tissue during repair and to promote granulation tissue development or parenchymal regeneration. In the final maturation phase, the provisional ECM is destroyed and reformed to restore the parenchymal tissue architecture. Constant stimulation to myofibroblasts or repeated or chronic injury prevent ECM to be resolved leading to the formation of fibrotic tissue with subsequent organ failure [19].

There are three isoforms of TGF beta (1, -2 and -3) transcribed from various genes but attached to one receptor and signaling via the same canonical (Smad-based) and non-canonical pathways (non-Smad-based). All three TGF-β isoforms bind as homodimers (their active form) to TGF-β receptor 2 (TGFR2), which subsequently recruits and promotes TGFR1 to initiate receptor signaling. TGF-2 has a role in tumor development by while TGF-3 participates in the development of the palate and lungs, it may also play a role in wound healing in wounded skin [20]. Experimentally, overexpression of the active form of TGF-β1 in the liver of an animal is enough to induce fibrosis in organs [20, 21] (Fig. 12.3).

The action of each isoform is controlled by several mechanisms including ECM-associated Small Leucine-Rich Proteoglycans (SLRPs), latent TGF beta growth factors (produced by specific cells in the organs), interactive cytokine-growth factor receptor systems, basement membrane which can change the activity of the isoforms and others as PDGF and Connective Tissue Growth Factor (CTGF) [21–24].

Because TGF beta-3 controls fibrotic gene expression in many more ways than TGF beta-1 and TGF beta-2, and the differences are typically modest, it was hypothesized that TGF beta-1 and TGF beta-2 are pro-fibrotic while TGF beta-3 is anti-fibrotic. TGF beta-3, on the other hand, thought to be a fibro-modulatory partner for the other two isoforms, permitting for more response to damage. The interaction between the three isoforms and other proteins regulates the process of fibrotic and nonfibrotic healing in response to infections, radiation, and toxins in an organ, as well as the regression of fibrosis when it occurs [23].

In conclusion, TGF β1 stimulates fibrogenesis in several ways; it prevents ECM breakdown by decreasing matrix metalloproteinases (MMPs) and increasing TIMP, a natural inhibitor. It also promotes myofibroblast development via tubular EMT. Lastly, it stimulates matrix synthesis via SMAD3-dependent or non-SMAD-related pathways [24–26].

Syndecans Amplifying the Angiotensin II and TGFβ1 Signaling

Syndecans are a type of heparin sulphate proteoglycan found on the surface of all adherent and some nonadherent cells. It acts as stabilizer to maintain the morphology

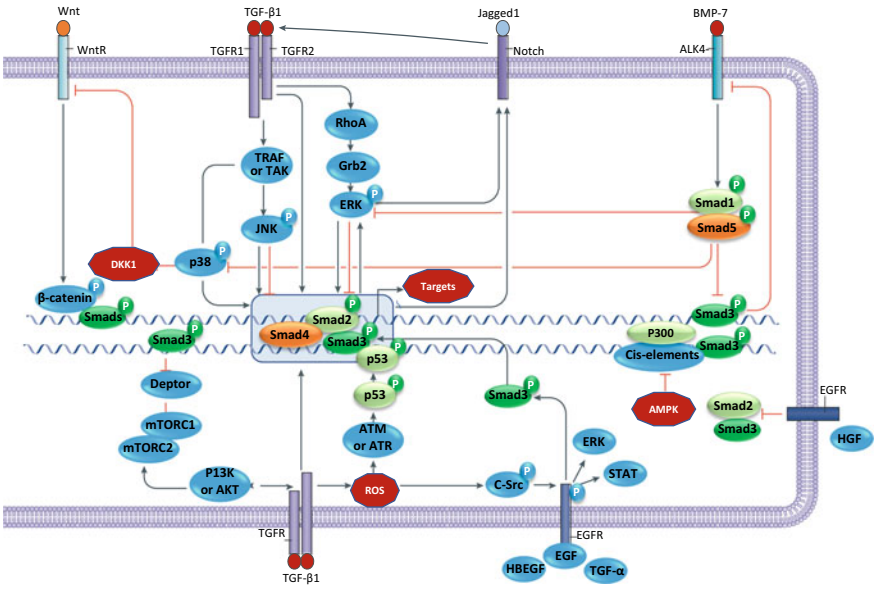


Fig. 12.3 Crosstalk between TGF β/Smad and other pathways in tissue fibrosis. TGF 1 interacts with other signaling pathways in addition to controlling transcription by phosphorylating Smad2 and Smad3 and permitting the development of a Smad2/3/4 complex that stimulates transcription. In a Smad-independent manner, it can stimulate the mitogen-activated protein kinases (MAPKs), p38, JNK, and ERK. Smad3 transcriptional activity can be modulated by MAPKs phosphorylating the linker region of Smad proteins. Other signaling pathways, such as angiotensin II, oxidative stress, and hyperglycemia, can also activate MAPKs and affect Smad phosphorylation. Wnt ligand-induced -catenin stabilization can make it easier for -catenin to form complexes with Smad proteins and boost profibrotic gene transcription. TGF-/Smad facilitates the activation of reactive oxygen species (ROS), hypoxia-responsive element activity, and hypoxia-inducible factor 1 expression through the mammalian target of rapamycin complex 1 (mTORC1). TGF- may activate the p53 tumor suppressor, which can then form complexes with Smad2/3 to control gene transcription. TGF- also can transactivate the epidermal growth factor receptor (EGFR) through a ROS-dependent mechanism. Smad1 and Smad5, which are negative regulators of Smad3-based gene transcription, are activated by bone morphogenic protein 7 (BMP 7) [26]. Reproduced after author and journal permission

of epithelial sheet through connection the ECM to the intracellular cytoskeleton. Syndecan-1 is a matrix receptor that transmits information between the ECM and the cell's outside.

It has a specific function:

1. Its ectodomain connects to various interstitial matrix components,
2. It is found on the basolateral surface of epithelial cells in culture and in simple epithelia in vivo, and
3. In polarized epithelial cells, it colocalizes with cytoskeletal actin filaments. By boosting angiotensin II and TGF1 signaling in an HS-dependent manner, Syndecan-1 remodulates matrix formation and promotes fibrosis. Syndecan-1

on the cell surface appears to promote fibrosis by inhibiting epithelial repair and upregulating fibrotic factors, whereas syndecan-1 on the ectodomain appears to promote fibrosis by inhibiting epithelial repair and upregulating fibrotic factors [27, 28].

Targeting Wingless-Integrated/β-Catenin (WNT/β-Catenin) Signaling Pathway

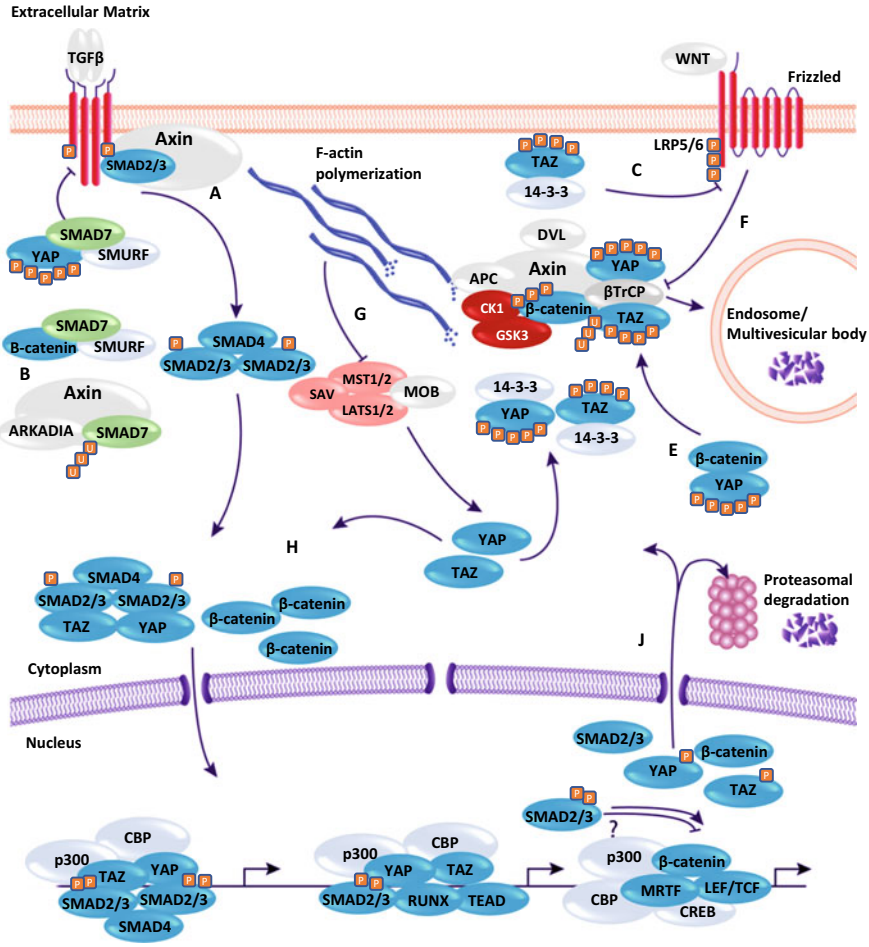
WNT is a glycoprotein secreted in human and present in nineteen ligands. The word comes from a mix of proto-oncogene integrated or (int-1) and Drosophila gene wingless (Wg). According to the pathway that will be initiated, WNTs stimulate both noncanonical (β-catenin independent) and canonical (β-catenin dependent) pathways [7, 28]. The WNT/β-catenin pathway is important in the liver and lung development, repair and regeneration after sickness or malignancy. Its activation results in regeneration process while its signaling dysregulated results in delayed regeneration and this is observed in organ disease [7, 29].

Canonical WNT Signaling

The nuclear translocation of β-catenin is caused by WNT signaling, which involves molecular interactions. WNT has been demonstrated to be a diversified growth factor in both homeostasis and illness and is implicated in the development and polarity of the main body axis. The receptors called Frizzled (Fz) are the site receptors where soluble WNT ligands bind to it. Multiple Fz receptors can be influenced by a single WNT ligand [3, 23, 30].

When the concentration of endogenous WNT antagonists exceeds the concentration of WNT ligands, cytoplasmic β-catenin is phosphorylated by two components of the β-catenin destruction complex, and -catenin is degraded (casein kinase (CK)one and glycogen synthase kinase (GSK)3) what is called a WNT-off state [31, 32].

When WNT ligands bind to Fz receptors, they enhance the signaling cascade by interacting with density-lipoprotein-receptor-related proteins (LRP)5/6 co-receptors. GSK3 and CK1 proteins phosphorylate LRP, which then interact with Disheveled (DVL), Axin, and GSK3 via Pro-Pro-Pro-(Ser/Tyr)-Pro repetitions, resulting in the WNT-on state. Axin interacts with -catenin, GSK3, CK1, APC (adenomatous polyposis coli), and the ubiquitin ligase-TrCP in the preceding complex. Because the Fz/LRP complex sequesters Axin and GSK3 to the plasma membrane, β-TrCP is excluded from the destruction complex, limiting β-catenin ubiquitination and degradation. When LRP5/6 aggregates with the destruction complex in so-called multivesicular bodies, this activates WNT. Catenin cannot interact with the destruction complex and so escapes the ubiquitylation process inside multivesicular bodies. To regulate the transcription of target genes, stabilized β-catenin accumulates in the



nucleus and interacts with TCF/Lef-1 transcription factors. WNT signaling can be abnormally activated by increased expression of WNT agonists or suppression of endogenous WNT antagonists, such as proteins from the Dikkopf (DKK) and secreted frizzled-related protein (sFRP) [23] (Fig. 12.4).

Experimental Research recommend that canonical WNT signaling can activate the program in fibroblast. It was observed that consumption of β-catenin cannot stop fibrosis process completely and it is not enough for the upregulation of specific genes of myofibroblasts-in other research on pulmonary tissue. The complicated expression pattern of WNT ligands during fibrogenesis demonstrates the complexity of β-catenin cytoplasmic/nuclear shuttling regulation [23, 33].

◀**Fig. 12.4 The TGF-β, WNT, and YAP/TAZ signaling pathways converge. Schematic overview of the molecular crosstalk between components of the TGFβ, WNT, and YAP/TAZ pathways**. **a** Axin promotes the tail-phosphorylation of Smad3 whenever TGF-β stimulated. **b** Axin also increases the breakdown of inhibitory Smad7, which boosts the TGF- signal even more. Both YAP and -catenin can interact with Smad7. When YAP binds to Smad7, it increases the type I receptor's affinity and the restrictive effects on TGF signaling. Smad7 binding to -catenin can cause -catenin breakdown as well as stability. **c** TAZ prevents casein kinase (CK)one from phosphorylating disheveled (DVL), producing either positive or negative feedback depending on the WNT ligand present. **d** Both YAP and TAZ are phosphorylated by the active Hippo core kinase complex, resulting in a phosphodegron. 14-3-3 proteins sequester phosphorylated YAP and TAZ, or they connect with the -catenin destruction complex. The docking of -TrCP to the destruction complex requires the presence of YAP and TAZ. **e** Phosphorylated YAP also binds to -catenin, preventing it from translocating to the nucleus and promoting its destruction. **f** The destruction complex is inhibited when WNT is activated because YAP and TAZ separate from the complex. The LRP/Frizzled/DVL receptor complex sequesters the destruction complex, which is then targeted for breakdown in the microvascular bodies. **g** The core kinase complex is inactivated by F-actin polymerization, resulting in the dephosphorylation of YAP and TAZ. In addition, because the inactive destruction complex does not breakdown -catenin, newly produced -catenin accumulates in the cytoplasm. **h** Smad complexes that have been activated connect with YAP or TAZ and translocate to the nucleus. -Catenin, both free and stabilized, translocates to the nucleus. **i** Depending on the context, transcription factors in the nucleus may co-localize at chromatin to regulate transcription of myofibroblast-related genes. **j** Transcript factors are either destroyed in the nucleus or translocated back to the cytoplasm for degradation or a new round of activation at the end of the transcription cycle [23]. Reproduced after journal permission

YAP/TAZ Signaling

A signaling Hippo pathway has numerous biological functions in tissue regeneration, and growth. The essential components of this pathway are:

1. Yes-associated protein (YAP) and its paralog, transcriptional coactivator with PDZ-binding motif (TAZ), also known as WW-domain containing transcription regulator-1 (WWTR1),
2. MST1/2 (mammalian Sterile 20-like kinase 1/2),
3. SAV1 (Salvador family WW domain containing 1),
4. MAP4K4 (Misshapen homolog),
5. LATS1/2 (large tumor suppressor kinase 1/2),
6. MOB1 (MPs one binder 1), If Hippo signaling pathway is activated, the YAP/TAZ will be phosphorylated by LATS1/2 kinase, this phosphorylated sensor will bind to cytoplasmic protein inside the cell which is then degraded by β-TrCP [23, 34–37] (Fig. 12.5).

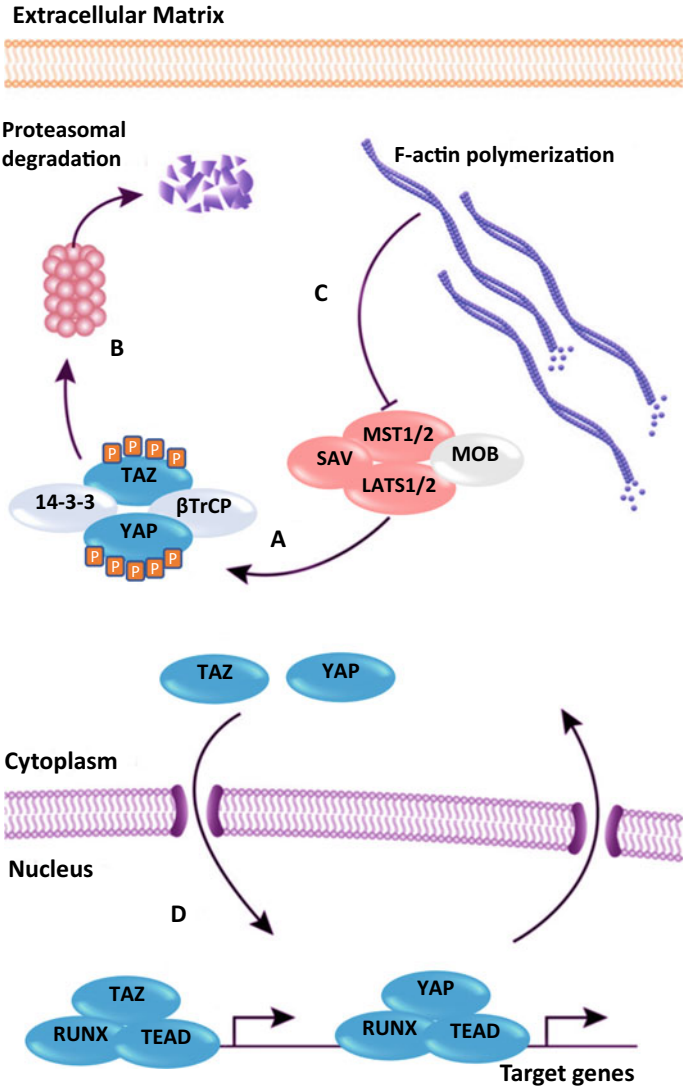


Fig. 12.5 YAP and TAZ signaling. Simplified scheme showing activation of YAP and TAZ. a When the Hippo kinase complex [MST1/2, MOB kinase activator 1 (MOB1), Salvador (SAV), and serine/threonine-protein kinases (LATS1/2)] is active, YAP and TAZ become phosphorylated on several sites, forming a phosphodegron. **b** 14-3-3 proteins sequester YAP and TAZ in the cytoplasm or TrCP targets them for destruction. **c** The F-actin cytoskeleton polymerization limits MST1/2 activity, rendering the core kinase complex inert (several other upstream activators of the core kinase complex are not shown). **d** YAP and TAZ now translocate to the nucleus, where they influence transcription by interacting with transcription factors such Runt-related transcription factor (RUNX) and TEA domain family member (TEAD) [23]. Reproduced after journal permission

YAP/TAZ in Lung and Liver Fibrosis (Fig. 12.6)

While the structures, regulations, and functions of YAP and TAZ are remarkably similar, their roles are distinct and non-overlapping. Both fibrosis and cancer are caused by YAP/TAZ signaling in collaboration with other signaling pathways e.g., TGF-β and WNT. It acts as mechanical force sensors, modulating both the fibrotic response and the way cancer cells behave. This signaling YAP/TAZ has shown abnormal activation in both the epithelial compartment and fibroblasts/myofibroblasts which is related to the pathophysiology of fibrosis [36]. The active mutant version of TAZ was reported to increase the expression of connective tissue growth factor (CTGF) and plasminogen activator inhibitor (PA1) in lung fibroblasts on a soft matrix, but not ECM proteins like collagen and fibronectin. Overexpression of TAZ, on the other hand, caused these ECM proteins to be expressed in cells cultivated on a rigid matrix [34].

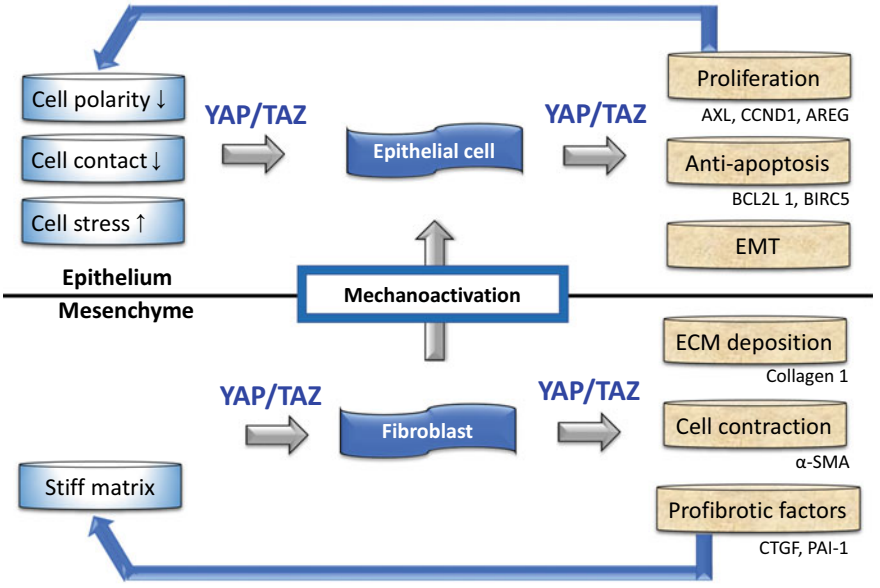


Fig. 12.6 The activation of Yes-associated protein (YAP) and the transcriptional coactivator with PDZ-binding motif (TAZ) in epithelial cells and fibroblasts. In epithelial cells, the disturbance of cell polarity, loss of cell contact, and increased cell stress signals activate YAP/TAZ, which promotes cell proliferation and epithelial-mesenchymal transition (EMT) and inhibits apoptosis. In contrast, in fibroblasts, YAP/TAZ function as sensors of extracellular matrix (ECM) stiffness through the mechano-transduction pathway. YAP/TAZ also incites the production of fibrogenic factors and ECM proteins and enhance cell contraction. This process stimulates tissue stiffness, thus forming a feed-forward loop of fibroblast activation and tissue fibrosis. YAP/TAZ can also be triggered in epithelial cells of fibrotic tissue due to increased ECM stiffness [40]. Reproduced after author and journal permission

Many findings imply that mechanical stimuli are required for full TAZ activation to enhance the production of fibrosis-related genes. According to gene expression profiling by RNA-sequencing, TAZ-regulated genes in lung fibroblasts were associated to cell migration and motility, and partially overlapped with those controlled by TGF-β, the known mediator of fibrogenesis [35]. Acceleration of the fibrotic process are accelerated by YAP/TAZ form (feed forward model). There is a direct proportional relation between the activity of YAP/TAZ and the fibrosis process. As the fibrosis process progresses the activity of YAP/TAZ stiffness sensors is increased. Also, it stimulates ECM proteins in collaboration with TGF signaling with increased production of fibrogenic factors (CTGF and PAI-1), so fibrosis process will be promoted. YAP/TAZ signaling controls the proliferation and differentiation of epithelial progenitor cells in both embryonic and adult lungs [35].

In patients with chronic liver diseases, the most common source of fibrogenic myofibroblasts is hepatic stellate cells (HSCs). In the stellate cells of mouse fibrotic livers induced by carbon tetrachloride exposure and in human cirrhotic livers caused by hepatitis C virus infection, nuclear localization of YAP was identified [36].

Verteporfin (benzoporphyrin derivative) can induce inactivation to YAP or silencing through siRNA, this will prevent YAP/TAZ/TEAD interaction and inhibits the expression of YAP target genes and myofibroblast development in HSCs. In mice, this drug can decrease liver fibrogenesis which prove the role of YAP hepatic fibrosis. HSC differentiation to myofibroblasts is directed by the hedgehog (HH) pathway-mediated activation of YAP, according to a recent study [37, 38].

According to Wang et al. [39] TAZ activation in hepatocytes increases the changing from steatosis to non-alcoholic steatohepatitis (NASH). The expression of TAZ was observed to be greater in NASH patients' hepatocytes. In mice experimentation inhibition of TAZ prevents hepatic inflammation and subsequent fibrosis. TAZ stimulation increased the expression of profibrotic genes such as collagen 1 and metallopeptidase inhibitor 1 (TIMP1) secondary to increased Indian hedgehog (IHH).

Adrenergic Receptor

They are G-protein coupled receptors (GPCRs) found in human tissues. Smooth muscle contractions are signaled by α1-adrenoceptors via G protein coupled receptors and intracellular calcium influx. Inhibition of adrenoceptors, via the use of α- and β-adrenoceptor antagonists, is used in the treatment of many of the urologic diseases. In addition, there is evidence that adrenoceptor antagonists have potential antitumor results in urology. It acts by diminishing the vascularization of the tumor with subsequent decrease in its size via regulation of the phenotypic epithelial-mesenchymal transition (EMT) [6].

α-2 Adrenergic Receptor (AR) in Liver Fibrosis and Antifibrotic Effect of the α2-AR Blockers

The noradrenergic system is thought to be important in liver fibrosis pathogenesis. Both α1- and β-adrenergic receptors (ARs) have been implicated in a variety of profibrogenic actions. Schwinghammer et al. [41] found that induced liver fibrosis by ligation of the bile duct or by CCL4 has been improved by using α2-AR blocker mesedin. The mRNA of α2a-, α2b-, and α2c-AR subtypes were increased in CCL4-treated mice (chronic form) and only α2b-AR was increased in response to liver injury in bile duct-ligated group (acute form).

In HSCs, the α2b-AR blocker mesedin reduced expression of α-smooth muscle actin, TGF-β, and α2a-AR. They believe that blocking α2-AR inhibits HSC activation and increases the permeability of liver sinusoids after liver damage [42]. This may be revealing the involvement of that α2a-AR and α1-AR in promoting fibrogenic action of norepinephrine which released by HSCs. α1-AR levels are lower in individuals with liver cirrhosis and portal hypertension, whereas β3-AR levels are greater in a CCl4 fibrosis model and in cirrhosis patients.

The protective qualities of α1 and β2 blockage have been demonstrated in studies utilizing α1-AR and β-AR antagonists, indicating the function of these receptors in the advancement of liver fibrosis [41, 43]. α2-AR blocker (Mesedin) exert its antifibrogenic action by blocking α2-AR and decreased production of α2-AR which leads to HSC deactivation. Also, it decreases the TGF β—together with the deactivation of HSCs which proved by decrease α-SMA, which was shown at the protein level and RNA. Mesedin did not influence PDGF production in HSCs, but it reduces the potency of α1-AR in hLSECs [41].

A study showed that in reaction to the NO donor chemical S-nitrosoglutathione, the density of α1-AR in rat lung membranes decreased. So, it is possible that there is a relationship between endothelial nitric oxide synthase (eNOS) and α1-AR expression levels in endothelial cells. The interaction between Adrenergic Receptor via NO may be confirmed in future investigations using NOS inhibitors [44].

Metabolic Processes

Metabolic Signature of Fibrosis

Fibrosis pathogenesis associated with metabolic changes include amino acids, lipids, glucose, etc., Evidence suggests that hepatic fibrosis alters amino acid, carbohydrate, and lipid metabolism in all liver cells. There are common pathways for lung and liver fibrosis. Their signaling showed either activation or downregulation among the liver and lung fibrosis samples. These pathways could be fibrosis-related "core pathways," which are required to turn an initial stimulus into fibrosis. Regulatory routes, which

can impact these pathways are versatile pathways particular to each of the studied organs if it does not turn the first stimuli into fibrosis [45, 46].

Metabolic Disturbance in Fibrosis Disease

The mechanisms of fibrosis are in resemblance to wound healing. It starts with inflammation, in response to damage that happened, with stimulation of the cells of immune system and fibroblasts, with secretion of ECM and increased secretion of chemokines, cytokines, and angiogenic factors to stimulate the regeneration process. Whenever this action is repeated or disturbed, scarring occurs as seen with chronic fibrosis. Other cell types contributing to fibrosis, include signaling, cell-matrix interactions, motility changes, cytoskeletal and, transcription. All these activities necessitate energy carriers to be fulfilled. Metabolic biosynthetic and bioenergetic pathways provide a supply for these activities and the pathological cells in will adapt to be able to conduct their synthetic activities and proliferation [10]. These pathways include:

1. **Fibrosis and Glycolysis**:

The metabolism of glucose begins with its intracellular transport and breakdown resulting in formation of 2 molecules of ATP and pyruvate. Several cellular enzymes e.g., phosphofructokinase, hexokinase, and pyruvate kinase control its flux. Pyruvate is converted by the mitochondria to acetyl-CoA or lactic acid to be part of the tricarboxylic acid (TCA) cycle. This process could occur either anaerobically (which is the most common) or aerobically which occurs in some cases of cancer and is called the Warburg Effect (WE). A hypothesis claimed that these proliferating cells activities were supplied by an increase in glycolytic intermediates through secondary pathways [47]. In fibrosis, a similar reverse has been suggested, in which aerobic glycolysis occurs in fibroblasts and epithelial cells and macrophages were influenced by glycolytic metabolites (or vice versa), contributing to this pathology. Aerobic glycolysis has been shown to occur in fibrosis of several organs as kidney fibrosis [48], lung fibrosis [49] and liver cirrhosis [50]. Glycolysis process is increased by Hypoxia-inducible factor 1-alpha (HIF1α) stabilization through activation of glycolytic enzymes and glucose transporters, inhibit the pyruvate dehydrogenase and mitochondrial TCA cycle [10].

Nuclear translocation, transcriptional activity and inhibition of degradation are pathways identified in HIF-1 stabilization. The phosphatidylinositol-3-kinase; protein kinase B, mammalian target of rapamycin pathway participates in increasing HIF1 transcription, and its influence of other pathways, such as TGF1 and WNT/-catenin, is particularly relevant to fibrosis. TGF1 can stimulate the 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase3 enzyme in to increase phosphofructokinase-1-mediated glycolysis stimulation found that treatment of renal anemia by HIF stabilizers does not cause fibrosis despite the evidence implicating HIF-1 in fibrogenesis [51, 52]. Glycolysis not only contributes to pro-fibrotic pathways, but it also provides amino acids for collagen synthesis. Collagen with glycine, proline,

and lysine are structural proteins found in the ECM of fibrotic tissue. Lactic acid, a byproduct of glycolysis, participates in collagen stability through promotion of proline hydroxylase activity [10].

2. **Fibrosis and amino acid metabolism**

Amino acids are a component of cellular weight and participate in cellular metabolism that are important for cellular living. They serve as a precursor for biosynthetic processes, maintaining tissue homeostasis, and as a source of energy. Glutamine is considered as an evidence link between fibrosis and altered amino acid metabolism. It is amino acid, circulating free and serves as a donor for nitrogen and carbon to synthesize macromolecule and arrange cellular signaling. Furthermore, glutaminolysis byproducts such as succinate can stabilize HIF-1α, which in turn boosts glycolysis [49, 53].

Studies on the metabolic process in patients with fibrotic diseases such as liver cirrhosis, IPF, and systemic sclerosis have revealed changes in glutamine-glutamate metabolism [54]. Some studies have confirmed the idea of changes in glutamine-glutamate metabolism, through some enzyme activity such as glutaminase and glutamate dehydrogenase. Elevated glutaminase levels were increased in experimental TGF β1-stimulated lung fibroblasts and in patient's fibroblast with idiopathic pulmonary fibrosis [55].

Glutaminolysis is stimulated by TGF β1which also leads to metabolic changes that promote differentiation in lung myofibroblast, whereas in the absence of glutamine, this will lead to decrease response of lung fibroblasts to TGFβ1. Glutaminolysis was required for collagen expression in TGFβ1-induced lung myofibroblasts, as glutaminase blockade led to reduction in the expression of first and third type of collagens, whereas increase release of glutaminase reverse the process [56, 57]. Ketoglutarate-dependent the mammalian target of rapamycin (mTOR) signaling pathway has a role in promoting hydroxylation of proline. Another researcher was concerned more with the production of glycine and proline through aldehyde-dehydrogenase 18A1 and cytoplasmic glutamate-consuming enzymes phosphoserine aminotransferase especially in lung fibrosis and liver cirrhosis [54, 56, 58].

Fibrosis and Lipid Metabolism

It is known that fibrosis is a character of adipose tissue in obesity. Chronic increased energy production encourages the tissue hyperplasia, hypertrophy, and neovascularization, resulting in pathological ECM reshaping, primarily by pre-adipocytes. It was noticed that ECM of the adipose tissue influence resident cells, change the metabolism of adipocyte, including lipolysis and insulin response [59]. Oxidation of Fatty acid (FA) is an essential energy source for the cells to function. Decreased FA oxidation has been linked to lung fibrosis [60].

The peroxisome proliferator-activated receptor (PPAR) plays a key role in fatty acid oxidation. FA oxidation in muscle is regulated by Delta PPAR, an amplifier

of fatty acid beta oxidation in mitochondria and peroxisomes in the liver (PPARα), and the most often expressed fatty acid production and storage activity in adipose tissue (PPARγ). Proliferator-Activated Receptor-Gamma Coactivator-1 (PGC1), is another essential regulator of fatty acid oxidation. It not only binds to PPAR and boosts its activity, but it also controls the activity of transcription factors that boost protein expression. The electron transport chain includes TCA. AMPK oversees PGC1. AMPK detects a cell's energy condition, activates when the AMP/ATP ratio is high, and activates multiple catabolic signaling pathways to restore ATP levels. Increased FA production and decreased its oxidation are linked to fibrogenesis. The amount of essential enzymes in FA production pathways are elevated in diabetes-induced renal fibrosis, which correlates to a decrease in oxidation of FA [46].

The serine protease inhibitor PAI1 (influence the activity of Matrix metalloproteinases (MMPs) which participates in extracellular collagen degradation) was demonstrated to boost TGFβ1 levels and decrease ECM degradation when fatty acid production was increased. In response to TGFβ1, most fibrosed tissues were shown to have decrease PPAR signaling which means diminished oxidation of FA. Cluster of differentiation 36 (CD36), fatty acid transporter is act in response of PPAR signaling—that regulates type I collagen internalization and degeneration, was found to be decreased in skin fibrosis in a recent study, providing another connection between FA oxidation and ECM modulation. It is worth noting that PGC-1 deficiency causes mitochondrial breakdown, inflammation, and renal fibrosis upstream of PPAR signaling. The key cellular metabolism regulator, AMPK, is reduced in pulmonary fibrosis. AMPK usually suppresses fatty acid production via ACC1 activation and SREBP1c, in addition to increasing fatty acid oxidation. Using metformin to activate AMPK, resulted in the reversal of induced fibrosis in lung model [61, 62].

The mevalonate route, which is also known as the cholesterol production pathway, warrants special attention. Geranylgeranyl pyrophosphate and isoprenoids farnesyl pyrophosphate (the intermediate in mevalonate route), participate in protein prenylation, which is a post-translational modification of proteins. Prenylation is required for the function of small monomeric GTPases that mediate a variety of factors that promotes fibrosis, either stabilizing protein-protein interactions or by increasing protein hydrophobicity for cell membrane [46].

The Connective Tissue Growth Factor/hypertrophic chondrocyte-specific protein (CTGF/CCN2/)/TGFβ1 and Hippo signaling pathways are notable downstream pathways. found that the induction of CTGF/CCN2 in human lung fibroblasts by TGFβ1 and activation of YAP/TAZ needs RhoA isoprenylation (The Rho family is subfamily of Ras superfamily), transcriptional co-activators of the Hippo signaling pathway that are induced in lung and liver fibrosis. Inhibition of isoprenoid production by statins demonstrated promising anti-fibrotic benefits [63].

Targeting Metabolic Pathways for Fibrosis Therapy

Metabolic abnormalities in fibrosis play a key role to help researchers to discover appropriate therapy. Most attempts to treat fibrosis with metabolic modifications have been limited to the preclinical stage, therefore it is necessary to think about how we can treat human disease by an excellent metabolic target [46]. The field of cancer therapy using metabolic pathways can teach us a thing or two, particularly about toleration of normal tissues to manipulating metabolic pathways. It is important to recognize the difficulties in targeting glucose metabolism in cancer therapies, since they foreshadow the alternative techniques being examined for fibrotic disorders. Isoform-selective targeting of glycolytic enzymes is one such method. Oxidation of FA is assumed to be hindered in fibrosis in general and various treatment findings have backed up this theory [60].

Fatty acid synthase inhibitor (C75), a synthetic drug that inhibits FASN while increasing oxidation of FA (through the rate-limiting enzyme Cpt1), was discovered to reduce kidney and lung fibrosis [64].

It was found that caffeic acid induced stimulation of Peroxisome Proliferator-activated receptor that will lead to increase oxidation of FA and reduced glucose metabolism while downregulating type 1 collagen and fibronectin. PPARγ agonists proved to have antifibrotic activity in a variety of fibrosis models, e.g., the lung, with searching for its mechanism it seems to work as an antagonist to TGF-induced responses through both dependent and independent PPARγ. When addressing the independent ligand effects, it can reduce mitochondrial pyruvate carrier activity, potentially reducing fibrosis [46].

Oruqaj et al. [65] discovered that PPAR activators inhibited TGF-induced collagen formation and myofibroblast activation in IPF fibroblasts and hypothesized that this was due to enhanced peroxisomal activity [65].

Finally, epigenetic pathways must be taken into consideration. DNA hypomethylation has long been linked to the activation of fibrogenic genes. Recent research found a relationship between fibrogenic activation, epigenetic, and metabolic disturbance. They demonstrated that promotes fibrogenesis due to TGFβ1 activity and hypoxia were dependent on epigenetic variables. Glycolysis and lactate production induced by TGFβ1 induced were reduced, whereas the Proliferator-Activated Receptor-Gamma Coactivator-1 PGC-1α (the metabolic regulator), which had been reversed by TGβ1, was reactivated. It is a transcriptional coactivator that enhances oxidation of FA among other metabolic functions [66].

Conclusion

The exploration of the RAAS, signaling pathways, α2-AR blockers and metabolic disorders in some diseases such as IPF and liver cirrhosis is considered as an opportunity to find a cure which will be of benefit to millions of patients. Through this

review, we tried to reemphasize the role of RAAS in organ fibrosis specially in the era of COVID-19. Renin-angiotensin-aldosterone system (RAAS) is either circulating RAAS or local RAAS. The circulating RAAS has a well-known role in monitoring and controlling the blood pressure, systemic vascular resistance and fluid and electrolyte balance. Local RAAS and its precursor prorenin are highly expressed in tissues. It participates in the injury process, inflammatory reaction, and fibrogenic diseases of many organs, as the liver and lung by another method which is not related to blood derived RAAS. The MAS oncogene and the ACE-2 peptide product ANG1–7 receptor has demonstrated to have a synergistic role in the protection against lung disease as well as liver disease. In addition to acting as a receptor for SARS-CoV and SARS-CoV-2, ACE2 and MAS, counteracts the deleterious effects of the RAAS and has anti-inflammatory properties, as evidenced by the high expression of ACE2 in organs and tissues. The RAAS interacts with multiple pro-fibrotic pathways to mediate fibrosis in different cell types, including Transforming Growth Factor (TGF-β), Tumor Necrosis Factor (TNF), Platelet-Derived Growth Factor (PDGF), IL-6 and IL-13, to accelerate the proliferative phase of repair. Other than transforming growth factor (TGF-β), there are several signaling pathways involved in the process of fibrosis, including Wingless/Int (WNT) and Yes-associated protein 1 (YAP)/transcriptional coactivator with PDZ-binding motif (TAZ) signaling. Genes involved in myofibroblast cell increase the number of cellular component when the TGF-β-induced SMAD complex is engaged, and this mechanism is also regulated by long and short noncoding RNA molecules, as well as epigenetic alterations of DNA and histone proteins. The same thing happens when WNT-stabilized -catenin translocate to the nucleus and induces transcription of its target genes. YAP and TAZ are two transcriptional co-activators in the Hippo signaling pathway that rely on nuclear translocation to operate in addition to other signaling pathways. The use of new medications and inhibition of adrenoceptors, via the use of α-and β-adrenoceptor antagonists, is used in the treatment of many diseases, specially urological diseases. In addition, there is evidence that adrenoceptor antagonists have potential antitumor effects. It acts by diminishing the vascularization of the tumor with subsequent decrease in its size via regulation of the phenotypic epithelial-mesenchymal transition (EMT). All the activities and reactions in fibrosis necessitate energy carriers. Metabolic biosynthetic and bioenergetic pathways provide a supply for these activities and the pathological cells will adapt to be able to conduct their synthetic activities and proliferation which might lead to metabolic disturbance.

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Chapter 13
Role of Renin Angiotensin-Aldosterone System in Kidney Homeostasis

Ravi Kant, Sneha Gupta, Tanya Kumra, Rashmi Rana, and Nirmal Kumar Ganguly

Abstract The Renin-Angiotensin-Aldosterone System (RAAS), which regulates plasma sodium levels, arterial blood pressure, and extracellular volume, is a crucial component of the human body. Angiotensin II is a multifunctional effector peptide hormone that is created when the renin enzyme, which is produced by the kidneys, interacts with angiotensinogen. RAAS activation and the ensuing hypertension, cell proliferation, inflammation, and fibrosis affect every organ. Numerous acute and chronic illnesses can be brought on by an imbalance between renin and angiotensin II. The advancement of kidney disease is correlated with proteinuria and a decline in renal function. RAAS over-activity promotes the emergence of a variety of clinical diseases, including the development of chronic kidney disease (CKD). In order to reduce blood pressure and proteinuria in patients with chronic kidney disease (CKD), reno preventive treatment has long depended on inhibiting the renin-angiotensin-aldosterone system (RAAS). According to research, RAAS inhibitors play a preventive effect in both the early and late stages of kidney disease by preventing proteinuria, kidney fibrosis, and slow decline in renal function. An overview of the RAAS pathway, its function in the kidney, and RAAS pathway blocking techniques for enhancing long-term outcomes in CKD patients are covered in this chapter.

Keywords Renin · Aldosterone · Angiotensinogen · Kidney · Renin inhibitors · Chronic kidney disease

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Introduction

Renin-Angiotensin-Aldosterone System (RAAS) is an important and valuable body system in body [1]. To maintain vascular tonicity, this system controls arterial pressure and extracellular fluid volume [2]. The RAAS is in-charge of longer-term modifications in arterial pressure, whereas the baroreceptor reflex is in charge of short-term variations. Aldosterone, angiotensin II, and renin make up the three primary substances of RAAS. Raising arterial pressure is a result of increased salt transport to the distal convoluted tubule and decreased renal blood pressure [3–5]. The body may experience prolonged blood pressure elevation as a result of these actions. Disruption of this system can result in blood pressure fluctuations, which can lead to chronic or acute illnesses as well as abrupt death [6]. It is believed that the renin-angiotensin-aldosterone system (RAAS) originated as a means of conserving salt and water to maintain tissue perfusion at livable levels in a sodium-depleted environment. Despite providing a survival advantage in the ancient world, RAAS appears to have been a disadvantage in the high-salt environment of modern times [7]. The prevalence of chronic kidney disease (CKD) is rising due to an increase in overeating around the world. The link between CKD and an elevated risk of cardiovascular disease is not fully explained by conventional risk factors such diabetes, hypertension, and hyperlipidemia [8–10]. Increased RAAS activity has been associated with CKD [11–14]. In the US, 16.8% of the population is estimated to have CKD. Medicare spent close to $42 billion on treating individuals with CKD in total in 2005. Significant reductions in renal mass result in proteinuria, glomerulosclerosis, tubulointerstitial inflammation and fibrosis, and eventually ESRD. Controlling blood pressure and proteinuria in people with chronic kidney disease is crucial for maintaining renal function and preventing the effects of renal impairment (CKD). Blocking the renin-angiotensin-aldosterone system (RAAS) with either angiotensin-converting enzyme (ACE) inhibitors or angiotensin-receptor blockers is the most effective pharmacological treatment for this (ARBs). Despite the fact that this method of treatment has been demonstrated to be beneficial, there is still much to be done to provide full protection against adverse renal sickness. Insufficient RAAS blocking, which may be brought on by insufficient dose, compensatory feedback mechanisms, such as those that contribute to aldosterone breakthrough, or the involvement of signaling pathways that are not affected by RAAS blockade, may account for the significant residual incidence of such events despite cutting-edge therapy. It has been demonstrated that a number of methods for therapy intensification enhance patients' short-term RAAS blockade responses. Unknown is the best method for enhancing the long-term outcomes of CKD patients receiving RAAS blocking therapy. On the other hand, excessive salt consumption while using RAAS-blocking medicine seems to enhance renal and cardiovascular outcomes in those with CKD. In this chapter, we gave an summary of the RAAS Pathway and its components, as well as information on the pathway's function in the kidney and ways for blocking it in order to improve the long-term prognosis of CKD patients.

Renin Angiotensin Aldosterone System

The Renin Angiotensin Aldosterone System (RAAS) controls blood pressure and is essential for controlling Na metabolism, vascular tone, renal hemodynamics, and vascular modelling [15]. Renin-angiotensin system begins with the renin biosynthesis from the renal afferent arteriole juxtaglomerular cells. A single gene encode renin and renin mRNA is translated into preprorenin, containing 401 amino acids [16, 17]. A signal peptide of 20 amino acids is cleaved from preprorenin in the juxtaglomerular cells endoplasmic reticulum resulting in the synthesis of prorenin. In Golgi apparatus, prorenin is packaged into secretory granules and a 46-amino-acid peptide sequence is cleaved from the N-terminal region of the prorenin resulting in the synthesis of renin (M.W. 37 kDa), a glycosylated carboxypeptidase. By using stimulus-secretion coupling, the juxtaglomerular cell releases renin through the exocytosis process. Inactive prorenin, on the other hand, is released consistently across the cell membrane. A proteolytic activation process that resembles trypsin transforms prorenin into renin [18]. Renin serve as an enzyme which catalytically cleave angiotensinogen to form the decapeptide Angiotensin I. Angiotensin-converting enzyme (M.W. 180 kDa), a glycoprotein, with two active carboxyterminal enzymatic sites, exists into two antagonistic isoforms named as Angiotensin-converting enzyme 1 (ACE 1) and Angiotensin-converting enzyme 2 (ACE 2) and hydrolyzes the inactive Angiotensin I into biologically active Angiotensin II [19]. Angiotensin-converting enzyme is found on the plasma membranes of many different cell types which includes neuroepithelial cells, vascular endothelial cells, and the apical brush border of epithelial cells. Angiotensin-converting enzyme metabolises bradykinin, a potent vasodilator and natriuretic autacoid, to bradykinin fragment 1-7 in addition to cleaving angiotensin I to angiotensin II [20]. Angiotensin-converting enzyme, then, causes a decrease in the production of bradykinin while increasing the production of Angiotensin II (a vasoconstrictor) (vasodilator). Albuminuria and glomerular damage have been linked to ACE2 deficiency, which is essential for maintaining renal homeostasis [21]. As a result, ACE and ACE2 serve opposing purposes; whereas the former is detrimental for the kidney and heart, the latter is beneficial [22]. In comparison to renin and angiotensinogen, the half-life of angiotensin II and other angiotensin peptides is relatively brief, and they are broken down into pieces within seconds by peptidases called angiotensinases. Aminopeptidase A and aminopeptidase N (APN) transform angiotensin II into angiotensin III, which is then transformed into angiotensin IV. However, little is known about the functional significance of these two important enzymes and the functional role of the peptide fragments generated. Angiotensin type-1 (AT1) receptor, a seven-transmembrane G protein-coupled receptor that is broadly distributed in various tissues and related favorably to protein kinase C and negatively to adenylyl cyclase, mediates the majority of Angiotensin II's cardiovascular, renal, and adrenal activities [23]. AT1 receptors play a number of roles in the body, including the contraction of vascular smooth muscle cells, the production of aldosterone, the induction of thirst, the stimulation of the sympathetic nervous system, the reabsorption of Na^+ by the renal tubules, and the ionotropic

and chronotropic responses of the heart. The Angiotensin type-2 [AT2] receptor is a second cloned receptor that Angiotensin II binds to, but its cell signaling processes and activities remain unknown [23].

Renin Angiotensin Aldosterone System Components

There are various components of Renin Angiotensin Aldosterone System which play an essential part in the system's normal operating conditions. Each of these elements plays a critical part in cardiovascular function and sustaining the body's proper kidney functions. Components of this system includes Renin, Angiotensinogen, Angiotensin II, Angiotensin Converting Enzyme (ACE), and Aldosterone Synthase.

Renin: Renin (M.W. 37 kDa) a 340 amino acids long protein, secreted by kidney glandular cells, is located on the chromosome 1 long arm [24]. The length of Renin gene is 12 kb long and it contains 8 introns [25]. Renin regulates arterial vasoconstriction, blood pressure, and cardiovascular homoeostasis in addition to mediating extracellular volume, which includes blood plasma, lymph, and interstitial fluids [26]. Conditions of Renin secretion from kidney include the decrease in arterial blood pressure, decrease in NaCl level in the nephrons ultra-filtrate. Sympathetic nervous system also regulates blood pressure as it is also associated in the release of renin to regulate blood pressure [27]. Renin circulates in the blood stream and cleaves the angiotensinogen which is secreted by liver into angiotensin I [28].

Angiotensinogen: Angiotensinogen, precursor of angiotensin peptide, is a glycoprotein of 452 amino acids and production and circulation of Angiotensinogen is mainly done by liver. Along with liver, Angiotensinogen is also produced and secreted by heart, kidneys, vessels and adipose tissues in small amount. Structurally, Angiotensinogen is a family member of inhibitors of serine proteases known as serpins (SERPINA8). Renin converts Angiotensinogen into the Angiotensin I [29]. Corticosteroid, thyroid hormone, estrogen, and angiotensin II levels help to increase plasma level of angiotensinogen [28]. Angiotensin-converting enzyme (M.W. 180 kDa), a glycoprotein, with two active carboxyterminal enzymatic sites, hydrolyzes the inactive Angiotensin I by removing two C terminal amino acids of into biologically active Angiotensin II in the lungs capillaries, endothelial cells, and kidney epithelial cells [30, 31].

Angiotensin II: With its physiological involvement in salt regulation, water balance, renal function, and blood pressure maintenance, angiotensin II is a diverse effector molecule that dominates the Renin Angiotensin Aldosterone System. Angiotensin II increases sodium reabsorption when blood pressure drops in order to restore blood pressure. By secreting noradrenalin, angiotensin II also produces vasoconstriction [32]. All blood vessels (arteries and veins) respond to angiotensin II's vasoactive effects by constricting the smooth muscle. It raises prothrombotic potential, blood

pressure, and the rate at which the heart beats [33]. It also stimulates the plasminogen activator inhibitor proteins PAI-1 and PAI-2. Additionally, it causes the cortex of the adrenal gland to release aldosterone [34]. Aldosterone regulates sodium-potassium balance by causing the kidney's proximal tubules to absorb more sodium, which causes the body to retain sodium while losing potassium [35]. Additionally, angiotensin II causes hemodynamic effects as well as a string of oxidative and inflammatory responses that affect mesangial cell development and podocyte proliferation, all of which advance glomerular damage and tubulointerstitial fibrosis [36–39].

Angiotensin Converting Enzyme (ACE): Angiotensin-converting enzyme (M.W. 180 kDa), a glycoprotein, with two active carboxyterminal enzymatic sites, exists into two antagonistic isoforms named as Angiotensin-converting enzyme 1 (ACE 1) and Angiotensin-converting enzyme 2 (ACE 2). Inactive Angiotensin I is hydrolyzed into physiologically active Angiotensin II by ACE [19]. The apical brush border of epithelial cells, vascular endothelial cells, and neuroepithelial cells all have angiotensin-converting enzyme localised on their plasma membranes [40, 41]. Angiotensin-converting enzyme metabolises bradykinin, a potent vasodilator and natriuretic autacoid, to bradykinin fragment 1-7 in addition to cleaving angiotensin I to angiotensin II [20]. Albuminuria and glomerular damage have been linked to ACE2 deficiency, which is essential for maintaining renal homeostasis [21]. Angiotensin-converting enzyme (ACE) is found in endothelial vascular cells as well as endothelial and epithelial cells of several organs, including the kidneys, heart, and lungs [42–44].

Aldosterone Synthase: An enzyme complex called aldosterone synthase is found at the inner membrane of mitochondria [45]. It is a crucial enzyme in the production of aldosterone and is a member of the cytochrome P450 superfamily. Aldosterone is released by the kidney's adrenal cortex and has a role in sodium retention, potassium secretion, increased water retention, and blood pressure increases. Numerous investigations have demonstrated that the aldosterone synthase gene, which is involved in the control of blood pressure, is the cause of diabetes and hypertension. Figure 13.1 shows the different components and working of RAAS pathway.

Role of Renin Angiotensin Aldosterone System in Body

RAAS stimulate thirst reflexes by acting on hypothalamus and hypothalamus osmoreceptors recognize this thirst feeling and reduces urinary loss after ADH secretion [46–48]. Adrenocorticotrophic hormone, secreted from corticotropes of anterior pituitary, is also influenced by Renin which results in regulation of cortisol production from the adrenal glands [49]. Prostaglandins release is also influenced by Angiotensin which in turn can influence renal vasoconstriction. Angiotensin II can also stimulate lipogenesis, resulting in increase of adipose tissue mass [50]. Subsequently, Angiotensin II is also linked to other consequences like inflammation of adipose, glucose intolerance, and insulin resistance [51]. Angiotensin II interacts with G-protein-coupled receptor (GPCR) AT1 by stimulating the Gq protein in vascular smooth muscle cells

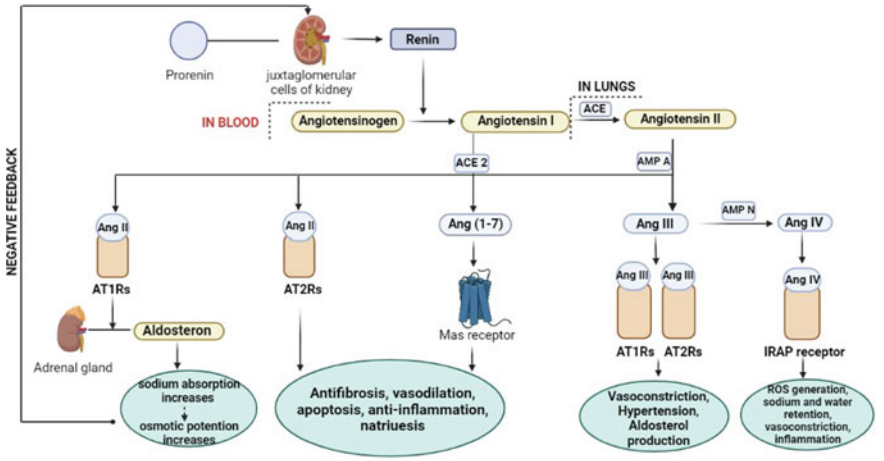

Fig. 13.1 Renin acts on angiotensinogen present in blood to convert it to Angiotensin I and the Angiotensin converting enzyme mainly expressed in lungs converts Angiotensin I to Angiotensin II. ACEII converts Angiotensin I to a heptapeptide Angiotensin (1-7) while the AMP-aminopeptidase A and N helps in conversion of Angiotensin II to III and IV, respectively. These angiotensin enzymes bind to various receptors to produce different conditions like hypertension, vasodilation, aldosterone production etc.

to activate phospholipase C, for increasing intracellular calcium [52]. Angiotensin II is hence an important blood volume regulator as well as Angiotensin II also regulate blood pressure, and pH of blood. There are several prorenin and renin receptors mainly includes mannose-6-phosphate receptor, ATPase H (+)-transporting lysosomal accessory protein 2 which is encoded by ATPase H (+)-transporting lysosomal accessory protein 2 gene [53, 54]. The binding of prorenin and renin enzyme with these receptors can affect their angiotensinogen-hydrolyzing ability. Renin activity is increased fourfold and the nucleophilic amino acids serine and tyrosine of the ATPase are phosphorylated by renin binding to the lysosomal accessory protein 2 receptor [55]. Circulating renin and prorenin cause the local production of angiotensin II and mediate angiotensin II-independent signaling cascades after binding to prorenin/renin receptors.

Role of Renin Angiotensin Aldosterone System in Kidney

The Renin Angiotensin Aldosterone System (RAAS) controls blood pressure and is essential in controlling sodium metabolism, vascular tone, renal hemodynamics, and vascular modelling. Kidney is the most affected organ of the body by renin imbalance. Renin level fluctuation can be measured by high serum potassium, low arterial pressure, high plasma creatinine level, anemia etc. In the 1970s, when it was shown that specific intrarenal RAS inhibition increased GFR and renal Na^+ and

water excretion, intrarenal RAS was initially identified [56, 57]. Since that time, the role of the intrarenal RAS in the regulation of Na^+ excretion and the long-term regulation of arterial blood pressure has been acknowledged [58, 59]. Studies shows that inappropriate activation of intrarenal RAS, results in abnormal Na^+ balance at normal arterial pressures which is an important factor for hypertension [58–60]. Several models like 2-kidney, 1-clip (2K1C) Goldblatt hypertension, Angiotensin-infused hypertension, etc. also supports the above-mentioned fact [58, 59, 61–64]. Another long-term effect of an inappropriately activated intrarenal RAS is increasing hypertension, fluid retention, Na^+ retention, renal, vascular, glomerular, and tubulointerstitial damage, as well as fibrosis [59, 65, 66]. Intrarenal RAS overactivation evidence for the hypertension has been accumulated for example, Retinal vascular and glomerular AT1 receptors are downregulated in angiotensin II-dependent hypertension, whereas proximal tubule receptors either are increased or not completely changed. Due to the AT1 receptor-mediated endocytosis, peptide uptake from circulation and also due to intrarenal production Angiotensin II content is found to be increased in some different hypertension models. Renin-induced increment in TGFb1 stimulates increase in fibronectin, PAI-1 and collagen I which indicates the renin role in renal fibrotic disease whereas kidney transplant is preferred in severe disorders [67, 68]. There have been reports of RAAS activation in dialysis patients and excessive renin production in failing kidneys [69]. Angiotensin II accumulation in the kidney is found to be increased by constant upsurge in circulating Angiotensin II. Renal interstitial Angiotensin II levels also have been reported as upsurged in many of the Angiotensin II-dependent models of HT, including the 2-kidney, 1-wrap etc. [70, 71]. Birth control pills, pregnancy [72], other hormones (oestrogen, testosterone, thyroid, cortisol), diuretics, vasodilators, blood pressure medicines, and other pharmaceuticals are only a few of the many variables that might affect RAAS. Additionally, several malignancies including Wilms' tumour, renal cell carcinoma, and reninoma, can increase renin levels [73, 74]. Due to the restricted pulmonary blood flow that prevents ACE, the foetus has high renin levels but considerably reduced angiotensin II levels [75].

Inhibitors of RAAS Pathway

Renin is the main component produced during RAAS pathway which is essential for body to regulate its function like blood flow in vessels but its overproduction causes various cardiovascular disorders such as high elevation of Blood pressure [76]. Inhibitors are used to treat such conditions by blocking certain pathway of enzymes responsible for overproduction of hormone [77]. First blockade enzyme to be used was Angiotensin-converting enzyme inhibitors (ACEIs) [78]. Currently, the RAAS inhibition is mainly done by the antihypertensive treatments. ACE inhibitors reduce the production of Angiotensin II by repressing the transformation of Angiotensin I to Angiotensin II by these enzymes [79]. **Figure 13.2 is showing different inhibitors and their effect on different components of RAAS Pathway**.

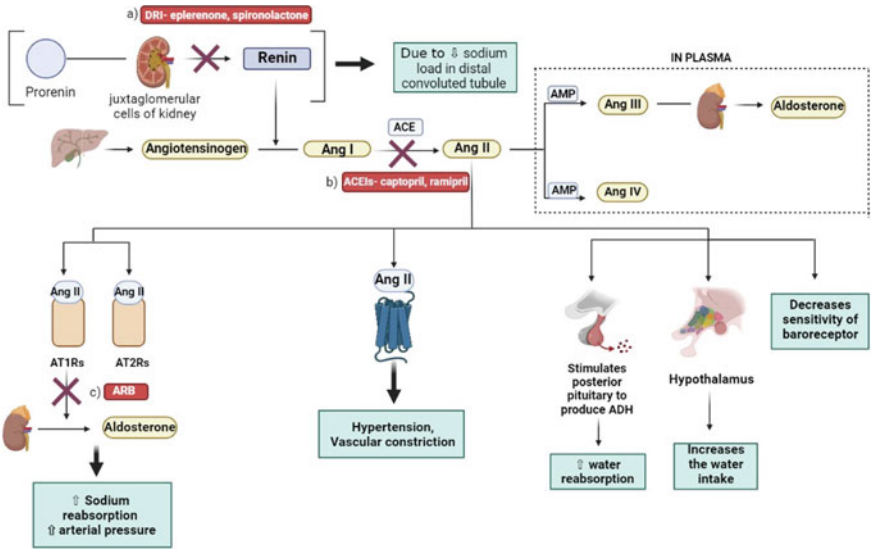

Fig. 13.2 **a** DRI-Direct renin inhibitors like eplerenone, spironolactone inhibits renin production from the juxtaglomerular cells of kidney. **b** ACEI-Angiotensin converting enzyme inhibitors, like captopril and ramipril blocks the conversion of Ang I to Ang II. **c** ARB-Angiotensin II receptor blockers, blocks AT1Rs-AngII complex from acting on adrenal gland to produce aldosterone

Mechanism of Inhibition

Angiotensin receptor blockers affect Angiotensin (AT1) receptors and restrict the forming of Angiotensin II from Angiotensin I with help of enzymes [80]. Nonetheless, ACE inhibitors and Angiotensin receptor blockers are related with an input circulation loop that expands plasma renin activity. AT1 receptors activation loss occurs on kidney juxtaglomerular cells which prompts a compensatory expansion in renin discharge. The subsequent expansion in plasma renin activity might restrict the organ protection assured by medications [81]. The entire RAAS is subsequently regulated despite the fact that AT1 receptor interceded impacts on effector molecule. Direct inhibitors of renin aiming the RAAS at its purpose in initiation, bringing about the decrease of plasma renin activity production [80, 81]. Use of both Angiotensin-converting enzyme inhibitors or ARBs result in blood pressure decrease in humans with diabetes and cardiovascular disease [82]. Direct inhibition of renin results in the effective suppression of RAAS [83]. The determined renin receptors perform an essential role in Angiotensin II production and responses of cell to Renin [84]. Renin-receptor binding triggers intracellular response and protein kinase 1 regulate extracellular response which might boom the activity of plasma activator inhibitor-1 which is a Pro-thrombotic agent which in turn affects RAAS mechanism [85]. Angiotensin II and AT1 receptor directly affects morphological modifications and regulates the gene expression of diverse bioactive materials and turns on more than

one intracellular signalling cascades in cardiac myocytes, muscle cells, fibroblasts, vascular endothelial, and renal mesangial cells [78, 85, 86]. Over activation of RAAS pathway with the help of angiotensin I receptor blocker causes hypertension, a consequence of chronic kidney disease, which may cause a high severity of chronic kidney disease development to end stage renal disease and mortality, diagnosed by means of excessive systolic blood pressure [87]. When Angiotensin-converting enzyme or Angiotensin 1 receptor blocker consistently works, they cause non-stop utilization of potassium levels which results in increased blood potassium levels [87, 88].

Types of Inhibitors

There are various kinds of blockers which work on different mechanisms; few of them act on smooth muscles of vascular system which results in suppression of aldosterone secretion. Inhibitors working is based on inhibition of linkage between Angiotensin I, II and AT1 receptor blocker. Some other inhibitors work by binding with mineralocorticoid receptor antagonist on kidney distal tubules resulting in hindrance of chemical secreted [89]. Some of the blockers and their mechanism of action are given in Table 13.1.

Combination Therapy

When two blockers are used in combination they produce more effective line of treatment as compare to mono-blockers. In current era combination therapy of using two blockers is more researched and used in treatments. Use of AT1 receptor blocker and ACE inhibitor simultaneously provide complete hindrage to RAAS pathway as compare to the single blocker. Better control of blood pressure, sugar level and albuminuria can be achieved through combination therapy. Doses of Losartan and Lisinopril help in reducing proteinuria approximately 45–70% respectively [89]. AT1 receptor blockers and direct renin inhibitors are used to lower down the plasma renin elevation levels. Usually angiotensin converting enzyme inhibitor is used but for better efficiency angiotensin II type-1 receptor blocker with direct renin inhibitor is used which lowers the level of renin 50%. Angiotensin Type-1 receptor blocker and mineralocorticoid receptor antagonist combination is used with other blockers which provide much sustainable treatment on chronic kidney disease patients by showing effective decrease in albuminuria levels [88, 89]. These inhibitors work by reacting on sodium and potassium balance with linkage to mineralocorticoid receptors and activate transcription genes [78].

Table 13.1 In the given table different categories of renin-angiotensin-aldosterone system blockers with their sub-types and working mechanism is shown

Category	Blockers name	Mechanism	Advantage	Disadvantage	References
Receptor blockers	ARB	Suppress aldosterone secretion	Reduces secretion of ADH	Renal failure	[83]
Mineralocorticoid blockers	ALDO	Binds on MRA receptor of kidney tubules	Absorb potassium and excrete sodium, lowers B.P	Hyperkalaemia, kidney stones	[82]
	ACEIs	Hinders vasoconstriction	Dilation of blood vessels, lowers B.P	Increase level of bradykinin level, angioedema	[83]
Direct renin inhibitors	Eplerenone Spironolactone	Aliskiren works on bond hindrage	Decrease chances of bronchoconstriction	Bradycardia, ineffective in tremors	[88]

Inhibitors Efficacy

Low sodium diet can be used for speedy improvement of patient condition. Sodium is responsible for elevating the blood pressure which directly affects the RAAS pathway. Usually foods with less protein and low sodium content are recommended for high blood pressure, Diabetic and non-diabetic patients [89]. Nutritional Na restriction improves blood pressure and proteinuria effectiveness and also have effect on RAAS-blocking dealers, such as ACE inhibitor. AT1 receptor blocked by enhancing the efficacy of blockers which results in renin-inhibition [78, 85]. Decreasing of Na consumption additionally improve the non-RAAS blocking receptor's anti-proteinuric efficacy which also includes neprilysin inhibitors as well as vitamin D receptor agonists [78, 89]. In long term, increased level of creatinine serum was observed in patients who were on medications based on RAAS blockers [88]. Low sodium diet patients are still at low risk as compare to high sodium diet patients [88]. In a study, high sodium diet group was seen with persistent elevation of proteinuria. Mechanism like artery stenosis or arterial stenosis in renal transplant is based on Angiotensin II with respect to working of renal artery. So when a patient vomit or have a condition like diarrhoea, extracellular volume is reduced from body which increase serum creatinine after a person is treated with AT1 receptor blocker as well as ACE inhibitor [78]. This condition is not permanent as after 14 days blockers settled down make themselves compatible with body and creatinine levels is maintained again [78]. The use of blockers can result in organ failure, electrolyte imbalances, and hypotension. Dual treatment is riskier than single blocker therapy. But dual therapy comprising of AT1 receptor blocker and ACE inhibitor are more effective or safer with less side effects as compare to other combinations or line of treatment [78].

Conclusion

Renin-Angiotensin-Aldosterone System is an important and valuable body system which regulates extracellular fluid volume and arterial pressure to maintain vascular tonicity. RAAS also play vital role in proper functioning of other body organs. But over activity of RAAS pathway might results in chronic kidney diseases and other severe problems. To inhibit the overactivity of RAAS pathway inhibitors are used. Presently, Dual blockage therapy is commonly used with respect to monotargeted therapy. However prolonged uses of this kind of therapy might result in high blood pressure, proteinuria and other related problems. So, future studies should focus to bypass the limitations of blockers strategies.

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Chapter 14
Renin-Angiotensin System in Chronic Kidney Disease: Implications in Stroke Outcome

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Abstract Stroke has an intricate pathophysiology and aggravation of that often leads to co-morbidities. Chronic kidney disease is devastating and plays an important role to exacerbate stroke outcomes. Renal impairment is an established risk factor that alters the hemodynamic balance. Various clinical studies investigated the impact of CKD in different pathologies reported unexpected neurological and cognitive impairment in the patients. On the other hand, renin-angiotensin-aldosterone system (RAAS) modification contributes towards maximum renal impairment. RAAS alteration can modify electrolyte balance and affect the sympathetic nervous system that can further alter the vasoregulation. These factors can cumulatively aggravate the progression as well as outcome of stroke. To date, management of CKD and stroke are limited and requires extensive study to investigate promising therapeutic strategies. Therefore, a thorough knowledge of RAAS, CKD and stroke inter-relationship is solicited.

Keywords Renin-angiotensin-aldosterone system · Chronic kidney disease · Dialysis · Stroke · Microbleeds · Vascular calcification · Hypertension · eGFR

Introduction

The Renin Angiotensin Aldosterone System (RAAS) is the major regulator of blood pressure, fluid balance, hydro mineral homeostasis and systemic vascular resistance

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[1]. The system mainly comprises of 3 components: Renin, Angiotensin II and Aldosterone that are involved in renal blood flow regulation [2]. The vasoactive peptide Angiotensin II (ANG-II) contributes to blood pressure regulation via binding to angiotensin receptors present in vascular smooth muscles and renal tubules. In classical RAAS system, renin is present in the inactive form (pro-renin), within juxtaglomerular (JG) cells of kidney, which in response to decreased blood pressure gets converted to renin (active form) and cleaves angiotensinogen (produced in liver and found in plasma), to angiotensin I (ANG I). Angiotensin Converting Enzyme (ACE) is released from lungs and convert ANG I to a potent vasoconstrictor, ANG II [3]. ANG II in turn binds with the type I ANG II receptors activating aldosterone, and anti-diuretic hormone (ADH), that regulates sympathetic vascular tone [3, 4]. The RAAS is regulated by a negative feedback loop in which ANG II inhibits renin gene transcription and tubular renin secretion by directly interacting with JG cells, lowering the flux of ACE release [5]. For decades, chronic kidney disease (CKD) and its neurological consequences are one of the major health concerns [6].

CKD involves altered kidney structure as well as function with effective glomerular filtration rate (eGFR) less than 60 ml/min/1.37 m^2 for more than 3 months [7]. The primary indications of CKD include glomerulosclerosis and tubulointerstitial fibrosis [8]. Apart from these, renal inflammation also has an important role in CKD generation [9, 10]. Protein markers such as cystatin C, cardiac troponin T, β-trace protein, β2 microglobulin, and C-reactive protein (CRP) are important biomarkers of vascular dysfunctions in CKD [11]. Elevated levels of albumin in urine (albuminuria), creatinine, uric acid, urea and other uremic toxins (UTs) in serum acts as biomarker of CKD [12]. CKD and stroke share a bi-directional interconnection of various cerebro-renal events, highlighting a dual cause association [13].

Stroke is one of the leading causes of morbidity and mortality worldwide imparting a huge economic burden to any nation [14]. The world health organization (WHO) defines stroke as clinical signs that are developed rapidly due to focal (or global) disturbance in cerebral function and lasted more than 24 h or lead to death, with an apparent cause of vascular origin [15]. It is further categorized to ischemic stroke, hemorrhagic stroke, and transient ischemic attack (TIA), often known as mini-stroke, which accounts for 87%, 12%, and 1% of all strokes, respectively [16].

There are some common risk factors of CKD and stroke, that include- hypertension, hyperlipidemia, age, metabolic syndrome, obesity, history of cardiovascular events, smoking, diabetes and genetics [11]. Factors such as bone mineral metabolism, UTs, and anemia, as well as the dialysis process in CKD patients, may contribute to an increased risk of stroke [17]. Blockade of RAAS results in upregulation of blood pressure, salt and water retention that ultimately leads to gradual renal dysfunction [18]. Studies suggest, regardless of stroke subtypes, renal impairment is linked to worsening of neurological outcome, functional outcome and higher mortality [19]. Proteinuria is linked to significant neurological impairment and a higher in-hospital death rate, whereas, CKD is reported to be an independent risk factor for cardiovascular disease [20]. Patients with End Stage Kidney Disease (ESKD) or on dialysis (renal replacement therapy) are 4 to 10 folds at higher risk of stroke as compared to normal population [21]. According to the study,

every 10 ml/min/1.73 m^2 decrease in GFR raises stroke risk around 7%, and every 25 mg/mmol rise in albuminuria raises stroke risk by 10% [22]. Kidney impairment increases the risk of stroke via modulating cerebral auto regulation, altering the cerebral vasculature, and decreasing cerebral blood flow (CBF) [23]. Recent reports suggests existence of two way correlation between CKD and stroke as in comparison to the healthy individuals, where renal dysfunction is present in 40% of stroke survivors, and stroke susceptibility increases by 43% in stage 3 or higher of CKD [17]. In clinical trials, inhibitors of the ACE (ACEI) and Angiotensin 1 (AT1) receptor blockers have been indicated to delay disease onset and progression of CKD. Despite the fact that they are now considered standard therapy [5].

Epidemiology

A systemic analysis to study the global burden of stroke between 1990 and 2019 reports a close relationship of stroke with aged population of World bank low income group where the risk and susceptibility of stroke remains high [24]. The fastest growing risk factor was high body mass index [24]. Stroke was the second-leading cause of mortality (11.6% of total deaths) as well as the third-leading cause of death and disability combined (5.7% of total disability adjusted life years or DALYs) globally in 2019 [24]. There were 12.2 million (95% uncertainty interval UI) stroke in 2019, 101 million prevalent cases of stroke, 143 million (DALYs) due to stroke, and 6.55 million deaths due to stroke in 2019. Figure 14.1 represents a detailed overview of epidemiological status of CKD and stroke between 1993 and 2017. According to the same study, the global burden of stroke and % change in stroke evidences between 1990 and 2019 has been tabulated in Table 14.1 [24].

eGFR is a well-known kidney factor that demonstrate an inverse step-wise relationship with kidney function and stroke [19]. Within CKD stage 3 to 5, patients undergoing dialysis with relation to general population are reported to have an increase stroke incident by 3, 4.1, 5.4, and 7.1 folds [25]. Apart from this, atrial fibrillation is a common consequence of CKD that has been reported as an important risk factor of stroke [26]. There is also an increased prevalence of atrial fibrillation in dialysis patients, which indicates an important temporal relationship between timing of dialysis and stroke risk [19]. Patients with advanced CKD (stage 4 or 5) are closely associated with a 30-day time window of kidney failure risk at a percentage of 4.68 and 25.7 respectively [27]. This 30-day before and after dialysis initiation is the maximum vulnerable period for stroke [27]. With regard to this, hemodialysis patients are more susceptible to stroke with compared to peritoneal and kidney transplant patients [21]. On the other hand, RAAS blockers such as mineralocorticoid receptor antagonists are reported to reduce cardiovascular event in pre-dialysis and on-dialysis patients, however, ACEI, AR blockers, direct renin inhibitors and their combination therapy were not proven to be effective in these patients [28].

The basic requisite of stroke care is related to its subtypes and understanding the pathophysiology of stroke [23]. All major categories of stroke risk are reported to

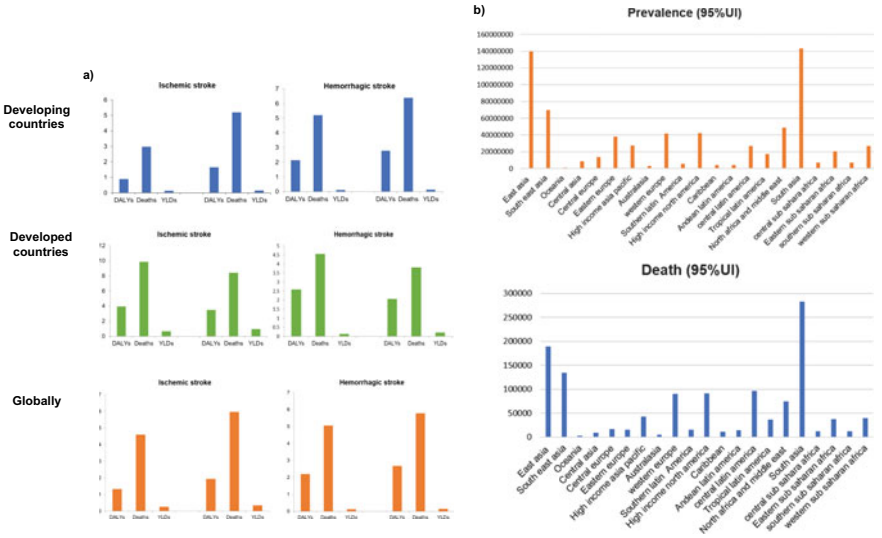

Fig. 14.1 Graphical representation of- (a) % stroke burden and % count of stroke within 1990–2013. **(b)** CKD prevalence as well as death rate in different countries are also represented (data taken from Saran et al. [71])

Table 14.1 Global burden of stroke within 1990–2019

Event	*Increased by*	*Reference*
Incidences of stroke	70%	Feigin et al. [24]
Prevalent cases of stroke	85%	
Fatalities	43%	
DALYs	32%	
Incidents among people under age of 70 year	*Increased by*	
Incidence rate	15%	
Prevalence rate	22%	
Age standardized rates	Fell by	
Incidences of stroke	17%	
Death	36%	
Prevalence	6%	
DALYs	36%	

be closely associated with CKD and dialysis [23]. Epidemiological reports related to different stroke subtypes and CKD stages are less explored, however, incidents of cardioembolic stroke is more frequent in CKD patients following dialysis. Apart from this, alteration of stroke subtypes in the advanced stages of CKD is not yet well reported [19].

Pre-clinical and Translational Studies Relating CKD and Stroke: RAAS Regulation

CKD influences the neuro-hormonal balance that activates the RAAS by activating the sympathetic connection in a sustained manner (Fig. 14.2; Table 14.2) [29]. RAAS is governed by ANG-II, which orchestrates the cerebro-renal functioning during CKD and instigates the ischemic risk [13]. ANG-II is known to up-regulate proinflammatory cytokines expression via nuclear factor kappa light chain enhancer of activated B cells (NF-κβ) activation through the angiotensin receptors 1 and 2 (AT1 and AT2) present in vascular smooth muscle cells of rats [30]. In an investigation with rodents, ANG-II is reported to escalate the capillary filtration pressure by efferent vasoconstriction and results proteinuria [13]. ANG-II regulates the activation of Nicotinamide adenine dinucleotide phosphate (NAPDH) oxidase (*NOX*) thus playing a pivotal role in generation of arterial hypertension [31]. In a hypertensive rat model of stroke, ischemia was attenuated by renal denervation due to inhibition of oxidative stress generation [32]. In an AT2 receptor knock-out mice model of ischemic stroke, severe stroke outcome following middle cerebral artery occlusion was reported. This same study also reported that treatment with AT1 antagonist to the mice could not render expected neuroprotection as compared to wild-type mice [33]. Stimulation of RAAS in CKD up-regulates the degradation of ANG-II into ANG-IV which initiates the release of profibrotic markers such as plasminogen activator inhibitor-1 (PAI-1) by activating AT4 receptors [34, 35]. PAI-1 hinders fibrinolysis by inhibiting tissue plasminogen activator and urokinase, thus prompting pro-thrombotic condition in CKD [11]. Therefore, pro-thrombotic condition during CKD may lead to alteration of RAAS associated with exacerbation of stroke.

Indoxyl sulfate, hippuric acid, p-cresyl sulfate, guanidino compounds, advanced glycation end products (AGEs), and activated NOX are some UTs that are reported to aggravate ROS production and neuroinflammation through endothelial dysfunction in CKD rodent models [36, 37]. Uric acid and P-cresyl sulfate are UTs that enhance the activation of RAAS by stimulating the cardiomyocytes [38]. Protein-bound gut-derived UTs such as indoxyl sulfate and p-cresyl phosphate were reported to activate various leukocyte adhesion molecules that leads to endothelial dysfunction in brain [39]. In a rat model of CKD where animals were subjected to 5/6 nephrectomy, blood brain barrier (BBB) disruption was reported to increase the transfer of UT in the brain [40]. UTs stimulate the bulbospinal neurons in the rostral ventrolateral medulla of Wistar rats, which regulates the blood pressure through the action of parasympathetic neurons, and causes hypertension [41, 42]. During CKD, increased activation of RAAS results in alteration of dimethylarginine dimethylaminohydrolase and arginine methyltransferase function, thus leading to increase in asymmetric dimethyl arginine (ADMA) accumulation [43]. Plasma concentration of ADMA contributes to arterial stiffness along with cerebral blood flow reduction by inhibiting nitric oxide production [44]. RAAS inhibitors such as ACEIs and AR blockers have been reported to decrease plasma ADMA. However, the mechanism by which it modulates the ADMA metabolism is yet to be explored [45]. Homocysteine is another UT

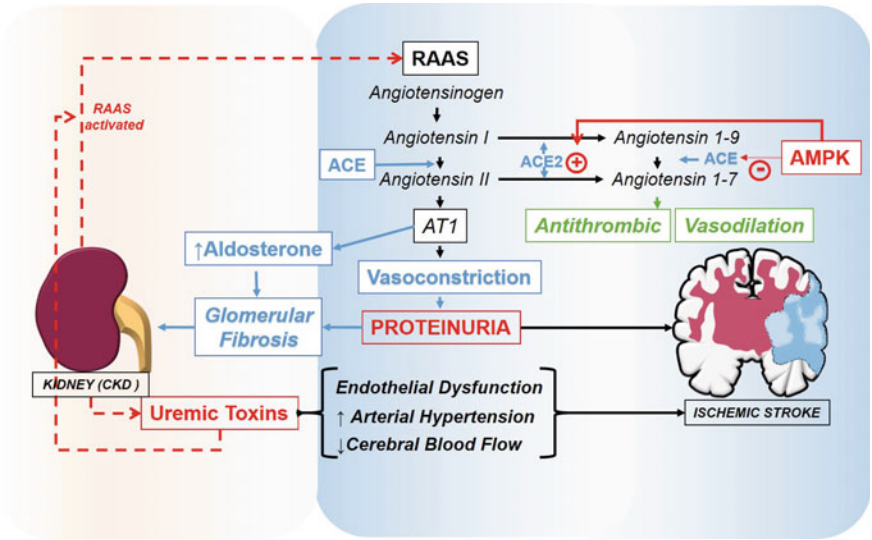

Fig. 14.2 Inter-relationship between Chronic Kidney Disease (CKD) and stroke mediated by Renin Angiotensin Aldosterone System (RAAS). Chronic kidney disease can aggravate stroke by initiating the accumulation of uremic toxins (UTs). This further results in endothelial dysfunction, arterial hypertension and reduction in cerebral blood flow. UTs activates RAAS cascade leading to proteinuria, that ultimately lead to stroke. AMP-activated protein kinase (AMPK) has been reported to antagonize angiotensin converting enzyme (ACE) while agonize ACE2, both being important enzymes in RAAS pathway leading to proteinuria. Proteinuria results in glomerular fibrosis, a pivotal factor for CKD progression. Adapted from Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License (https://smart.servier.com/)

that disrupts cerebral homeostasis by inducing cell death by activating the ERK/p38 MAPK pathway through the NMDA receptor activation, leading to apoptosis and necrosis [17]. This UT has been reported to participate in increasing blood pressure by activating AT1, which plays a pivotal role in RAAS [46]. The transcription factor aryl hydrocarbon receptor (AHR) activated by the ligand indoxyl sulphate, is known to induce vascular dysfunction thus contributing to the potential risk of stroke in CKD [47]. RAAS signaling was decreased in AHR-null animals leading to hypotension, thus indicating the involvement of RAAS in CKD-induced stroke [48]. Thus, the bidirectional relationship between stroke and CKD is augmented by the accumulation of UTs and the connection between both pathologies may be supported by the involvement of RAAS.

In a mice CKD-stroke complex induced via electro-cauterization of right kidney cortex, and total nephrectomy of left kidney followed by transient middle cerebral artery occlusion (tMCAO) at 6 weeks, reported neuronal loss and glial activation that are mediated by alteration in 5′ adenosine monophosphate-activated kinase (AMPK)-NF-κβ pathway [49]. Phosphorylation of AMPK can suppress ACE expression in a tissue or cell-type dependent manner and leads to activation of ACE2 which helps to initiate the anti-inflammatory, anti-fibrotic, and anti-proliferative activities [50].

Table 14.2 Preclinical studies representing involvement of RAAS in CKD induced stroke incidences

Hormones	Relation to RAAS	Relation to CKD	Relation to stroke	References
Angiotensin II	Main effector of RAAS	Up-regulate NF-κβ pathway (activated by stimulation of AT1 and AT2 receptors in rats and MDP-2 and TLR-4 in mice)	Pro-inflammatory response aggravate endothelial dysfunction	Chavda et al. [13], Wu et al. [30], Xu et al. [70]
		Proteinuria (by TGF-β1-mediated ROS production)	Increase in urinary albumin levels causes mortality in CKD patients with stroke	Chelluboina and Vemuganti [11], Chavda et al. [13]
		Activation of *NOX*	Arterial hypertension	Nguyen Dinh Cat et al. [31]
		Activating AT2 receptors	In mice AT2 receptor knockout model intensified brain injury was observed when subjected to ischemic stroke	Iwai et al. [33]
		Degradation of ANG-IV which acts through AT4 receptors	Release of profibrotic markers (PAI-1) causes pro-thrombotic condition in CKD	Chelluboina and Vemuganti [11], Matsumoto and Horie [34], Rüster and Wolf [35]
Uremic toxins	Uric acid and P-cresyl sulfate—activates RAAS that leads to arterioles contraction which leads to further accumulation of UTs	Decreased functionality of nephrons causes accumulation of UTs	Indoxyl sulfate and p-cresyl phosphate activation of leukocyte adhesion molecules that leads BBB disruption in CKD rat model	Tyagi et al. [36], Wautier et al. [37], Falconi et al. [38], Aseem et al. [39], Jing et al. [40], Ma et al. [43]
			UTs stimulates the bulbospinal neurons in rats leading to hypertension	Chelluboina and Vemuganti [11], Madden and Sved [41], Oshima et al. [42]

(continued)

Table 14.2 (continued)

Hormones	Relation to RAAS	Relation to CKD	Relation to stroke	References
			ADMA causes arterial stiffness and decrease in cerebral blood flow reduction	Ma et al. [43], Kielstein et al. [44], Be^{3}towski and Kêdra [45]
			Homocysteine activates AT1 thus increases blood pressure	Shah et al. [17], Li et al. [46]
			Indoxyl sulphate activates AHR and induces vascular dysfunction leading to potential risk of stroke in CKD	Vanholder et al. [47], Coelho et al. [48]
AMPK	Supresses ACE and activates ACE2	Anti-diabetic drugs (metformin and spironolactone), activators of AMPK, expressed positive outcomes in a pre-conditioned CKD mice under induced stroke condition		Hénaut et al. [49], Liu et al. [50], Li et al. [51], Grissi et al. [52], Lando et al. [53]

AMPK is well reported to block the pro-inflammatory microglia/macrophages M1 polarization and enhance anti-inflammatory M2 polarization [51]. Later investigations implicates the role of anti-diabetic drug metformin as an activator of AMPK in a pre-conditioning of CKD mice under induced stroke conditions by transient middle cerebral artery occlusion [51, 52]. Another anti-diabetic drug, spironolactone, a mineralocorticoid receptor antagonist expressed positive outcomes in stroke induced CKD male mice model [53]. Thus, presenting anti-diabetic drugs as adjuvant therapies to treat patients with CKD-induced stroke conditions.

Various Clinical Reports

CKD is a well-established risk factor for stroke and also has a direct correlation to subclinical cerebrovascular abnormalities and cognitive impairment. The possible reason behind the relationship that CKD shares various traditional and non-traditional factors with cerebrovascular diseases, including uremia-related and dialysis-related factors [54]. The impact of CKD on stroke varies according to geographical position as well as racial difference. Stroke risk associated with existing CKD is higher in Asian population than non-Asian population [55]. Currently, researchers apparently relate the limitation of effective pharmacotherapies as one of the main reasons of severe neurological outcomes as well as poor functional and vital outcomes

in stroke patients (both ischemic and hemorrhagic) with existing CKD [54]. The Fukuoka Stroke Registry report is the largest multicenter, cross-sectional study to date, included 3778 patients who had their first ever ischemic stroke, of which 1320 (35%) had pre-existing CKD [56]. It also reports that patients with CKD are 49% more susceptible to severe neurological outcome which correlates with at least a 2-point increase of National Institutes of Health (NIH) Stroke Scale score, and a 138% higher risk of in-hospital mortality, as well as a 25% higher risk score of 2 or more as per Modified Rankin Scale (mRS) at discharge [56]. Another study of the same registry reports a 73% more cardioembolic stroke recurrence risk is associated with CKD patients [56].

Several population-based cross-sectional surveys report that lower the eGFR higher the white matter lesion volume as well as silent brain infarcts detected in magnetic resonance imaging (MRI) scan [57–60]. Northern Manhattan study on 615 patients with low eGFR reports an increase in log-transformed white matter lesion volume [57]. The Rotterdam scan study reports a small deep white matter volume as well as silent brain infarction in aged population [58]. Lower eGFR is also associated with cerebral micro bleeding independent of age, gender and diastolic blood pressure. Nevertheless, eGFR less than 45 ml/min per 1.73 m^2 is reported to be independently associated with stroke mortality, morbidity, transient ischemic attack, and recurrence of any kind of stroke [54].

Earlier studies report suggest that blockade of RAAS can promote endothelial function and alleviate different inflammatory markers in severe vascular diseases such as stroke [61]. According to a multiple randomized control study, ACE inhibition and receptor blockade resulted in poor cardiovascular outcomes [61]. A meta-analysis of certain cohort and clinical trials derived from 33 prospective studies involving 284,672 people with 7863 stroke patients reported that patients with eGFR below 60 ml/min per 1.73 m^2 had a 43% increased risk of incident stroke [62]. In a meta-analysis of 11 prospective cohort studies encompassing 140,231 persons who encountered 3266 stroke occurrences, patients with proteinuria or other components of CKD, had a 71% (95% CI 39–110) higher stroke risk than healthy individuals [63].

The effects of dual RAAS blockade with various combination of drugs (an ACEI along with an ARB) were studied in the Telmisartan Alone and Combination with Ramipril Global Endpoint (ONTARGET) trial, in which 25,620 people with and without diabetes were randomly assigned to either telmisartan–Ramipril combination or placebo–Ramipril combination and followed for a mean of 56 months and reported no significance increase in stroke or cerebrovascular events [18]. Aliskiren Trial in Type 2 Diabetes Using Cardio-Renal Endpoints or ALTITUDE trial was designed to assess effect of renin inhibitors in diabetic patients, however, it was terminated early due to an increase in unexpected non-fatal stroke [18, 64]. Philip Masson et al., have found a linear relationship between decreasing GFR and the risk of stroke in the meta-analysis of 83 cohorts that reports over 30,000 stroke occurrences in 2,253,741 participants [22]. They reported that every 10 ml/min/1.73 m^2 decrease in GFR is linked with 7% increase in the relative risk of stroke and every 25 mg/mmol increase in albumin to creatinine ratio (ACR) is associated with a 10% increase in

the risk of stroke. According to their report, when GFR lowers and albuminuria rises, the risk of stroke increases exponentially and additively [22]. Another meta-analysis to investigate the substantial association of CKD and stroke. They analyzed 168 studies that include data of 5,611,939 people and 115,770 stroke patient. Out of those, 85 studies (with 3,417,098 people; 72,996 strokes) delivered ample data for meta-analysis of projected GFR and stroke risk [65]. Participants with eGFR level approximately 60 ml/min per 1.73 m^2 showed an elevated risk of stroke (RR = 1.73; 95% CI, 1.57–1.90; P0.001) however, there were significant heterogeneity between trials (P0.0001; I2, 78.5%). The adjusted blood pressure factors were: single baseline blood pressure measurement (RR = 1.63; CI, 1.34–1.99; P0.001); existing hypertension (RR = 1.35; CI, 1.24–1.46; P0.001); and multiple time blood pressure measurements over months to years (RR = 1.10; CI, 1.02–1.18; P = 0.01) [65]. After thorough adjustment of cardiovascular and progressive diminution of above hypertension factors the stroke outcome was seen to be reduced [65]. These findings not only show the inter-relationship of classical cardiovascular risk factors to play a significant role in high stroke susceptibility in patients with low eGFR, but also increase the possibilities of additional unconventional risk factors that may play an important role in low eGFR related stroke susceptibility [66].

Conclusion

Kidney and brain both are susceptible to similar kind of vascular injury as they share similarity in functional vasoregulation [67]. Limited management of stroke as well as CKD synergistically contribute to severe functional as well as cognitive outcomes [54]. CKD patients have high thromboembolic and hemorrhagic risk that are important predictors of ischemic as well as hemorrhagic stroke, therefore, risk-benefit ratio of antithrombotic treatment in stroke patients with pre-existing CKD is often difficult to anticipate [54]. The recovery rate of CKD patients after a stroke and alteplase therapy has been reported to be worsen along with higher risk of bleeding complications as compared to stroke patients without pre-existing CKD [54]. In addition to this, some special situation such as presence of hypertension in CKD-stroke patients can exhibit impaired endothelial tPA secretion that might obstruct reperfusion and worsen stroke outcome [68]. In the later stage of CKD, atrial fibrillation becomes prevalent, which an important risk factors of stroke [54]. On the other hand, warfarin treatment in dialysis patients are can lead to vascular calcification, therefore, it should be administered under close monitoring as per international standard [69]. Nevertheless, early preventive measures of CKD can attenuate stroke risk as well as its severity. However, strategy of CKD management often depends on the health care system of the particular countries. Renal transplant treatment is costly and requires high end technologies which are often limited in developing countries. Therefore, stroke gets further elevated in these countries [54].

Establishment of novel therapeutic strategy is critical in stroke patients with existing CKD and RAAS impairment as large clinical trials exclude patients with

higher renal impairment owing to safety issues [54]. Both neuroprotective as well as nephroprotective therapies may be promising strategy to manage such condition. Therefore, thorough understanding of the inter-relationship between RAAS, CKD and stroke is solicited to minimize the global health burden of stroke associated with existing CKD.

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Part III
Pathogenesis and Pharmacotherapy During Development of Cancer

Chapter 15
The Renin-Angiotensin System and Cancer

Sabrina P. Koh, Ethan J. Kilmister, Agadha C. Wickremesekera, Matthew J. Munro, Clint Gray, and Swee T. Tan

Abstract The renin-angiotensin system (RAS), classically known for its role in cardiovascular homeostasis, is increasingly recognized to be dysregulated in cancer. Epidemiological studies have largely demonstrated a cancer-protective role of RAS inhibitors (RASi), which is further supported by in vitro and xenograft models of cancer. The hierarchical model of cancer proposes the presence of cancer stem cells (CSCs), a small population of highly tumorigenic cancer cells sitting atop of a cellular hierarchy being responsible for sustaining tumor growth, intratumoral heterogeneity, loco-regional recurrence, distant metastasis, disease progression and treatment failure. CSCs have been identified in numerous cancer types, and the expression of components of the RAS by CSCs suggests CSCs may be a novel therapeutic target by modulation of the RAS. The classical view of the RAS centers around angiotensin II (ATII) as the main active peptide, which promote angiogenesis, fibrosis, inflammation, cellular proliferation, migration and invasion, and inhibition of apoptosis by activating angiotensin II receptor 1 (AT_1R), and its interaction with angiotensin II receptor 2 (AT_2R) antagonizes these effects. ATII signaling via AT_1R is thought to drive mitogenic processes in cancer and contribute to other pathological conditions including fibro-proliferative conditions and vascular anomalies. Blockade of the classical RAS by traditional RASi, including angiotensin-converting enzyme inhibitors

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(ACEIs), angiotensin receptor blockers (ARBs) and β-blockers, can be circumvented by enzymes such as cathepsins B, D and G which provide alternative pathways of ATII biosynthesis. The presence of these "bypass loops" that provide in-built redundancies, and converging signaling pathways, including the upstream Wnt/β-catenin pathway and the downstream NOX-ROS-NF-κB-COX signaling cascades, enables the RAS to continue functioning despite blockade by traditional RASi. This suggests that effective inhibition of the RAS may require a multi-step blockade. Further complexities to this model include the intimate interaction of CSCs with their surrounding dynamic tumor microenvironment (TME). The TME facilitates intercellular communication between CSCs and the surrounding *niche* to foster an environment favorable for sustaining tumorigenesis through cytokine networks, RAS signaling, and disruption of stem cell signaling pathways. Thus, effective modulation of CSCs requires additional consideration of the role of the surrounding TME. Instead of the long-standing pursuit of a 'silver-bullet', single point intervention in the treatment of cancer, future treatments will involve a combination of medications. This may be achieved by re-purposing existing, commonly used, low-cost, and safe medications that modulate the RAS, its "bypass loops" and convergent signaling pathways. There is currently a paucity of clinical trials assessing the effectiveness of this treatment approach for cancer.

Keywords Cancer · Renin-angiotensin system · Renin-angiotensin system inhibitor · Angiotensin-converting enzyme inhibitor · Angiotensin receptor blocker · β-blocker · Cancer stem cells · Tumor microenvironment · Drug repurposing

Introduction

Aspects of cancer biology remain a mystery. One modern concept of cancer attributes carcinogenesis to the presence of cancer stem cells (CSCs), a small subpopulation of highly tumorigenic cancer cells that contribute to intratumoral heterogeneity, disease progression, loco-regional recurrence, distant metastasis, treatment failure, and resistance to chemotherapy [1, 2], radiotherapy [1] and immunotherapy [3]. There is an emerging body of evidence demonstrating the presence of CSCs in numerous cancer types [4–21]. However, this small tumorigenic population remains an elusive target in the treatment of cancer.

The classical renin-angiotensin system (RAS) is known as an endocrine system with a fundamental role in cardiovascular physiology [22]. The contemporary view of the RAS recognizes the presence of a local paracrine RAS which is implicated in many pathological states, including cancer [23]. The RAS can be modulated by different classes of medications, which are commonly used for the treatment of hypertension, cardiac failure, renal disease and diabetes, including angiotensin-converting enzyme inhibitors (ACEIs), angiotensin receptor blockers (ARBs), and β-blockers.

Lever et al. first observed a protective role of RAS inhibitor (RASi) use in cancer outcomes [24]. This finding is supported by a plethora of subsequent epidemiological studies reporting a positive correlation between RASi use and reduced cancer risk and/or improved cancer outcomes [24–58], and investigations using in vitro and xenograft models of cancer [59–99]. Components of the RAS have been demonstrated in cancer and have been shown to be expressed by CSCs in numerous cancer types [100–110].

While the classical RAS focusses on angiotensin II (ATII) as the main active peptide, mediating its effects through interaction with ATII receptor 1 (AT_1R) and ATII receptor 2 (AT_2R), alternative pathways of ATII biosynthesis by enzymes such as cathepsins B, D and G, have been identified [111]. The presence of these "bypass loops", and converging signaling pathways including the upstream Wnt/β-catenin pathway and the downstream NADPH oxidase (NOX), reactive oxygen species (ROS), nuclear factor-kappa B (NF-κB) and cyclooxygenase-2 (COX-2) signaling pathways [111], create in-built redundancies that allow mitigation of blockade of the classical RAS using traditional RASi such as ACEIs, ARBs and β-blockers.

CSCs are regulated by a complex and dynamic tumor microenvironment (TME) playing an integral role in facilitating intercellular communication between CSCs and other cellular and non-cellular components of the TME. The cellular components of the TME include CSCs, inflammatory cells, mesenchymal stem cells (MSCs), stromal cells, cancer-associated fibroblasts, pericytes, adipocytes, vascular endothelial cells and other non-cancer cells [112, 113], and the non-cellular components form the extracellular matrix (ECM) [114]. Ultimately, there is multi-directional communication between CSCs and the surrounding TME to orchestrate a favorable *milieu* for tumorigenesis and regulating CSC stemness, growth and differentiation, which may be influenced by local paracrine RAS signaling [115–117], dysregulation of other stem cell regulatory pathways including Wnt/β-catenin, Notch, NF-κB, P13K and Jak/STAT pathways [118], and cytokine communication networks [118–120].

CSCs remain an elusive therapeutic target for the treatment of cancer which requires consideration of the surrounding TME, the RAS, its "bypass loops" and convergent signaling pathways. This may be achieved by re-purposing a combination of existing, low-cost and safe medications. However, there is a paucity of clinical trials, investigating re-purposing of RASi for the treatment of cancer [121–136].

Models of Cancer

The prevailing stochastic model of cancer, also known as the clonal evolution model of cancer, attributes carcinogenesis to the sequential accumulation of genetic mutations [1] (Fig. 15.1a). Based on the Darwinian principles of evolution, this concept proposes that cells acquiring advantageous mutations undergo clonal expansion to form a homogenous tumorigenic population, which continues to accrue genetic mutations and undergo further clonal expansions [137, 138].

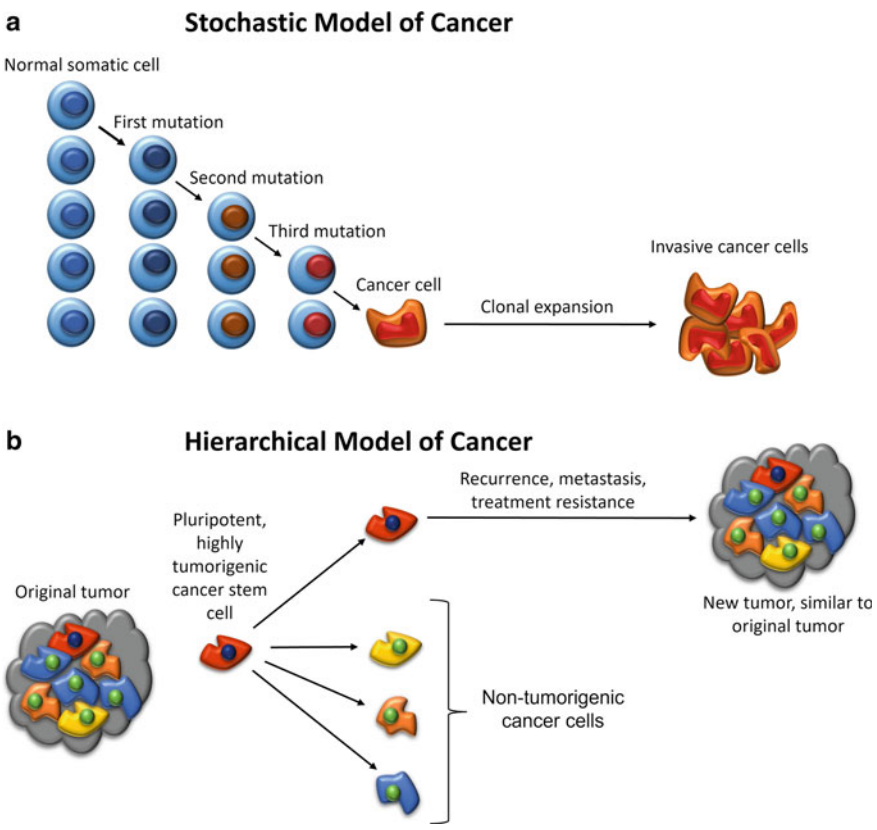

Fig. 15.1 Models of cancer. **a** A diagram illustrating the stochastic model of cancer: a normal somatic cell acquires oncogenic mutations in a stepwise manner and becomes a cancer cell that clonally expands to form a tumor. **b** A diagram illustrating the hierarchical model of cancer: a highly tumorigenic and pluripotent cancer stem cell, sitting atop the tumor cellular hierarchy, divides asymmetrically to form non-tumorigenic cancer cells that form the bulk of the tumor, and divides symmetrically to form cancer stem cells which are attributable for loco-regional recurrence, distant metastasis, and treatment resistance, and give rise to new tumors, similar to the original tumor. *Reproduced and modified with permission from the Atlas of Extreme Facial Cancer, Springer Nature* [293]

The new hierarchical model of cancer, also known as the CSC concept of cancer, proposes that carcinogenesis is sustained by a small subpopulation of cancer cells, known as CSCs, that possess embryonic stem cell properties [1, 139–146] and thus are imbued with pluripotency and self-renewal properties, aberrant growth, and the ability to evade normal tissue homeostatic mechanisms including apoptosis [1, 137, 143–147] (Fig. 15.1b). CSCs undergo asymmetric division to replenish the CSC population, and to form progenitor cells which undergo further differentiation to ultimately form mature, non-tumorigenic cancer cells [1, 141–144]. First characterized in acute myeloid leukemia [148, 149], CSCs have been identified in many

solid cancers including glioblastoma [4, 5] and other brain tumors [6, 7], cutaneous squamous cell carcinoma (cSCC) [8], head and neck SCC (HNSCC) [9] including oral cavity SCC (OCSCC) affecting different subsites [10–12], metastatic malignant melanoma (MM) [13, 14], renal clear cell carcinoma [15], breast [16], colorectal [17], gastric [18], liver [19], lung [20], and prostate [21] cancers.

The precise origin of the CSC remains unclear. Current theories hypothesize that CSCs may arise from normal resident stem cells which have accrued genetic and epigenetic changes [147]; by de-differentiation of differentiated cancer cells through epithelial-to-mesenchymal transition (EMT) [147]; or from reprogramming of adult somatic cells to a phenotypic embryonic stem cell state, commonly termed induced pluripotent stem cells [147, 150]. This hierarchical model of cancer provides an explanation for several phenomena observed in cancer which remain unexplained by the stochastic model of cancer, including (1) intratumoral heterogeneity—cancers are comprised of a heterogenous profile of cells with differing capacities for self-renewal [137, 151]; (2) the presence of a small subset of cells capable of initiating and sustaining tumor growth in in vitro and xenograft models [148, 152, 153]; (3) loco-regional recurrence and distant metastasis [139, 143]; and (4) resistance to chemotherapy [1, 2], radiotherapy [1] and immunotherapy [3].

Resistance to chemotherapy and radiotherapy may be explained by these therapies targeting rapidly dividing cells, while CSCs are generally in a quiescent state [111, 144, 145, 147]. Other mechanisms that confer resistance to chemotherapy include expression of drug efflux pumps, known as ATP-binding cassette (ABC) transporters [2, 154]; enhanced aldehyde dehydrogenase activity in CSCs [2]; up-regulated expression of anti-apoptotic B-cell lymphoma-2 (BCL2) proteins [2]; and activation of signaling pathways such as the Wnt/β-catenin, Notch, Sonic hedgehog, and NF-κB pathways [2]. The activation of DNA repair mechanism pathways may further facilitate the evasion of chemotherapy and radiotherapy induced cell death of CSCs [154]. Resistance to chemotherapy and immunotherapy may be facilitated by the presence of long non-coding RNAs (lncRNAs), regions of non-coding RNA consisting of more than 200 nucleotides which regulate gene expression at the epigenetic, transcriptional, post-transcriptional, translational and post-transitional levels [155–157]. Dysregulated expression of lncRNAs is observed in numerous cancer types, and is postulated to induce and maintain CSCs, promote EMT, facilitate communication with the surrounding TME through lncRNA export via exosomes [155], promote invasion, inhibit apoptosis [157], up-regulate the expression of ABC transporters thus contributing to chemotherapy resistance [155], and suppress the immune system thus contributing to immunotherapy resistance [155, 157].

The stochastic model of cancer and the hierarchical model of cancer are not mutually exclusive, and cancer biology is likely to encapsulate concepts from both models. As in normal tissues, cells within a tumor exhibit a phenotypic hierarchy, with CSCs sitting atop this hierarchy, replenishing the CSC population and also giving rise to downstream progenitor and differentiated cancer cells [1, 142, 144–147]. The presence of this hierarchy has been well demonstrated in many cancer types including leukemia [149], breast cancer [158], and glioblastoma [151, 159–161]. Added intricacies to the hierarchical model of cancer include the demonstrated ability

of non-CSCs to revert to a pluripotent state [162–165], underscoring the concept of plasticity within the CSC hierarchy—a phenomenon demonstrated in human cancers including glioblastoma [162], breast [163–165] and colorectal cancer [166]. The presence of a plastic and dynamic CSC hierarchy may further confound the concept of therapeutic resistance by allowing cancer cells to adapt to the TME. CSCs, the small subset of cells that possess tumorigenic potential, sustain tumor growth, and are responsible for treatment failure, loco-regional recurrence and distant metastasis, remain an elusive therapeutic target in the treatment of cancer.

The Renin-Angiotensin System

The Systemic Renin-Angiotensin System

The systemic (endocrine) RAS (Fig. 15.2) is classically recognized for its integral role in cardiovascular homeostasis. The basic foundation of the RAS involves the cleavage of angiotensinogen by renin to form angiotensin I (ATI), which is then converted to the active octapeptide ATII, by angiotensin-converting enzyme (ACE) [167]. The effects of ATII are mediated through its interactions with AT_1R and AT_2R [168]. Actions of ATII mediated through AT_1R include the physiological effects of sodium and water retention, vasoconstriction, promotion of aldosterone release from adrenal glands and anti-diuretic hormone release from the pituitary gland, subsequently driving an increase in blood pressure. Pathological effects of ATII mediated through AT_1R include promotion of cellular proliferation, hypertrophy, fibrosis, inflammation, and oxidative stress [169], with antagonistic effects mediated through AT_2R including vasodilation, inhibition of cellular proliferation and growth [170], and neurotrophic effects [171].

While the RAS classically centers on ATII as the main active peptide, the contemporary view of the RAS recognizes the role of other active peptides within this system (Fig. 15.2). Angiotensin III (ATIII) is generated from ATII by aminopeptidase A and exerts its actions by binding with AT_1R and AT_2R, similar to ATII. Angiotensin IV (ATIV) is generated from ATIII by aminopeptidase M and acts via insulin-regulated aminopeptidase receptors, inducing downstream effects including activation of NF-kB, vasodilation through the expression of cytokines including interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), and hypertrophy [170]. Angiotensin 1-7 (Ang1-7), generated from ATII by angiotensin-converting enzyme 2 (ACE2), acts via the MAS receptor to exert vasodilatory, anti-hypertrophic, anti-fibrotic, anti-thrombotic, anti-proliferative [170, 172], and anti-angiogenic effects to oppose those of ATII, and further reduces cell migration, invasion, and EMT [173].

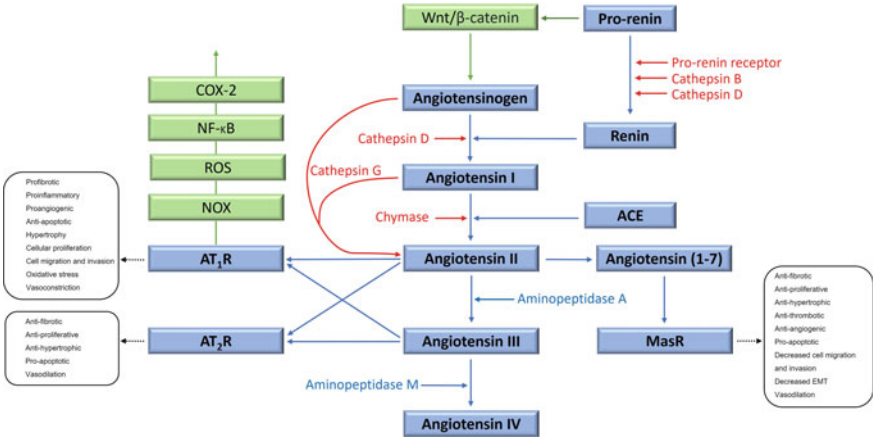

Fig. 15.2 The renin-angiotensin system (RAS), its "bypass loops", and convergent signaling pathways. In the classical RAS (blue), pro-renin is activated by pro-renin receptor, to form renin. Angiotensinogen is converted to angiotensin I (ATI), by renin. ATI is is further cleaved by angiotensin-converting enzyme (ACE) to form angiotensin II (ATII). ATII exerts its physiological effects via ATII receptor 1 (AT_1R) and ATII receptor 2 (AT_2R). ATII is further cleaved to form angiotensin 1-7 (Ang1-7) which exerts its actions via Mas receptor (MasR). ATII is converted to angiotensin III (ATIII) by aminopeptidase A, and acts via AT_1R and AT_2R. ATIII is converted to angiotensin IV (ATIV) by aminopeptidase M. Proteases including cathepsins B, D, and G, and chymase facilitate alternative pathways of ATII biosynthesis, thus constituting "bypass loops" of the RAS (red), providing inbuilt redundancies to this system. Cathepsin B converts pro-renin into renin, cathepsin D converts angiotensin to ATI, and cathepsin G generates ATII from angiotensinogen and ATI. Other signaling pathways converge on the RAS (green) including the upstream Wnt/β-catenin signaling pathway which is activated by pro-renin, and the downstream inflammatory signaling NOX-ROS-NFκB-COX2 axis, induced by activation of AT_1R. EMT, epithelial-to-mesenchymal transition; NOX, NADPH oxidase; ROS, reactive oxygen species, NF-κB, nuclear factor-kappa B; COX-2, cyclooxygenase-2. *Reproduced and modified with permission from the Journal of Histochemistry and Cytochemistry* [294]

The Local Paracrine Renin-Angiotensin System

A self-sufficient local tissue paracrine RAS has been identified in many types of tissues and organs including the brain, heart, gonads, pancreas, placenta, kidney, adipose tissue and vessels, and has the capacity to generate ATII locally [174]. A local paracrine RAS has been identified in numerous pathological states including cancer [22], fibroproliferative conditions [175, 176], and vascular anomalies [177–179]. Within these conditions, the pathological effects of the RAS are similarly mediated through the interaction of ATII with AT_1R resulting in the evasion of apoptosis, promotion of angiogenesis, inflammation, cellular proliferation, invasion and metastasis, and increased oxidative stress through the generation of reactive oxygen species (ROS) which helps maintains a hypoxic *milieu*, further contributing to cellular damage [23, 180, 181]. These pathological effects are antagonized by the interaction of ATII with AT_2R [181].

Renin-Angiotension System in Cancer

There is an emerging body of evidence demonstrating the presence of a local tissue paracrine RAS influencing pathological states such as cancer [180, 181]. The role of the RAS in cancer is underscored by several layers of evidence, including a correlation between RASi use and reduced cancer risk and improved cancer outcomes in epidemiological studies, the identification of expression of components of the RAS in numerous cancer types, and protective effects of RASi in cancer demonstrated in studies using in vitro and xenograft models of cancer.

Components of the RAS are commonly targeted by classes of drugs used for the treatment of hypertension, including ACEIs, which inhibit ACE to prevent conversion of ATI to ATII, ARBs which block AT_1R, and β-blockers which are classical β-adrenergic receptor antagonists that inhibit conversion of pro-renin to renin [111] (Fig. 15.3).

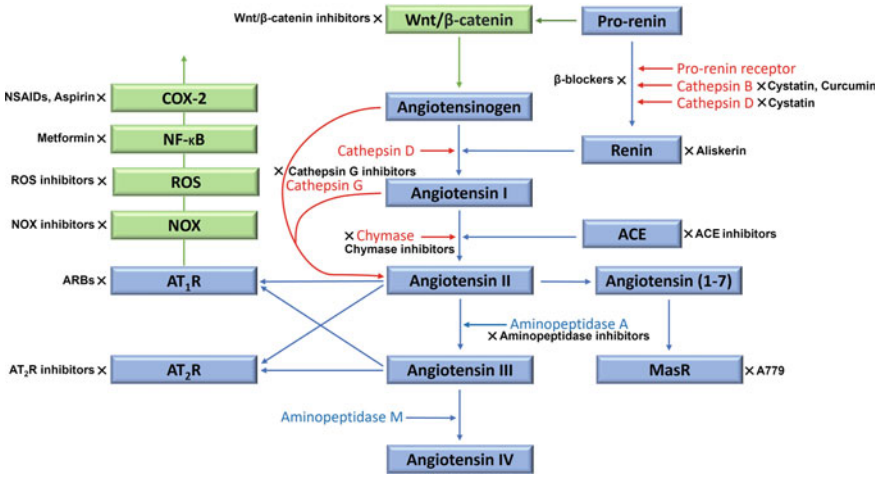

Fig. 15.3 Inhibitors of the renin-angiotensin system, its "bypass loops", and convergent signaling pathways. The classical renin-angiotensin system (blue), its "bypass loops" (red) and convergent signaling pathways (green), can be modulated by various inhibitors (x). ACEI, angiotensin-converting enzyme; MasR, Mas receptor; AT_1R, angiotensin II receptor 1; AT_2R, angiotensin II receptor 1; ARB, angiotensin receptor blocker; NOX, NADPH oxidase; ROS, reactive oxygen species; NF-κB, nuclear factor-kappa B; COX-2, cyclooxygenase-2. *Reproduced and modified with permission from the Journal of Histochemistry and Cytochemistry* [294]

Epidemiological Evidence of the Role of the Renin-Angiotensin System in Cancer

ACE Inhibitors

A seminal retrospective cohort study by Lever et al. in 1996 first demonstrated a cancer-protective effect of RASi use, with ACEIs being associated with a significantly reduced incidence of cancer [24]. The beneficial effect of RASi in cancer has been further supported by a plethora of subsequent epidemiological studies (Table 15.1).

A protective association between ACEI use and reduced risk of cancer has been observed in several retrospective cohort studies [25, 26]. ACEI use has been correlated with a significantly reduced incidence of cSCC and basal cell carcinoma (BCC) but not cancer-related deaths [28], and a reduced incidence of breast cancer [29], cancer recurrence, increased disease-free survival [30], and breast-cancer specific deaths [31]. ACEI use is associated with a reduced incidence of colorectal cancer [32], reduced cancer progression and cancer-related hospitalizations, and improved overall survival when used in conjunction with a β-blocker [33]. Protective effects of ACEIs have been observed in metastatic pancreatic cancer, with improved progression-free survival and overall survival [34, 35]. ACEI use is correlated with increased 5-year metastasis-free survival in urothelial cancer [36], and increased disease-specific survival and overall survival in urothelial cancer [36] and esophageal cancer [37]. Lung cancer patients taking ACEIs have greater overall survival [38], significant reductions in lymph node metastasis and risk of advanced pathological state, and longer progression-free survival when used in conjunction with adjuvant chemotherapy [39].

However, not all epidemiological studies observe a protective effect of RASi in cancer, and some studies have observed no difference in the risk of breast [182], pancreatic [183], and prostate [184] cancers, and an increased risk of renal cancer [185], in those taking ACEIs. The variable results of observational studies on ACEI use in cancer could be attributed to discrepancies in the duration and type of ACEI used, variable sample size, and other confounding factors of the study populations including cancer stage, adjuvant treatments and patient comorbidities, amongst the studies.

Angiotensin Receptor Blockers

The effect of ARB use on cancer risk has been investigated in numerous epidemiological studies. A large retrospective cohort study observes a significantly reduced incidence of cancer in patients taking ARBs, regardless of the duration of treatment [42]. Other cohort studies have similarly observed a protective correlation between ARB use and reduced cancer risk [25–27]. ARB use is associated with a significantly reduced incidence of cSCC, BCC [28], and colorectal cancer [32], reduced tumor progression and cancer-related hospitalizations, and increased overall survival

Table 15.1 Epidemiological studies on the effect of renin-angiotensin system inhibitors on cancer risk and cancer outcomes

Authors and year	Type of study	Time period	Sample size	Cancer types	Class and type of RASi	Outcomes of treatment with RASi
Lever et al. [24]	Retrospective cohort study	1980 - 1995	5,207	All types	ACEI (n = 1,559) Captopril, enalapril, lisinopril, other	Reduced risk of cancer and incidence of fatal cancer Increased cancer-free survival and overall survival
Kong et al. [25]	Retrospective cohort study	1996 - 2005	6,103	All types	ACEI or ARB (n = 1,796) (type not specified)	ACEI or ARB use: Reduced risk of cancer
Chiang et al. [26]	Retrospective cohort study	2000 - 2008	297,688	All types	ACEI (n = 4,988) or ARB (n = 6,960) (type not specified)	ACEI or ARB use: Reduced risk of cancer
Wang et al. [27]	Retrospective cohort study	1997 - 2009	85,842	All types	ARB (n = 42,921) Candesartan, irbesartan, losartan, olmesartan, telmisartan, valsartan	Reduced risk of all cancers: breast, colorectal, liver, lung, gastric, and prostate cancers
Huang et al. [42]	Retrospective cohort study	1998 - 2006	109,002	All types	ARB (n = 40,124) Candesartan, losartan, irbesartan, telmisartan, valsartan	Reduced risk of all cancers: oral cavity, pharyngeal, GI, lung, bone, connective tissue, skin, breast, lymphoma, hematopoietic and genitourinary cancers
Friis et al. [185]	Prospective cohort study	1989 - 1995	17,897	All types	ACEI (n = 17,897) Captopril, enalapril, lisinopril, other	No reduction in overall risk of cancer Increased risk of renal cancer No difference in risk of cancer depending on type of ACEI
Sun et al. [197]	Meta-analysis	1990 - 2015	358,601	All types	ACEI or ARB	ACEI or ARB use: Increased overall survival with specific increased survival in renal cell, gastric, pancreatic, hepatocellular, upper-tract urothelial and bladder cancers, but no difference in colorectal, lung, prostate, oropharyngeal, esophageal, breast and biliary tract cancers, glioblastoma, head and neck SCC, or malignant melanoma Increased overall survival with ARB use, but no difference with ACEI use, when stratified by type of RASI Increased progression-free survival, disease-free survival and metastasis-free survival

(continued)

Table 15.1 (continued)

Authors and year	Type of study	Time period	Sample size	Cancer types	Class and type of RASi	Outcomes of treatment with RASi
Song et al. [198]	Meta-analysis	2007 - 2012	4,964	All types	ACEI or ARB	ACEI or ARB use: Increased overall disease-free survival, with analysis by type of cancer showing increased disease-free survival for renal tract, colorectal, pancreatic and prostate cancers, but no difference in disease-free survival for breast and hepatocellular cancers Increased overall survival
Yoon et al. [199]	Meta-analysis	1994 - 2010	Not specified	All types	ACEI or ARB	ACEI or ARB use: No difference in overall risk of cancer, but significant heterogeneity between studies Reduced risk of esophageal cancer Increased risk of malignant melanoma and renal cancer
Christian et al. [28]	Prospective cohort study	1998 - 2003	1,051	Cutaneous SCC and BCC	ACEI or ARB (n = 532) ACEI: Captopril, enalapril, fosinopril, lisinopril ARB: Candesartan, irbesartan, losartan, telmisartan, valsartan	ACEI or ARB use: Reduced risk of cutaneous SCCs and BCCs No difference in depth of SCCs No difference in cancer-related deaths for both cutaneous SCC and BCC
Tang et al. [207]	Meta-analysis	1996 - 2017	Not specified	Malignant melanoma	ACEI or ARB or β-blockers	ACEI or ARB use: No difference in risk of malignant melanoma β-blocker use: Increased risk of malignant melanoma
Tang et al. [208]	Meta-analysis	2008 - 2017	Not specified	Cutaneous SCC and BCC	ACEI or ARB or β-blockers	ACEI or ARB use: No difference in risk of BCC or SCC, but significant heterogeneity between studies Reduced risk of BCC and SCC in high risk individuals β-blocker use: Increased risk of BCC No difference in risk of SCC

(continued)

Table 15.1 (continued)

Authors and year	Type of study	Time period	Sample size	Cancer types	Class and type of RASi	Outcomes of treatment with RASi
Chae et al. [30]	Retrospective cohort study	1999 - 2005	703	Breast cancer	ACEI or ARB (n = 168) ACEI: Benazepril, enalapril, lisinopril, quinapril, other ARB: Eprosartan, irbesartan, losartan, omesartan, valsartan, other	ACEI or ARB use: Reduced risk of cancer recurrence Increased disease-free survival No difference in overall survival
Santala et al. [31]	Retrospective cohort study	1995 - 2013	73,170	Breast cancer	ACEI (n = 20,742) or ARB (n = 16,552) or β-blocker (n = 33,611) (type not specified)	Reduced risk of cancer-specific deaths with ACEI, ARB and β-blocker use post-diagnosis of breast cancer Reduced risk of cancer-specific deaths with ARB use but not ACEI or β-blocker use pre-diagnosis of breast cancer
Raimondi et al. [201]	Meta-analysis	2010 - 2014	46,265	Breast cancer	ACEI or ARB or β-blockers	ACEI or ARB use: No difference in overall survival or disease-free survival β-blocker use: Improved overall survival Improved disease-free survival but non-significant
Xie et al. [202]	Meta-analysis	2000 - 2020	3,726,281	Breast cancer	ACEI or ARB or β-blockers	ACEI or ARB use: No difference in overall risk of breast cancer, reduced risk with use of less than 5 years and greater than 10 years No difference in breast-cancer specific mortality, overall survival, recurrence or disease-free survival β-blocker use: Increased risk of cancer No difference in breast-cancer specific mortality, overall survival, recurrence or disease-free survival
Makar et al. [32]	Retrospective cohort study with nested case-control study	1987 - 2002	2,847	Colorectal cancer	ACEI or ARB (n = 722) (type not specified)	ACEI or ARB use: Reduced risk of colorectal cancer

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Table 15.1 (continued)

Authors and year	Type of study	Time period	Sample size	Cancer types	Class and type of RASi	Outcomes of treatment with RASi
Engineer et al. [33]	Retrospective cohort study	2000 - 2009	262	Colorectal cancer	ACEI or ARB (n = 52) or β-blocker (n = 32) (type not specified) Combination of ACE or ARB with β-blocker (n = 39)	Monotherapy with either ACEI, ARB or β-blocker only: No difference in overall survival, risk of tumor progression or cancer-related hospitalizations Combination therapy of ACEI or ARB with β-blocker: Increased overall survival Reduced risk of tumor progression, cancer related hospitalizations, and mortality
Chen et al. [204]	Meta-analysis	2008 - 2014	2,847,597	Colorectal cancer	ACEI or ARB	ACEI or ARB use: 6% reduction in risk of colorectal cancer per year of RASI use Reduced cancer-specific mortality
Dai et al. [205]	Meta-analysis	2001 - 2014	1133,048	Colorectal cancer	ACEI or ARB	ACEI or ARB use: Reduced risk of colorectal cancer No difference in cancer-specific mortality, but significant heterogeneity between studies
Nakai et al. [34]	Retrospective cohort study	2001 - 2009	155	Pancreatic cancer	ACEI or ARB (n = 27) ACEI: Enalapril, lisinopril, temocapril ARB: Candesartan, losartan, olmesartan, valsartan	ACEI or ARB use compared to other anti-hypertensives or no anti-hypertensives: Increased progression-free survival and overall survival ACEI compared to ARB use: No significant difference in progression-free survival and overall survival
Nakai et al. [35]	Retrospective cohort study	2001 - 2013	349	Pancreatic cancer	ACEI (n = 15) or ARB (n = 180) ACEI: Enalapril, temocapril, lisinopril, other ARB: Candesartan, irbesartan, losartan, olmesartan, telmisartan, valsartan	ACEI or ARB use: No difference in progression-free survival and overall survival Increased progression-free survival and overall survival in never smokers and those receiving gemcitabine therapy

(continued)

Table 15.1 (continued)

Authors and year	Type of study	Time period	Sample size	Cancer types	Class and type of RASi	Outcomes of treatment with RASi
Mandilaras et al. [183]	Retrospective case control study with nested case-control study	1995 - 2009	866	Pancreatic cancer	ACEI (n = 402) or ARB (n = 112) (type not specified)	ACEI or ARB use: No difference in risk of developing pancreatic cancer
Zhou et al. [206]	Meta-analysis	2007 - 2018	Not specified	Pancreatic, hepatic, gastric, colorectal and esophageal cancers	ACEI or ARB	ACEI or ARB use: Increased overall, cancer-specific and recurrence-free survival No difference in progression-free or disease-free survival
Tanaka et al. [36]	Retrospective cohort study	1995 - 2009	279	Urothelial cancer	ACEI (n = 5) or ARB (n = 43) ACEI: Enalapril, cilazapril, perindopril ARB: Candesartan, losartan, olmesartan, telmisartan, valsartan	ACEI or ARB use: Increased 5-year metastasis-free survival, disease-specific survival and overall survival
Chen et al. [37]	Retrospective cohort study	1996 - 2011	141	Esophageal SCC	ACEI (n = 5) or ARB (n = 15) ACEI: Enalapril, fosinopril, imidapril ARB: Irbesartan, losartan, telmisartan, valsartan	ACEI or ARB use: Increased disease-free survival and overall survival
Wilop et al. [38]	Retrospective cohort study	1996 - 2007	287	Lung cancer	ACEI (n = 43) or ARB (n = 9) ACEI: Captopril, enalapril, lisinopril, ramipril ARB: Candesartan, losartan, valsartan	ACEI or ARB use: Increased median survival
Wei et al. [39]	Retrospective cohort study	2016 - 2018	678	Lung cancer	ACEI (n = 97) or ARB (n = 117) (type not specified)	ACEI or ARB treatment compared to other anti-hypertensives: Reduced lymph node metastasis and advanced pathological stage Increased progression-free survival with adjuvant chemotherapy ACEI compared to ARB: No difference lymph node metastasis or pathological stage No difference in progression-free survival with adjuvant chemotherapy
Rao et al. [40]	Retrospective cohort study	1999 - 2010	1,228,960	Lung cancer	ARB (n = 78,075) Candesartan, irbesartan, losartan, valsartan	Reduced risk of lung cancer

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Table 15.1 (continued)

Authors and year	Type of study	Time period	Sample size	Cancer types	Class and type of RASi	Outcomes of treatment with RASi
Zhang et al. [200]	Meta-analysis	2011 - 2013	298,000	Lung cancer	ARB	Reduced risk of lung cancer, with significant reduction with use of less than 5 years, and no significant difference in risk with use over 5 years
Rao et al. [40]	Retrospective cohort study	2003 - 2009	543,824	Prostate cancer	ARB (n = 33,989) (type not specified)	Reduced risk of prostate cancer
Cardwell et al. [182]	Retrospective case control study with nested case-control study	1998 - 2006	4,130	Breast, colorectal and prostate cancer	ACEI (n = 859) or ARB (n = 266) (type not specified)	ACEI or ARB use: No difference in breast cancer-related deaths Reduced cancer-related death for colorectal cancer and prostate cancer with ACEI use No difference in cancer-related deaths for colorectal cancer and prostate cancer with ARB use
Ronquist et al. [184]	Retrospective cohort study with nested case-control study	1995 - 1999	1,013	Prostate cancer	ACE (n = 890) or β-blocker (n = 777) ACE: Captopril, enalapril, lisinopril β-blocker: Atenolol, propranolol	No difference in risk of prostate cancer with ACEI or β-blocker use No difference in risk of prostate cancer depending on type of ACEI
Asgharzadeh et al. [203]	Meta-analysis	2014 - 2018	Not specified	Renal cancer	ACEI or ARB	ACEI or ARB use: Reduced risk of mortality
Algazi et al. [43]	Prospective cohort study	1962 - 1996	839	All types	β-blocker (n = 326) (type not specified)	Reduced risk of all cancers
Lin et al. [44]	Retrospective case-control study	2000 - 2010	13,542	All types	β-blocker (n = 6,771) Carvedilol	Reduced incidence of cancer with significant reductions in upper GI and lung cancers
Chang et al. [45]	Retrospective cohort study	2000 - 2011	24,238	All types	β-blocker (n = 12,119) Propranolol	Reduced risk of head and neck, esophageal, gastric, colorectal, and prostate cancers
Choi et al. [209]	Meta-analysis	1993 - 2013	20,898	All types	β-blocker	Increased overall survival, with specific increased survival in breast, ovarian, and non-small cell lung cancers, and no difference in survival in prostate cancer or melanoma Increased disease-free survival

(continued)

Table 15.1 (continued)

Authors and year	Type of study	Time period	Sample size	Cancer types	Class and type of RASi	Outcomes of treatment with RASi
Yap et al. [210]	Meta-analysis	Prior to 2017	67,244	All types	β-blocker	No difference in pooled disease-free survival, but increased disease-free survival in melanoma, and ovarian cancer (with non selective β-blocker use), and reduced disease-free survival in endometrial cancer No difference in pooled overall survival, but increased overall survival in melanoma, and reduced overall survival in head and neck and prostate cancer No difference in recurrence
De Giorgi et al. [46]	Prospective cohort study	1993 - 2009	121	Malignant melanoma	β-blocker (n = 30) (type not specified)	Increased disease-free survival Reduced positive sentinel lymph nodes, death and risk of recurrence with 36% reduction in risk of recurrence per year of β-blocker use
De Giorgi et al. [47]	Prospective cohort study	1993 - 2009	741	Malignant melanoma	β-blocker (n = 79) Atenolol, betaxolol, bisoprolol, carvedilol, labetalol, metoprolol, nadolol, nebivolol, propranolol, sotalol, timolol	Increased overall survival Reduced lymph node metastasis, death and risk of recurrence with 38% reduction in risk of recurrence per year of β-blocker use
De Giorgi et al. [48]	Prospective cohort study	1993 - 2009	121	Malignant melanoma	β-blocker (n = 30) (type not specified)	Increased disease-free, progression-free and overall survival
Kokolus et al. [49]	Retrospective cohort study	2000 - 2015	195	Metastatic melanoma	β-blocker β1-selective β-blocker (n = 45) Non-selective β-blocker (n = 17) (type not specified)	Improved overall survival with non-selective β-blockers use No difference in overall survival with β1-selective blocker use
Livingstone et al. [186]	Retrospective cohort study	1998 - 2010	709	Malignant melanoma	β-blocker (n = 203) Acebutolol, atenolol, bisoprolol, carvedilol, labetalol, metoprolol, nebivolol, oxprenolol, pindolol, propranolol, sotalol	No difference in overall survival
McCourt et al. [187]	Retrospective cohort study with nested case-control study	1998 - 2010	242	Malignant melanoma	β-blocker (n = 49) Atenolol, bisoprolol, metoprolol, propranolol, sotalol	No difference in cancer-specific survival
Barron et al. [50]	Case control study	2001 - 2006	5,801	Breast cancer	β-blocker (n = 595) Atenolol, propranolol	Reduced risk of T4 tumor, N2/N3 nodal involvement, metastatic disease and 5-year cancer-specific deaths with propranolol but not atenolol use

(continued)

Table 15.1 (continued)

Authors and year	Type of study	Time period	Sample size	Cancer types	Class and type of RASi	Outcomes of treatment with RASi
Botteri et al. [51]	Retrospective cohort study	1997 - 2008	800	Breast cancer	β-blocker (n = 74) Atenolol, betaxolol, bisoprolol, carvedilol, metoprolol, nebivolol, sotalol	Reduced risk of cancer recurrence, distant metastasis and cancer-related deaths
Powe et al. [52]	Retrospective cohort study	1987 - 1994	466	Breast cancer	β-blocker (n = 43) Atenolol, bisoprolol, propranolol, timolol	Increased disease-free survival Reduced risk of distant metastasis, cancer recurrence and cancer-specific deaths
Melhem-Bertrandt et al. [53]	Retrospective cohort study	1995 - 2007	1,413	Breast cancer	β-blocker (n = 102) (type not specified)	Increased overall survival Reduced risk of cancer recurrence
Sakellakis et al. [188]	Retrospective cohort study	1983 - 2013	610	Breast cancer	β-blocker (n = 63) (type not specified)	No difference in disease-free survival
Cardwell et al. [189]	Retrospective cohort study with nested case-control study	1998 - 2007	1,435	Breast cancer	β-blocker (n = 271) Atenolol, bisoprolol, carvedilol, metoprolol, propranolol, sotalol	No difference in breast cancer-specific deaths No difference in breast cancer-specific deaths depending on type of β-blocker
Ganz et al. [190]	Retrospective cohort study	1997 - 2000	407	Breast cancer	β-blocker and ACEI use (n = 66) β-blocker use only (n = 204) ACEI use only (n = 137) β-blocker: Atenolol, metoprolol, propranolol ACEI: Lisinopril, prinivil	ACEI use only: Increased risk of recurrence β-blocker use only: No difference in risk of recurrence Combination ACEI and β-blocker use: No difference in risk of recurrence No difference in cancer-specific deaths
Kurtis Childers et al. [211]	Meta-analysis	2010 - 2013	22,988	Breast cancer	β-blocker	Reduced breast cancer specific deaths No difference in risk of recurrence or overall mortality
Kim et al. [212]	Meta-analysis	2010 - 2013	18,118	Breast cancer	β-blocker	No difference in overall mortality, cancer-specific deaths or recurrence
Grytli et al. [54]	Retrospective cohort study	2004 - 2009	3,561	Prostate cancer	β-blocker (n = 1,115) (type not specified)	Reduced cancer-specific deaths

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Table 15.1 (continued)

Authors and year	Type of study	Time period	Sample size	Cancer types	Class and type of RASi	Outcomes of treatment with RASi
Grytli et al. [55]	Prospective cohort study	2000 - 2010	6,515	Prostate cancer	β-blocker (n = 776) (type not specified)	No difference in cancer-specific deaths Reduced prostate cancer-specific deaths when used with concurrent androgen deprivation therapy
Assayag et al. [195]	Retrospective cohort study	1998 - 2009	6,270	Prostate cancer	β-blocker (n = 854) (type not specified)	No difference in cancer-specific deaths and overall survival
Lu et al. [214]	Meta-analysis	2013 - 2014	16,825	Prostate cancer	β-blocker	Reduced prostate cancer-specific mortality No difference in overall mortality
Herrera et al. [57]	Prospective cohort study	2006 - 2007	173	Hepatocellular cancer	β-blocker (n = 73) (type not specified)	Reduced risk of developing hepatocellular cancer in those with cirrhosis
Ahl et al. [58]	Retrospective cohort study	2007 - 2016	22,337	Colon cancer	β-blocker (n = 8,072) Atenolol, bisoprolol, metoprolol	Reduced mortality at 90 days, 1 year and 5 years following surgery
Jansen et al. [191]	Retrospective case control study	2003 - 2007	1,762	Colorectal cancer	β-blocker (n = 309) Cardio-selective: Acebutalol, atenolol, acebutalol, betaxolol, bisoprolol, celoprolol, esmolol. Metoprolol, nebivolol Non-selective: Carvedilol, carteolol, nadolol, penbutalol, pindolol, propranolol	No difference in risk of colorectal cancer Long term use of 6 years or more may be associated with an increased risk of stage IV colorectal cancer
Hicks et al. [192]	Retrospective cohort study with nested case-control study	1998 - 2007	1,559	Colorectal cancer	β-blocker (n = 334) Atenolol, bisoprolol, carvedilol, metoprolol, propranolol, sotalol	Reduced overall mortality No difference in risk of cancer specific deaths
Diaz et al. [56]	Retrospective cohort study	1996 - 2006	248	Ovarian cancer	β-blocker (n = 23) (type not specified)	Increased progression-free, disease-specific and overall survival
Johannesdottir et al. [194]	Retrospective cohort study	1999 - 2010	6,166	Ovarian cancer	β-blocker (n = 373) (type not specified)	No difference in overall survival

(continued)

Table 15.1 (continued)

Authors and year	Type of study	Time period	Sample size	Cancer types	Class and type of RASi	Outcomes of treatment with RASi
Wen et al. [215]	Meta-analysis	2012 to 2020	20,274	Ovarian cancer	β-blocker	No difference in overall mortality, cancer-specific mortality or progression-free survival
Cata et al. [193]	Retrospective cohort study	2004 - 2006	435	Non-small cell lung cancer	β-blocker (n = 193) β1-selective and non-selective (types not specified)	No difference in recurrence-free survival and overall survival with both β1-selective and non-selective β-blocker use
Lei et al. [213]	Meta-analysis	2011 - 2020	30,870	Lung cancer	β-blocker	No difference in pooled overall survival, but increased overall survival in those with stage III lung cancer or not receiving surgical resection Non-selective β-blockers reduced overall survival
Kim et al. [196]	Retrospective cohort study	2001 - 2012	1,274	Head and neck SCC	β-blocker (n = 114) (type not specified)	Reduced overall survival Increased cancer-specific mortality

RAS, renin-angiotensin system; RASi, renin-angiotensin system inhibitor; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BCC, basal cell carcinoma; SCC, squamous cell carcinoma; GI, gastro-intestinal

in colorectal cancer [33]. For lung cancer, ARB use is associated with increased overall survival [38], improved progression-free survival when used with adjuvant chemotherapy [39], reduced pathological stage, metastasis to lymph nodes [39] and incidence of cancer [40]. In breast cancer, ARB use is associated with a reduced risk of cancer recurrence and cancer-specific death [31], and increased disease-free survival [30]. ARB use is also associated with improved progression-free survival in pancreatic cancer [34], increased five-year metastasis-free survival, disease-specific survival and overall survival in urothelial cancer [36], increased overall survival and disease-free survival in esophageal cancer [37], and reduced risk of prostate cancer [41].

Two small cohort studies have observed no significant reduction in the incidence of pancreatic cancer [183] and no difference in disease-free survival and overall survival [35], in ARB users compared to non-users. Similar to ACEIs, factors contributing to these discrepancies may include variations in sample sizes, type, dose and duration of ARB use, and other confounding factors amongst the study populations.

β-Blockers

Epidemiological studies have demonstrated a cancer-protective role of β-blockers with a significant reduction in the risk of cancer [43–45], including head and neck, esophageal, stomach, colorectal and prostate cancer in those taking propranolol for at least 6 months [45]. In MM, β-blocker use has been associated with increased disease-free survival and overall survival [46–48] and a 36–38% reduction in the risk of recurrence per year of β-blocker use [46, 47], decreased disease progression [48] and lymph node metastasis [46, 47]. Interestingly, the protective effects of β-blockers may be restricted to non-selective β-blockers, with no difference in the overall survival in MM patients treated with β_1-selective blockers [49]. In breast cancer, β-blocker use is correlated with a significantly reduced risk of distant metastasis [50–52], cancer-specific mortality [31, 50–52] and recurrence [51–53], and increased overall survival [53] and disease-free survival [52]. Similarly, the protective effects of β-blockers observed in breast cancer are limited to the use of propranolol, a non-selective β-blocker, and are not reproduced by atenolol, a β_1-selective blocker [50]. β-blocker use in prostate cancer patients is associated with a significant reduction in overall mortality [54], and cancer-specific mortality when used in conjunction with androgen deprivation therapy [55]. In ovarian cancer, β-blocker use is associated with increased progression-free survival, disease-specific survival, and overall survival [56]. β-blocker use is also associated with a reduced risk of developing hepatocellular carcinoma in those with cirrhosis [57], and reduced mortality in colorectal cancer [58].

While many observational studies have demonstrated a protective role of β-blockers in cancer, some studies have reported no significant improvement of outcomes in patients taking β-blockers in MM [186, 187], breast [188–190], colorectal [191, 192], lung [193], ovarian [194] and prostate [195] cancers. A harmful role of β-blockers has been observed in HNSCC with reduced overall survival and

increased cancer-specific mortality [196]. This discrepancy could be attributed to the small sample size of some studies, other confounding factors such patient demographics and cancer stage of the study population, adjuvant treatments, differences in the duration and type of RASi use, and the duration of follow-up.

Meta-analyses of ACE Inhibitor, Angiotensin Receptor Blocker, and β-Blocker Use and Cancer Outcomes

Meta-analyses exploring the role of ACEIs and ARBs in cancer have yielded mixed findings. A large meta-analysis of 55 studies including 358,601 patients, shows ACEI and ARB use is associated with an increased overall survival in renal cell, gastric, pancreatic, hepatocellular, upper-tract urothelial and bladder cancers, and no difference in overall survival in lung, prostate, oropharyngeal, esophageal, breast and biliary cancers, glioblastoma, HNSCC or MM [197]. Interestingly, when stratified by individual RASi use, increased overall survival is observed only with ARB use, and a non-significant increase in overall survival is observed with ACEI use. This study also observes a correlation between ACEI or ARB use and increased progression-free, disease-free and metastasis-free survival [197]. These results are supported by a meta-analyses by Song et al., that similarly demonstrates an association between ACEI or ARB use with increased overall survival and disease-free survival [198]. Another meta-analysis observes no difference in the overall risk of cancer, however, significant variability amongst the results of individual studies has been noted [199].

A meta-analysis of ARB use in lung cancer observes a significant reduction in the risk of lung cancer in those using ARBs for less than five years, but not with ARBs use beyond five years [200]. Meta-analyses of ACEI or ARB use in breast cancer noted no differences in overall survival, disease-free survival [201, 202], cancer-specific mortality, risk of recurrence [202], or overall risk of cancer, although treatment of less than five years or greater than 10 years associated with a reduced risk of cancer [202]. Other meta-analyses of ACEI and ARB use have demonstrated a reduced risk of mortality in renal cancer [203] and risk of developing colorectal cancer [204, 205], with a 6% reduction in risk per year of ACEI or ARB use [204]. ACEI or ARB use has also been associated with increased overall survival, cancer-specific survival and recurrence-free survival, but no difference in progression-free survival or disease-free survival in pancreatic, liver, stomach, colorectal and esophageal cancers [206]. There have been no observed associations between ACEI or ARB use and the risk of MM [207], cSCC or BCC [208]; however, there is significant variability in the results between individual studies. Interestingly, in those at high risk of skin cancers, ACEI or ARB use is associated with a reduced risk of developing cSCCs and BCC [208].

Meta-analyses of β-blocker use in cancer also demonstrate mixed findings. In a meta-analysis of 20,898 patients, β-blocker use is associated with an increased overall survival and disease-free survival in breast, ovarian and non-small cell lung cancers [209]. However, these findings are not reproduced in a meta-analysis by Yap et al. which observes no difference in disease-free survival, overall survival

or cancer recurrence [210]. Meta-analyses of β-blocker use in breast cancer have demonstrated improved overall survival [201] and reduced cancer-specific deaths [211], but no difference in the risk of recurrence [211, 212] or overall mortality [211, 212]. Other meta-analyses of β-blocker use have observed increased overall survival in those with stage III lung cancer and those lung cancer not requiring surgical resection [213], reduced cancer-specific mortality [214] but no difference in overall mortality in prostate cancer [214] and ovarian cancer [215], and no difference in cancer-specific deaths or progression-free survival in ovarian cancer [215]. In skin cancer, β-blocker use is associated with no difference in risk of cSCC, but increased risk of BCC [205] and MM [207].

The mixed findings of meta-analyses could be attributed to the variable results of individual cohort studies included in these analyses, and may be further explained by the variations in type, dose and duration of RASi use and confounding factors of the individual study populations. While epidemiological studies have observed a largely cancer-protective role of RASi, stronger evidence of the role of RAS in cancer is underscored by in vitro and in vivo studies. Gold-standard randomized control clinical trials are warranted to confirm the observed benefit of RASi.

In Vitro and In Vivo Evidence of the Role of the Renin-Angiotensin System in Cancer

Considering the positive association between RASi use and cancer outcomes demonstrated by epidemiological studies, numerous studies have investigated the effects of RAS inhibition using in vitro and xenograft models of cancer (Table 15.2).

ACE Inhibitors

The cancer-protective effect of ACEIs have been strongly demonstrated in studies using in vitro and xenograft models. In a xenograft model of renal clear cell cancer, treatment with the ACEI captopril, results in a significant reduction in tumor growth compared to non-treated controls [59]. Similarly, in a murine lung cancer model, captopril treatment leads to a significant reduction in tumor growth but a non-significant reduction in axillary nodal involvement [60]. In murine models of liver metastases from colorectal cancer, captopril-treated mice exhibit reduced number, size and percentage of liver affected by metastases compared to non-treated controls [62, 63]. These mice also display areas of microvascular filling defects, suggesting tumor disruption [63]. Up-regulated expression of AT_1R and ACE compared to adjacent non-metastatic liver tissue and the healthy liver of control mice has been further observed in a murine model of liver metastases from colorectal cancer, with captopril treatment resulting in a significant reduction of expression of angiotensinogen, ACE, and AT_1R [64]. These findings allude to differences in the local RAS in cancer

Table 15.2 Studies on the effect of inhibition of the renin-angiotensin system on cancer using in vitro assays and xenograft models

Authors and year	Type(s) of cancer	Type of model	Class of RASi	Type of RASi	Outcomes of RASi use
Chen et al. [37]	Esophageal cancer	In vitro assay	ACEI and ARB	ACEI: captopril (up to 30mM) ARB: Losartan (up to 2000μM), irbesartan (up to 800μM)	ARB and ACEI treatment: Reduced VEGF expression and cellular proliferation in dose-dependent manner
Hii et al. [59]	Renal cell cancer	In vitro assay and xenograft model	ACEI	Captopril (19mg/kg/day, 94mg/kg/day)	Reduced tumor growth, tumor volume, and cellular proliferation
Miyajima et al. [61]	Renal cancer pulmonary metastasis	Xenograft model	ARB	Candesartan (10mg/kg/day)	Reduced pulmonary metastatic nodules, angiogenesis, and expression of TGF-β and VEGF
Wysocki et al. [216]	Renal cell cancer, sarcoma	Xenograft model	ACEI	Captopril (25mg/kg/day, 60mg/kg/day)	Decreased survival in a dose-dependent manner
Koh et al. [62]	Colorectal cancer liver metastasis	Xenograft model	ACEI	Captopril (250mg/kg/day)	Reduced liver metastasis and tumor volume and increased apoptosis No difference in cellular proliferation
Neo et al. [63]	Colorectal cancer liver metastasis	Xenograft model	ACEI and ARB	ACEI: Captopril (250mg/kg/day, 375mg/kg/day, 750mg/kg/day) ARB: Irbesartan (50mg/kg/day)	Captopril treatment: Reduced liver metastasis, tumor volume and angiogenesis at all doses No difference in overall survival Irbesartan treatment: Reduced liver metastasis, tumor volume and angiogenesis No difference in overall survival
Neo et al. [64]	Colorectal cancer liver metastasis	Xenograft model	ACEI	Captopril (750mg/kg/day)	Increased expression of ACE and AT_1R but not AT_2R in tumors Reduced liver metastasis and expression of ACE and AT_1R but not AT_2R
Attoub et al. [60]	Lung cancer	In vitro assay and xenograft model	ACEI	Captopril (2.8mg/mouse)	Reduced tumor volume and cellular proliferation Increased apoptosis No difference in axillary lymph node metastasis and angiogenesis
Zhang et al. [65]	Lung cancer	In vitro assay	ARB	Telmisartan (up to 20μM)	Reduced cellular proliferation, cell invasion and migration Increased apoptosis
Rasheduzzaman et al. [66]	Lung cancer	In vitro assay	ARB	Candesartan (up to 10μM)	Increased apoptosis

(continued)

Table 15.2 (continued)

Authors and year	Type(s) of cancer	Type of model	Class of RASi	Type of RASi	Outcomes of RASi use
Fendrich et al. [67]	Pancreatic cancer	Xenograft model	ACEI and COXI	ACEI: Enalapril 0.6mg/kg/day COXI: Aspirin 20mg/kg/day	AT_1R and NF-κB are expressed in pancreatic cancer but not normal pancreatic tissue Enalapril or aspirin treatment: Reduced progression, ductal involvement, and expression of VEGF and NF-κB genes Combined treatment with enalapril and aspirin: Reduced progression and ductal involvement
Noguchi et al. [68]	Pancreatic cancer	Xenograft model	ARB	Losartan (30mg/kg/day)	Losartan treatment only: Reduced tumor development, angiogenesis and VEGF expression Losartan treatment with adjuvant chemotherapy (Gentamicibe): Reduced tumor development, angiogenesis and VEGF expression compared to losartan treatment alone
Fendrich et a. [69]	Pancreatic neuroendocrine tumors	Xenograft model	ACEI and COXI	ACEI: Enalapril (0.6mg/kg/day) COXI: Aspirin (20mg/kg/day)	AT_1R and NF-κB are expressed in tumor cells Enalapril treatment or aspirin treatment: Inhibited expression of AT_1R and NF-κB Increased survival Reduced tumor volume, tumor number and cellular proliferation
Yoshiji et al. [70]	Hepatocellular cancer	In vitro assay and xenograft model	ACEI and ARB	In vitro assay: ACEI: Perindopril (20mg/kg/day), Temocapril (20mg/kg/day), Captopril (20mg/kg/day) ARB: Candesartan (20mg/kg/day), Losartan (20mg/kg/day) Xenograft model: ACEI: Perindopril (2mg/kg/day)	ACEI treatment: Reduced VEGF expression, angiogenesis and tumor growth, with perindopril exerting the strongest effect on suppression of tumor growth with no dose-dependent relationship ARB treatment: No effect on tumor growth
Yoshiji et al. [71]	Hepatocellular cancer	Xenograft model	ACEI	Perindopril (2mg/kg/day)	Reduced number and size of pre-neoplastic lesions, angiogenesis and VEGF expression
Noguchi et al. [72]	Hepatocellular cancer	In vitro assay and senograft model	ACEI	Perindopril (2mg/kg/day)	Reduced tumor growth, angiogenesis and VEGF expression No effect on cellular proliferation

(continued)

Table 15.2 (continued)

Authors and year	Type(s) of cancer	Type of model	Class of RASi	Type of RASi	Outcomes of RASi use
Isobe et al. [73]	Uterine leiomyoma	In vitro assay	ARB	Telmisartan (10μM), losartan (10μM)	AT_1R and AT_2R are expressed by leiomyoma cells, and ATII induces cellular proliferation in dose-dependent manner ARB treatment: Reduced ATII-induced cellular proliferation, but no effect on cellular proliferation in cells not treated with ATII AT_2R agonist treatment: No effect of ATII induced cellular proliferation
Funao et al. [74]	Prostate cancer	In vitro assay	ARB	Telmisartan (100μM)	Reduced cellular proliferation and increased apoptosis
Renziehausen et al. [75]	Malignant melanoma	In vitro assay	ARB	Losartan (dose not specified)	Increased serum levels of AT_1R in patients with metastatic melanoma compared to those without metastatic disease and healthy controls ATII treatment: Increased cellular proliferation via AT_2R in a dose-dependent manner and AT_2R blockade leads to inhibition of cellular proliferation and angiogenesis Losartan treatment: Increased cellular proliferation
Chen et al. [76]	Breast cancer	In vitro assay and xenograft model	ARB	In vitro assay: Losartan (10μmol, 100μmol) Xenograft model: Candesartan (5mg/kg/day, 10mg/kg/day)	AT_1R is expressed in breast cancer cells with positive correlation to axillary node metastasis ATII treatment: Increased cellular proliferation and expression of VEGF Losartan treatment: In vitro reduced cellular proliferation and VEGF expression when losartan administered to ATII treated cells, but no difference in cellular proliferation compared to non-treated controls Candesartan treatment: Reduced tumor volume and VEGF expression in vivo

(continued)

Table 15.2 (continued)

Authors and year	Type(s) of cancer	Type of model	Class of RASi	Type of RASi	Outcomes of RASi use
Kinoshita et al. [77]	Gastric cancer	In vitro assay and xenograft model	ARB	In vitro assay: Candesartan (up to 10μM) Xenograft model: Candesartan (10mg/kg/day)	Increased ATII concentration in tumor tissue and increased expression of AT_1R in gastric cancer cells compared to normal gastric mucosa ATII treatment: Increased NF-κB activity, expression of anti-apoptotic gene (survivin) and cellular proliferation in dose-dependent manner Candesartan treatment: Inhibited ATII induced cellular proliferation Increased survival in animal model compared to non-treated controls
Amaya et al. [78]	Angiosarcoma	In vitro assay and xenograft model	β-blocker	In vitro assay Non-selective: Propranolol (10μM, 25μM, 50μM, 100μM, 200μM) β_1-selective: Atenolol (up to100μM), esmolol (up to 100μM) $\beta_1$2-selective: Butoxaine (up to 100μM), ICI-118551 (up to 100μM) Xenograft model: Propranolol (15mg/kg/day)	Non-selective β-blocker (propranolol) treatment: Reduced cell viability in dose-dependent manner in vitro Increased expression of apoptotic proteins and reduced expression of mitogenic proteins in xenografts β_1- and β_2- selective blocker treatment: Reduced cell viability, but less effective compared to propranolol
Stiles et al. [79]	Hemangioendothelioma, angiosarcoma	In vitro assay and xenograft model	β-blocker	In vitro assay: Propranolol (up to 200μM) Xenograft model: Propranolol (10mg/kg/2 days)	Reduced cellular proliferation in vitro in a dose-dependent manner Increased apoptosis in vitro at dose of 100μM or greater Reduced cellular migration in vitro Reduced tumor size in vivo
Wrobel et al. [80]	Malignant melanoma	In vitro assay and xenograft model	β-blocker	In vitro assay: Propranolol (100μM), metoprolol (100μM) Xenograft model: Propranolol (0.5g/L in drinking water, approx. 1.7mg/day)	Propranolol treatment: Inhibited cellular proliferation, increased cell death in vitro Decreased tumor growth and volume, cellular proliferation and angiogenesis, and increased apoptosis in vivo Metoprolol treatment: Inhibited cellular proliferation, but to a lesser extent than propranolol No effect on cell death

(continued)

Table 15.2 (continued)

Authors and year	Type(s) of cancer	Type of model	Class of RASi	Type of RASi	Outcomes of RASi use
Zhou et al. [81]	Malignant melanoma	In vitro assay and xenograft model	β-blocker	In vitro assay: Propranolol (up to 400μM) Xenograft model: Propranolol (2mg/kg/day, 10mg/kg/day)	Reduced cell viability in a time-dependent and dose-dependent manner, increased apoptosis and cell cycle arrest at G0/G1 and S phase, reduced cellular proliferation, in vitro Reduced tumor volume and cellular proliferation and increased apoptosis, in vivo
Wrobel et al. [82]	Malignant melanoma	Xenograft model	β-blocker	Propranolol (0.5g/L in drinking water)	Delayed time until occurrence of primary tumor and metastasis Increased survival and B-cell, cytotoxic CD8 + T cell and NK cell infiltration to the primary tumors Reduced angiogenesis
Maccari et al. [83]	Malignant melanoma	In vitro assay and xenograft model	β-blocker	In vitro assay: Propranolol (up to 10μM), Isoprenaline (up to 10μM) Xenograft model: Propranolol (10mg/kg/day to 40mg/kg/day)	Propranolol treatment: No effect on cellular proliferation in vitro Reduced tumor growth in bi-phasic dose-response manner in vivo, with maximum inhibition at 20mg/kg/day Reduced arteriogenesis at 40mg/kg/day but not at low doses of 10mg/kg/day and 20mg/kg/day in vivo No effect on capillary density in vivo No effect on expression of inflammatory cytokines or vascular growth factors Isoprenaline treatment: No effect on cellular proliferation in vitro
Zhang et al. [84]	Pancreatic cancer	In vitro assay	β-blocker	Non-selective β-blocker: Propranolol (up to 200μM) β_1-selective blocker: Metoprolol (100μM) β_2-selective blocker: Butoxamine (100μM)	Propranolol treatment: Reduced cellular proliferation, and increased apoptosis compared to metoprolol, but less than butoxamine

(continued)

Table 15.2 (continued)

Authors and year	Type(s) of cancer	Type of model	Class of RASi	Type of RASi	Outcomes of RASi use
Guo et al. [85]	Pancreatic cancer	In vitro assay	β-blocker	Propranolol (1μM)	Reduced invasion of pancreatic cancer cells, expression of MMP-2 and MMP-9 and VEGF
Al-Wadei et al. [86]	Pancreatic cancer	Xenograft model	β-blocker	Propranolol (0.3mg/100mg/kg 5 times weekly)	Increased survival and reduced risk of developing pancreatic cancer and VEGF expression
Pasquier et al. [87]	Breast cancer, glioblastoma, neuroblastoma, non-small cell lung cancer	In vitro assay and xenograft model	β-blocker	In vitro assay: Propranolol (up to 100μM) Xenograft model: Propranolol (10mg/kg/day five times weekly)	Propranolol treatment: Reduced cellular proliferation and angiogenesis in a dose-dependent manner in all cell lines (breast cancer, lung cancer, neuroblastoma, glioblastoma) in vitro Propranolol treatment with chemotherapy (5-fluorouracil or paclitaxel): Increased anti-proliferative and anti-angiogenic effects of chemotherapy in vitro Reduced angiogenesis and increases survival in vivo
Pasquier et al. [88]	Neuroblastoma	In vitro assay and xenograft model	α/β-blocker and β-blocker	In vitro assay: Mixed α/β blocker: carvedilol (up to 100μM), labetalol (up to 100μM) Non-selective: Propranolol (up to 100μM) β_1-selective: nebivolol (up to 100μM), atenolol (up to 100μM), metoprolol (up to 100μM) β_2-selective: butoxamine (up to 100μM) Xenograft model: Mixed α/β blocker: carvedilol (10mg/kg/day 5 times weekly) Non-selective: propranolol (50mg/kg/day 5 times weekly) β_1 - selective: nebivolol (10mg/kg/day 5 times weekly)	Reduced cellular proliferation with potent effects from carvedilol and nebivolol, intermediate effects from propranolol and labetalol, and weak effects from atenolol, metoprolol and butoxamine in vitro Reduced angiogenesis with most potent effects from carvedilol, nebivolol and propranolol in vitro. No effect on angiogenesis with atenolol, labetalol, metoprolol and butoxamine Carvedilol, nebvilol or propranolol treatment: Potentiated anti-angiogenic and anti-proliferative effects of chemotherapy in vitro No effect on tumor regression and delayed tumor progression in vivo Induced tumor regression, delayed tumor regrowth, and increased survival when used in combination with chemotherapy

(continued)

Table 15.2 (continued)

Authors and year	Type(s) of cancer	Type of model	Class of RASi	Type of RASi	Outcomes of RASi use
Liao et al. [89]	Gastric cancer	In vitro assay	β-blocker	Propranolol (up to 300μM)	Reduced cellular proliferation and increased apoptosis in dose-dependent manner Promoted cell cycle arrest in G0/G1 phase and in G2/M phase Reduced expression of NF-κB, COX2 and VEGF
Coelho et al. [90]	Colorectal cancer	In vitro assay	β-blocker	Mixed α/β blocker: Carvedilol (up to 100μM) Non-selective: Propranolol (up to 100μM) β_1-selective: atenolol (up to 100μM) β_2-selective: ICI 118,551 (up to 100μM)	Propranolol, carvedilol and atenolol but not ICI 118,55 reduced cellular proliferation
Wong et al. [91]	Colorectal cancer	Xenograft model	β-blocker	β_1-selective: Atenolol (5mg/kg/day or 10mg/kg/day three times weekly) β_2-selecxtive: ICI 118,551 (5mg/kg/day or 10mg/kg/day three times weekly)	Nicotine treatment increased levels of β_1- and β_2- adrenoceptors and COX2, and promoted tumor growth Atenolol or ICI 118,551 treatment: Reduced COX-2 and VEGF expression and angiogenesis
Masur et al. [92]	Colorectal cancer	In vitro assay	β-blocker	Non-selective: Propranolol (10μM) β_1-selective: Atenolol (10μM)	Propranolol treatment: Reduced cellular migration Atenolol treatment: No effect on cellular migration
Montoya et al. [93]	Breast cancer	In vitro assay	β-blocker	Mixed α/β blocker: Carvedilol (up to 200μM) Non-selective: Propranolol (up to 200μM) β_1-selective: Atenolol (up to 200μM), nebivolol (up to 200μM), esmolol (up to 200μM) β_2-selective: Butoxamine (up to 200μM)	Propranolol treatment: Reduced cellular proliferation β_1- or β_2- blocker treatment: Less effective at reducing cellular proliferation compared to non-selective β-blockers
Xie et al. [94]	Breast cancer	In vitro assay	β-blocker	Non-selective: Propranolol (up to 400μM) β_1-selective: Metoprolol (up to 400μM) β_2-selective: ICI 118,551(up to 400μM)	Propranolol and ICI 118,551 treatment: Reduced cell viability and COX-2 expression Increased apoptosis and cell cycle arrest at G0/G1 with reduced cells in S-phase Metoprolol treatment: No effect on cell viability
Wilson et al. [95]	Breast cancer	In vitro assay	β-blocker	Propranolol (up to 50μM)	Reduced cellular migration
Wang et al. [96]	Liver cancer	In vitro assay	β-blocker	Propranolol (up to 160μM)	Reduced cellular proliferation in a dose-dependent and time-dependent manner Increased apoptosis

(continued)

Table 15.2 (continued)

Authors and year	Type(s) of cancer	Type of model	Class of RASi	Type of RASi	Outcomes of RASi use
Schuller et al. [97]	Lung cancer	Xenograft model	β-blocker	Propranolol (1mg/100g three times daily)	Reduced incidence of lung cancer and number of tumors
Brohée et al. [98]	Prostate cancer	In vitro assay and xenograft model	β-blocker	In vitro assay: Propranolol (100μM) Xenograft model: Propranolol (10mg/kg/day)	Reduced cellular proliferation and increased apoptosis in vitro Reduced metastasis in vivo Reduced tumor growth and volume only when used in conjunction with a glycolysis inhibitor in vivo
Palm et al. [99]	Prostate cancer	In vitro assay and xenograft model	β-blocker	In vitro assay: Propranolol (10μM) Xenograft model: Propranolol (1μM/100g over 2 weeks)	No effect on cellular proliferation and migration in vitro Reduced lymph node metastases in vivo

ACE, angiotensin converting enzyme; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; AT1R, ATII, angiotensin II; angiotensin II receptor 1; AT2R, angiotensin II receptor 2; COXI, COX inhibitor; EGFR, epidermal growth factor receptor; MMP, matrix metalloproteinases; NF-κB, nuclear factor kappa B; RAS, renin-angiotensin system; RASi, RAS inhibitor; TGF-β, transforming growth factor-β; VEGF, vascular endothelial growth factor

tissue compared to normal tissues. Administration of the ACEI enalapril results in a significant reduction of invasive pancreatic cancer compared to non-treated controls [67]. Similarly, in a murine model of pancreatic neuroendocrine neoplasms, enalapril treatment significantly reduces tumor size and number, increased survival and inhibited expression of AT_1R and NF-kB [69]. In hepatocellular cancer, perindopril-treated xenografts display reduced tumor growth and suppression of angiogenesis and vascular endothelial growth factor (VEGF) expression [70–72]. One xenograft model observes that treatment with temocapril, captopril, and to a greater extent, perindopril, significantly inhibits tumor growth compared to controls [70]. In contrast to these studies, a murine model of renal cell cancer observes a decrease in survival following captopril treatment, in a dose-dependent manner [216].

Angiotensin Receptor Blockers

In a murine model of pulmonary metastasis arising from renal cell cancer, administration of ARB candesartan significantly reduces the number of pulmonary metastases, transforming growth factor-β (TGF-β) expression, and angiogenesis [61]. Other in vitro and animal studies have reported protective effects of ARBs, including increased apoptosis in lung cancer [66] and prostate cancer [74], reduced cellular proliferation in lung [65], prostate [74], pancreatic [68], gastric [77] and esophageal [37] cancers and leiomyoma [73]; inhibition of angiogenesis in breast [76], pancreatic [68] and esophageal [37] cancers, and liver metastases from colorectal cancer [63]; decreased invasion in lung cancer [65]; decreased metastasis in colorectal cancer [63]; and increased survival in a murine gastric cancer model [77]. In vitro studies of gastric cancer [77] and leiomyoma [73] observe an increase in expression of AT_1R in tumor tissues compared to normal tissues, with a dose-dependent increase in cellular proliferation in response to ATII, and subsequent reduced cellular proliferation in response to addition of ARBs. Interestingly, this effect is not reproduced upon inhibition of AT_2R [73], suggesting that these pathological effects may be mediated via AT_1R. Contrary to these results, the use of the ARB losartan increases tumor cell proliferation in an in vitro model of MM [75].

β-Blockers

The effects of β-blockers in cancer have been investigated using in vitro and xenograft models. In both in vitro and animal models of angiosarcoma, propranolol use leads to increased apoptosis [78, 79] and reduced cellular proliferation in a dose-dependent manner [79], with propranolol, a non-selective β-blocker, showing superior efficacy in reducing cell viability compared to β_1- and β_2-selective inhibitors [78]. In vitro models of MM show that β-blocker use reduces cellular proliferation [80, 81] and increases apoptosis [80, 81] and cell cycle arrest [81]. In murine models of MM, β-blocker use inhibits tumor growth [82, 83], increases survival [82] and apoptosis [80, 81], and reduces metastasis [82], cellular proliferation [80–82] and angiogenesis

[80, 82]. In pancreatic cancer, propranolol treated cells exhibit and display reduced cellular proliferation [84], invasion [85] and VEGF expression [85, 86], and increased apoptosis [84]. In a hamster model, propranolol use reduces the incidence of pancreatic cancer [86]. Treatment with propranolol reduces cellular proliferation and angiogenesis in breast cancer [87] and neuroblastoma [88], in vitro. Furthermore, in a murine model of neuroblastoma, treatment of β-blockers leads to increased survival and tumor regression, and delayed tumor recurrence, when used in conjunction with the chemotherapy vincristine [88]. In a gastric cancer study, propranolol treatment reduces cellular proliferation and increase apoptosis in a dose-dependent manner in vitro, and potentiates the efficacy of radiotherapy, with increased cell death, apoptosis, and reduced expression of NF-κB, COX-2 and VEGF, compared to cells treated with radiotherapy alone [89]. β-blocker use significantly reduces cellular proliferation [90], angiogenesis, VEGF expression [91], and cellular migration [92] in in vitro models of colorectal cancer. In in vitro models of breast cancer, propranolol treatment reduces cellular proliferation [93], viability [94] and migration [95], and increased apoptosis [94]. Treatment of liver cancer cell lines with propranolol reduces cellular proliferation and increases apoptosis [96], and reduces the incidence and number of tumors in a xenograft model of lung cancer [97]. Prostate cancer cell lines treated with propranolol display reduced cellular proliferation, increased apoptosis [98], and reduced metastasis a murine xenograft model [99].

While most in vitro and animal studies have demonstrated a protective effect of RASi use in cancer, a small group of studies have observed a non-protective and potentially harmful effect. A study on MM demonstrates no effect on cellular proliferation, although the dosage of propranolol used is lower compared with other studies [83]. Other factors contributing to a variation in outcomes include small sample sizes, difference in the type of β-blockers used, and duration of treatment. Furthermore, in vitro and animal models may not fully recapitulate the complexities of the TME, which plays a vital role in tumor development.

Cancer Stem Cells Express Components of the Renin-Angiotensin System

Epidemiological studies have largely demonstrated a cancer-protective effect of the RASi, which is further supported by the findings from in vitro and murine models of cancer. The expression of components of the RAS has been demonstrated in numerous cancer types such as HNSCC [100, 101] including OCSCC [102, 103], metastatic MM [104, 105], glioblastoma [106], liver metastases from colorectal cancer [107], and renal clear cell cancer [108]. Components of the RAS have been shown to co-localize with markers used to identify CSCs, including OCT4, SOX2 and NANOG [100–109].

While there is documented co-localization of components of the RAS with markers of CSCs, there is a paucity of literature investigating the functional role of the

RAS on the CSC population. Expression of ACE, ACE2, pro-renin receptor (PRR) and AT_2R has been observed on colorectal adenocarcinoma tissues, with localization of these components of the RAS to the phenotypic CSCs [110]. Colorectal adenocarcinoma derived primary cell lines treated with RASi including propranolol, timolol and losartan result in reduced expression of pluripotency markers [110]. These findings suggest that components of the RAS are expressed by the CSC population, with RAS inhibition directly targeting this CSC population. Further functional studies on the effects of RAS modulation on CSCs, using xenograft and in vitro models such as tumorspheres and organoid systems, are warranted. Organoids generated from patient-specific CSCs, may provide novel three-dimensional models that offer the possibility of modeling the pathogenesis of cancer and could be used to investigate responses to RASi and other therapies [217].

"Bypass Loops" of the Renin-Angiotension System and Convergent Signaling Pathways

Enzymes such as cathepsins B, D and G, and chymase provide alternative pathways for biosynthesis of ATII, that enable the RAS to continue functioning despite blockade by traditional RASi [111]. Additionally, various signaling pathways converge onto the RAS, including the upstream Wnt/β-catenin signaling pathway, and the downstream NOX, ROS, NF-κB COX-2 [111] signaling pathways. Effective modulation of the RAS would necessitate inhibition of these "bypass loops" and converging signaling pathways.

Cathepsins B, D and G

Cathepsin B, a lysosomal cysteine protease, catalyzes the conversion of pro-renin into active renin [218]. Cathepsin D, an aspartic protease, shares significant homology with renin, and like renin, promotes conversion of angiotensinogen into ATI [218]. Cathepsin G, a serine lysosomal protease, increases ATII yield either from ATI or directly from angiotensinogen [219]. Consequently, cathepsins B, D and G provide alternative pathways for ATII biosynthesis by bypassing components of the classical RAS. Expression of cathepsins B, D and G have been observed in numerous cancer types including glioblastoma [220], oral tongue SCC [221], primary [222] and metastatic [223] HNSCC, MM [224] and liver metastases from colon adenocarcinoma [225]. Cathepsins B and D are localized to the phenotypic CSC population, while cathepsin G expression is co-localized to chymase+ phenotypic mast cells. The expression of cathepsins B, D and G is significant, as blockade of the RAS

by traditional RASi may be circumvented by these enzymes that represent alternative pathways for ATII generation. This may provide an explanation for the variable cancer outcomes associated with RASi use observed in epidemiological studies.

Pro-Renin Receptor and Wnt/β-Catenin

PRR is a transmembrane protein that is abundant in normal brain, liver, cardiac, pancreatic, placental and renal tissues [226]. Expression of PRR is increased in numerous human malignancies including glioma [227], pancreatic adenocarcinoma [228, 229], colorectal [230, 231], breast [227], renal [232], urothelial [233] and prostate [234] cancers.

PRR has an established role in mediating the conversion of pro-renin to renin [226]. PRR also acts as an oncoprotein in the Wnt/β-catenin signaling pathway which is initiated through the binding of the Wnt ligands to Frizzled in the Wnt receptor complex [226]. This results in the phosphorylation of lipoprotein receptor-related protein 5/6 (LRP5/6) preventing degradation of β-catenin and facilitating the translocation of β-catenin to the nucleus, where it binds the T-cell factor/lymphoid enhancing factor transcription factor complex to promote transcription of various oncogenes including c-MYC, cyclin D1 and AXIN2 [226]. The Wnt/β-catenin signaling pathway also plays an integral role in regulating stem cells and embryonic development, differentiation of pluripotent stem cells, and maintenance of stem cells within their tissue microenvironment [235]. Actions of PRR in the Wnt/β-catenin pathway may function synergistically with actions mediated by V-ATPase, which is expressed in the cell membranes of numerous cancer cells, and plays an essential role in maintaining the acidity of the surrounding environment which is crucial for LRP5/6 phosphorylation [218, 226]. PRR is also involved in the mitogen-activated protein kinase (MAPK)/extracellular signal-related kinase (ERK) signaling pathway, resulting in up-regulation of ERK1/2 which promotes cellular proliferation and induces production of TGF-β, influencing tumorigenesis and metastasis [218, 226].

NADPH Oxidase

NOX enzymes are a family of enzymes involved in the generation of ROS in response to numerous cellular stimuli. NOX1 and NOX4 are of relevance in the RAS, with expression up-regulated by ATII in in vitro and xenograft models [236]. NOX activation results in the generation of ROS, which are involved in Ras oncogene effects including cellular proliferation and differentiation, and up-regulation of VEGF and thus tumor angiogenesis [237]. In oral SCC cell lines, inhibition of NOX1 has been associated with significant reduction in the generation of ROS, increased apoptosis, and reduced cell viability [238]. Overexpression of NOX1 and NOX4 has

also been observed in colorectal cancer, with expression of NOX4 being associated with markers of poor prognosis and increased tumor angiogenesis, EMT, and Notch signaling [239]. Overexpression of the NOX family has been observed in gastric [240], prostate [241] and lung [242] cancers.

NF-κB Signaling

The NF-κB family of transcription factors are involved in inflammation and the immune system. There is emerging evidence for the role of NF-κB in cancer and the RAS, through its role as a convergent signaling pathway. NF-κB is activated by various growth factors, cytokines, TNF receptors, insulin growth factor receptor, and signaling pathways such as Ras/MAPK and phosphatidylinositol 3-kinase (PI3K)/Akt [243]. NF-κB can be activated via the canonical 'classical' NF-κB pathway, or the alternative pathway. Canonical signaling is activated by pro-inflammatory cytokines including interleukin IL-6 and IL-8, and viral infections, resulting in phosphorylation of the IkappaB (IkB) proteins which bind NF-κB dimers, causing subsequent ubiquitin-mediated degradation of IkB proteins, allowing translocation of NF-κB to the nucleus to exert its effects as a transcription factor. The alternative signaling pathway is initiated by TNF activating IKKα, subsequent phosphorylation of p100 and generation of mature p52, which migrates to the nucleus to act as a transcription factor [243] for numerous cytokines including IL-6 and IL-8 [118]. NF-κB may play a role in cancer through its anti-apoptotic effects and promotion of proliferation and cellular migration [243]. In human breast cancer cells, ATII treatment activates NF-κB signaling, enhances the expression of matrix metalloproteinases (MMPs)-2-9 and cancer cell migration, which is reduced by inhibition of NF-κB, AT_1R, and PI3K [244].

COX-2

COX-2 is a pro-inflammatory enzyme involved in the synthesis of prostaglandins (PGs) from arachidonic acid [245]. The COX family consists of two isoforms, COX-1 which is expressed abundantly by most tissues [245], and COX-2 which is absent in most tissues and is up-regulated by pro-inflammatory cytokines including IL-1β, IL-2 and TNF-α, growth factors including epidermal growth factor (EGF) and platelet-derived growth factor, NF-κB, and oncogenes [245–247]. Up-regulation of COX-2 is observed in numerous malignancies including HNSCC [248], lung [249, 250], colon [251, 252], prostate [253], breast [254] and skin [255] cancers. Dysregulated expression of COX-2 results in accumulation of its downstream product PGE_2 [256].

COX-2 has been shown to be related to the RAS, with increased expression in high renin states, including during the use of ACEIs [257] COX-2 increases renin via a positive feedback loop involving PGE_2, and thus increases ATII levels [258].

Mitogenic properties of COX-2 are likely to be mediated by PGE_2 through evasion of apoptosis [245, 256], increased cellular proliferation and growth [256], promotion of angiogenesis via induction and production of pro-angiogenic factors including VEGF, NOS, IL-6 and IL-8 [245, 256], tumor invasion and metastasis through up-regulation of metalloproteinases [247] and EGFR-P13K-Akt pathway [245], and suppression of immune responses through the down-regulated activation of T cells and B cells [245, 256].

COX enzymes are non-selectively inhibited by aspirin and certain non-steroidal anti-inflammatory drugs including ibuprofen and diclofenac, while COX-2 is selectively inhibited by celecoxib [245]. COX-2 inhibition in colorectal cancer is associated with reduced cellular proliferation [259, 260] and migration [252] and increased apoptosis in vitro [259–261], and reduced tumor metastasis in vivo [260]. In breast cancer, COX-2 inhibition has been demonstrated to inhibit cell migration and invasion in vitro [262], and reduce metastasis in vivo [263].

The identification of the redundancies within the RAS through the presence of cathepsins B, D and G constituting the "bypass loops", and convergent signaling pathways involving the up-stream Wnt/β-catenin pathway and the down-stream PRR, NOX, NF-kB and COX-2 signaling cascade, offers a better appreciation of the complexities within the RAS. This may explain the variable effects of single point inhibition of the RAS using different classes of traditional RASi as demonstrated in epidemiological studies and confirmed in investigations using in vitro and xenograft models of cancer. Thus, effective modulation of the RAS may require concurrent inhibition of the RAS, its "bypass loops" and convergent signaling pathways at multiple points (Fig. 15.3).

The Renin-Angiotensin System and the Tumor Microenvironment

Cancer is a complex system consisting of CSCs and cancer cells interacting intimately with the surrounding complex and dynamic TME comprised of cellular and non-cellular components. Immune cells form a fundamental component of the TME and function dichotomously both by suppressing tumor growth or promoting tumorigenesis in pro-inflammatory states as demonstrated in cervical, colorectal and hepatocellular cancer [264]. Other cellular components of the TME include CSCs and their downstream progenies, MSCs, stromal cells, cancer-associated fibroblasts, pericytes, adipocytes, vascular and lymphatic endothelial cells, and non-cancer cells [112, 113]. Non-cellular elements of the TME include collagen, laminin, hyaluronan and fibronectin [114]. The TME is further regulated by the local paracrine RAS [115–117] which coordinates intercellular communication, and regulates the complex ECM to support tumor growth.

Cancer Stem Cells and the Tumor Microenvironment

Residing within the TME, and central to tumorigenesis, are CSCs, which are orchestrated by extrinsic signals from the surrounding TME [118]. The communication of CSCs with the surrounding TME is multi-directional, and while the TME can influence CSC stemness, growth, differentiation and metastasis, CSCs can reciprocally communicate with the surrounding TME to maintain a favorable tumorigenic *niche*. Communication between CSCs and the surrounding MSCs, cancer-associated fibroblasts, endothelial cells and immune cells are mediated through various growth factors and cytokine networks [118]. This communication further regulates the plasticity of the CSC hierarchy [265]. Numerous stem cell regulatory pathways including the Wnt/β-catenin, Notch, NF-κB, PI3K, and Jak/STAT pathways can be disrupted by signals from the surrounding TME [118].

The relationship between CSCs and the surrounding TME has been demonstrated in in vitro and animal models of cancer. In breast cancer, CSCs maintain a synergistic relationship with the surrounding MSCs through cytokine communication networks [119, 120] involving C–C motif chemokine ligand 5 (CCL5) [119], and CXC chemokine ligand 7 (CXCL7), IL6 and IL8 [120] expression. Concurrent xenografting of MSCs and breast cancer cells results in up-regulated secretion of CCL5 by MSCs which enhances the invasive and metastatic potential of breast cancer cells, which is not reproduced when MSCs are xenografted into areas separate from breast cancer cells [119]. A positive feedback loop involving CXCL7 and IL-6 further promotes tumor invasion and metastasis [120]. IL-8, secreted by MSCs and immune cells, stimulates self-renewal and metastasis of breast CSCs, with inhibition of IL-8 reducing tumorigenicity and metastasis [266]. Secretion of IL-6 and IL-8 is regulated through the NF-κB pathway, with IL-6 engaging in a positive feedback loop with NF-κB, thus sustaining a chronic inflammatory state within cancer [118]. This highlights the synergistic role of breast CSCs and cells within the surrounding TME and the paracrine role of cytokines in the local TME in sustaining tumor growth and dissemination. In brain tumors, including glioblastoma, CSCs are sustained by co-culture with endothelial cells in vitro, and tumor growth is enhanced by concurrent xenografting of endothelial cells and reduced by subsequent treatment with anti-angiogenic drugs [267]. While the TME is integral in regulating CSCs, this communication is multidirectional, and brain tumor CSCs similarly communicate with cells in the surrounding TME to sustain a favorable tumorigenic *niche* [268]. Similarly, in skin cancers, CSCs rely on the surrounding vascular *niche* and VEGF signaling, which sustains CSC stemness, with inhibition of VEGF receptor 2 resulting in tumor regression through reduction of angiogenesis and depletion of the CSC pool [269].

The Renin-Angiotensin System and the Tumor Microenvironment

In conjunction with cytokine communication networks, the RAS plays a crucial role in regulating the TME. Components of the RAS are expressed in various cancers, and are expressed by cells within the TME including phenotypic CSCs [111], immune cells such as monocytes, macrophages, neutrophils, dendritic cells and T cells, endothelial cells and fibroblasts [117]. The role of a local paracrine RAS in the TME has been proposed in lung cancer [270], breast cancer [271] and glioblastoma [116]. ATII-mediated signaling via AT_1R in these cells facilitates pathological processes including promoting cellular proliferation, migration and invasion, metastasis, inflammation, angiogenesis, and inhibition of apoptosis [117]. Within the TME, the ATII/AT_1R axis acts through several mechanisms to maintain an overall immunosuppressive *milieu.* Firstly, ATII, via its interaction with AT_1R, promotes up-regulation of pro-fibrotic pathways, resulting in the deposition of a dense desmoplastic stroma. This forms a barrier limiting the physical migration of T-cells and reduces vascular perfusion within the tumor, thus contributing to and maintaining a hypoxic and acidic *niche* which further retards the function of immune cells [117]. Secondly, the ATII/AT_1R axis promotes up-regulation of VEGF-mediated angiogenesis, with the formation of aberrantly hyperpermeable vessels, which in conjunction with ATII-mediated vasoconstriction of vessels further enhances the hypoxic, acidic, immunosuppressive conditions of the TME, and also contributes to the dissemination of tumors cells [117]. Thirdly, the ATII/AT_1R axis promotes the production of pro-inflammatory cytokines including, monocyte chemoattractant protein (MCP)-1, macrophage colony-stimulating factor, COX-2 and C-reactive protein, some of which exert immunosuppressive effects, thus further impairing the recruitment of immune cells [117].

The paracrine RAS is involved in the maintenance of an immunosuppressive environment within the TME, with subsequent inhibition of the RAS, resulting in immunomodulatory properties. In a murine model of colorectal cancer, ARB administration reduces expression of immunosuppressive factors including IL-6, IL-10, and VEGF and up-regulates expression of CD8+ cytotoxic T lymphocytes [272]. These findings are supported by other murine models of breast cancer and colorectal cancer, with ARB administration reversing the immunosuppressive properties mediated by ATII within the TME, with increased expression of CD8+ T cells, and reduced expression of T regulatory cells (Tregs) [273]. In this model angiotensinogen silencing results in up-regulated expression of immune-activating cytokines, and reduced expression of immunosuppressive cytokines, reversing the immunosuppressive actions mediated by the presence of a hypoxic TME [273]. In a xenograft model of pancreatic adenocarcinoma, ARB administration attenuates the expression of TGF-β, resulting in reduced fibrosis, the number of Tregs, invasion, metastasis and vascular permeability [274].

CSCs and tumorigenesis are governed by a complex and dynamic TME (Fig. 15.4) with multi-directional communication between CSCs and the surrounding TME

through cytokine networks, disruptions in stem cell regulatory pathways, and signaling via the RAS to sustain an overall tumorigenic *niche*. It is important to recognize that CSCs do not function as a static entity and are subject to dynamic intercellular communication with the surrounding TME, which hitherto cannot be fully recapitulated by existing in vitro and animal models of cancer. Insights into this complex and dynamic TME will provide an improved understanding of tumor development and progression and treatment failure, and underscore effective modulation of CSCs in the treatment of cancer.

Therapeutic Targetting of the Renin-Angiotension System

Modulation of the Renin-Angiotensin System in the Treatment of Infantile Hemangioma

Infantile hemangioma (IH), the most common tumor of infancy affecting up to 10% of infants, is characterized by rapid post-natal proliferation followed by spontaneous involution. Approximately 15% of IH are problematic and require intervention during infancy due to threat to function such as airway and visual obstruction, tissue distortion or ulceration [275, 276]. Historically, the first-line treatment of problematic proliferating IH was high-dose systemic corticosteroids [277], with interferon and vincristine being used for refractory cases [278], all of which are associated with significant side-effects.

The treatment of IH was revolutionized following the serendipitous discovery of accelerated involution of IH induced by the non-selective β-blocker propranolol [279] and the selective β_1-blocker acebutolol [280] by two independent French groups in 2008. Propranolol is now the mainstay treatment of problematic proliferating IH [275, 281], However, interaction with the β_2-adrenergic receptor due to its non-selective nature mediates side effects including bronchial hyperactivity and hypoglycemia, and due to its lipophilic properties, propranolol can cross the blood brain barrier and cause intolerable side effects of agitation and sleep disturbance [275]. Many studies have investigated the efficacy of other β-blockers, including non-selective β-blockers such as nadolol, selective β_1-blockers such as atenolol and acebutalol, local administration of β-blockers by intralesional propranolol injections, and topical propranolol and timolol in the treatment of IH [275] (Fig. 15.5). Similarly, the ACEI captopril has been shown to be effective for the treatment of IH [282–284] (Fig. 15.6). While these alternative treatments show early promise, these studies are limited by the small sample sizes and there is significant scope for further studies, particularly in the form of randomized control trials, to explore the safety and efficacy of these therapeutic alternatives to propranolol.

The demonstration of a phenotypic hemogenic endothelium [285] that expresses ACE, AT_2R [177], and PRR [286] provides a plausible explanation for the

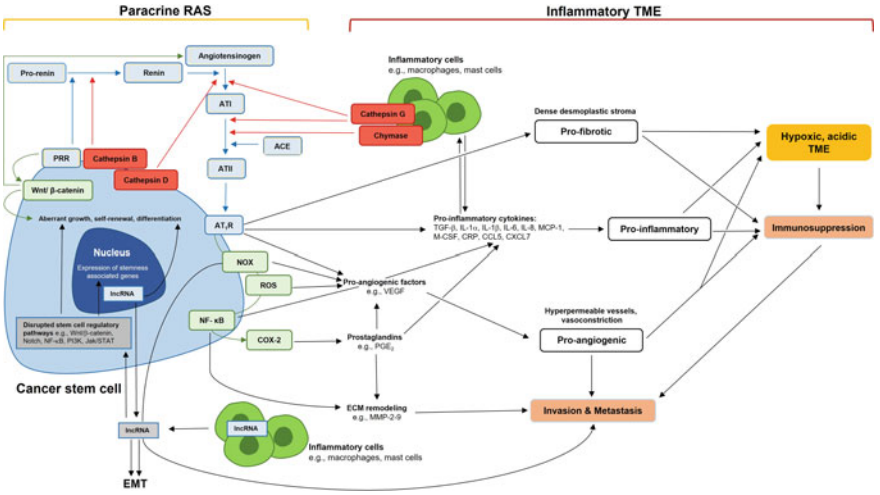

Fig. 15.4 A proposed model demonstrating the interaction between cancer stem cells (CSCs), the local paracrine renin-angiotensin system (RAS), and the tumor microenvironment (TME). Central to tumorigenesis is the CSCs, which are imbued with pluripotency and self-renewal capacity, aberrant growth, and differentiation. There is multi-directional communication between CSCs and the surrounding TME, to create an environment favorable for tumorigenesis. Communication between CSCs and the surrounding TME can be mediated through local paracrine RAS signaling. Angiotensin II (ATII) can be generated through the RAS (*blue arrows*) and exerts its actions via ATII receptor 1 (AT_1R), with downstream pro-fibrotic, pro-angiogenic, pro-angiogenic effects. AT_1R and pro-renin receptor (PRR) are expressed by CSCs. Cathepsins B and D, expressed by CSCs, and cathepsin G and chymase on inflammatory cells, form alternative pathways of ATII biosynthesis, thus acting as "bypass loops" (*red arrows*) of the RAS. Other signaling pathways (*green arrows*) converge on the RAS including the upstream Wnt/β-catenin pathway, which can be activated by PRR, and the downstream NADPH oxidase (NOX), reactive oxygen species (ROS), nuclear factor-kappa B (NF-κB), cyclooxygenase-2 (COX-2) pathways. NF-κB promotes generation of pro-inflammatory cytokines, and matrix metalloproteinases (MMP). COX-2 facilitates synthesis of prostaglandin E_2 which exerts pro-inflammatory and pro-angiogenic effects, and supports tumor invasion and metastasis through the up-regulation of MMPs. The pro-fibrotic, pro-inflammatory, and pro-angiogenic actions of the RAS within the TME, results in the formation of a hypoxic and acidic TME which contributes to immunosuppression and supports aberrant CSC growth, invasion, loco-regional recurrence and metastasis. Communication between CSCs and cells within the TME may also be facilitated through expression of long non-coding RNAs (lncRNAs), which may contribute to disruption of stem cell regulatory pathways, treatment resistance, promotion of epithelial-to-mesenchymal transition (EMT), invasion and metastasis. ATI, angiotensin I; ACE, angiotensin-converting enzyme; ncRNA, long non-coding RNA; NOX, NADPH oxidase; VEGF, vascular endothelial growth factor; PGE_2, prostaglandin E_2; TGF-β, transforming growth factor-β; IL-1α, interleukin-1α; IL-1β, interleukin-1β; IL-6, interleukin-6; IL-8, interleukin-8; MCP-1, monocyte chemoattractant protein-1; M-CSF, macrophage colony-stimulating factor; CRP, C-reactive protein; CCL5, C–C motif chemokine ligand 5; CXCL7, CXC chemokine ligand 7

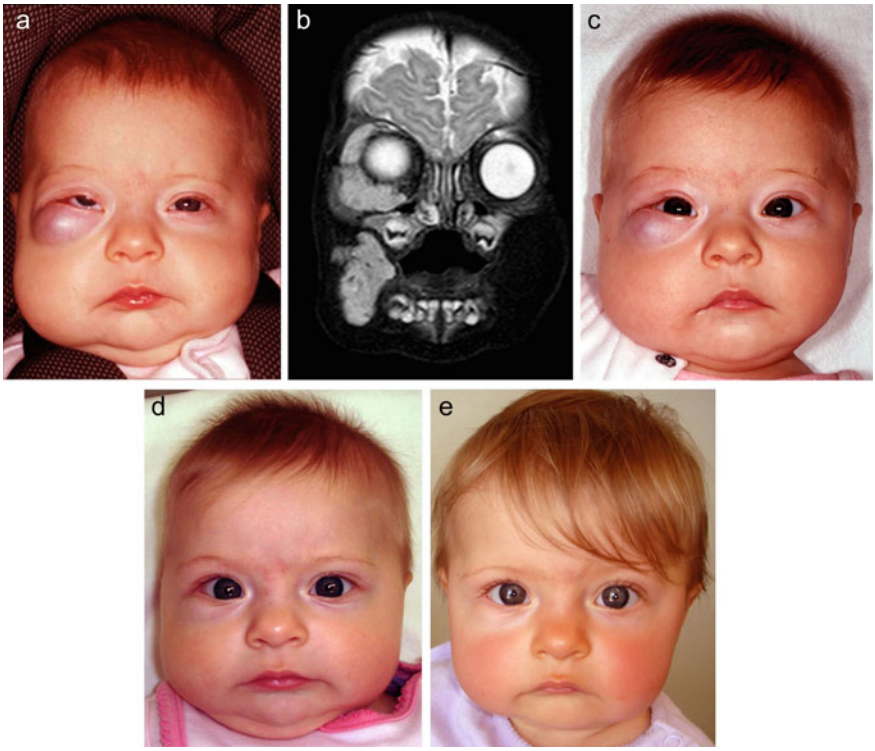

Fig. 15.5 A 4-month-old girl presented with a rapidly growing infantile hemangioma on the right cheek, lower lid and orbit causing ocular dystopia (**a**), as seen on a T_2-weighted magnetic resonance imaging scan (**b**). Accelerated involution of the lesion with equalization of the globes **c** 7 days, **d** 4 weeks and **e** 5 months following institution of propranolol therapy at 2 mg/kg/day. *Reproduced with permission from Plastic and Reconstructive Surgery* [278]

programmed biologic behavior and accelerated involution of IH induced by β-blockers and ACEIs [275, 278, 287]. ATII promotes the generation of blast-like structures from IH-derived cells, in a dose-dependent manner in vitro [177], likely through actions mediated by interaction of ATII with AT_2R [288]. Furthermore, reduced serum levels of renin, ACE and ATII are observed in IH patients treated with surgical excision and propranolol [289]. Expression of cathepsins B and D has also been demonstrated in proliferating IH, localized to the ESC-like population on the endothelium and the interstitium [290].

Propranolol formulations are comprised of a racemic mixture of S-propranolol, the isomer with potent β-adrenergic antagonistic properties, and R-propranolol, which does not exhibit activity against β-adrenergic receptors [291, 292]. The effects of propranolol may be mediated by the action of R-propranolol, through a β-adrenergic independent pathway via inhibition of SOX18 [292]. Interestingly in a study of colorectal adenocarcinoma, propranolol in its racemic form, and R-isomer and S-isomer forms are all capable at reducing cellular metabolism [110]. Thus, propranolol

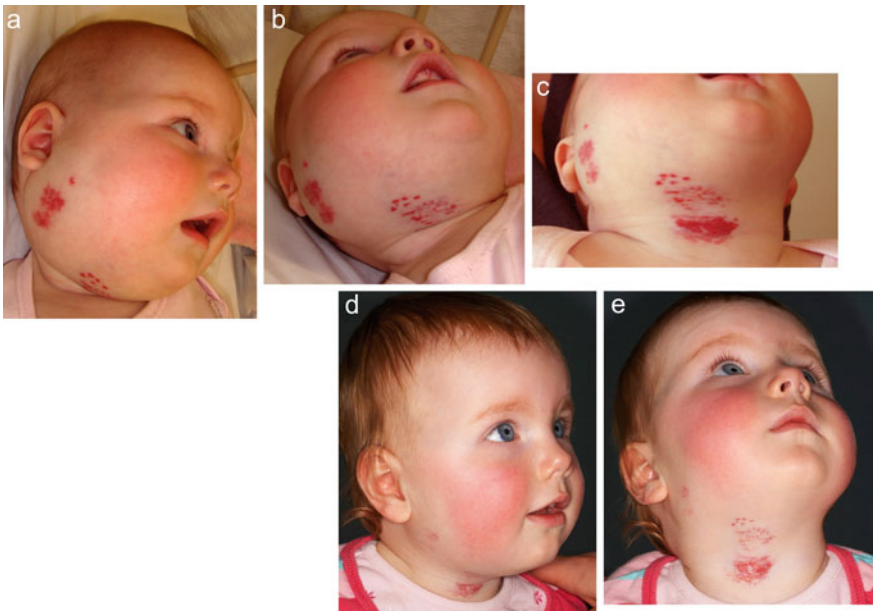

Fig. 15.6 A 22-week-old girl with a 7 × 10 cm proliferating infantile hemangioma in the right cervicofacial area causing significant tissue distortion before (**a**, **b**), 3 weeks (**c**) and 6 months (**d**, **e**) following administration of captopril at 1.5 mg/kg/day. *Reproduced with permission from the British Journal of Dermatology* [282]

may also function independently of its role as a non-selective β-blocker, and instead directly target the phenotypic stem cell populations.

Future studies should focus on elucidating the mechanism by which propranolol and other β-blockers exert their effects on IH, and the possibility of harnessing the beneficial features of propranolol through its R-propranolol isoform, while mitigating the risk of β-adrenergic effects. This may provide improved insight into the biology and thus effective treatment of IH, other vascular anomalies, and cancer.

Modulation of the Renin-Angiotensin System, Its "Bypass Loops" and Convergent Signaling Pathways

CSCs remains an elusive target in the treatment of cancer. There is an increasing body of evidence demonstrating the presence of a local paracrine RAS, with components of the RAS localized to the CSC population. The effectiveness of inhibition of the RAS with traditional RASi can be circumvented by the presence of (1) "bypass loops" consisting of enzymes such as cathepsins B, D and G that provide alternative pathways for ATII biosynthesis despite blockade of the classical RAS, and (2) convergent

signaling pathways such as the upstream Wnt/β-catenin signaling pathway, and the downstream NOX, ROS, NF-kB and COX-2 signaling pathways.

While epidemiological studies and studies based on in vitro and animal models on RASi use and cancer have largely shown a protective role of ACEIs, ARBs and β-blockers, a small number of studies show no such benefit or a harmful effect of RASi. Possible explanations for this discrepancy include study design and confounding factors such as the dose, duration and specific type of RASi used, smoking status, comorbidities, concurrent medications, stage and type of cancer, sample size and the duration of follow-up. The use of single agents such as ACEIs, ARBs or β-blockers, inhibits the RAS at single points, and this blockade may be mitigated by the presence of "bypass loops" and convergent signaling pathways, and may further explain the variable results reported in the epidemiological studies. Effective modulation of CSCs may require simultaneous blockade of the RAS, its "bypass loops" and convergent signaling pathways (Fig. 15.3).

Clinical Trials on the Use of Renin-Angiotensin System Inhibitors in Cancer

While in vitro and animal studies support a protective role of RASi in cancer as observed in epidemiological studies, there is a paucity of clinical trials investigating the effects of RASi in human cancers (Table 15.3). Most are phase I clinical trials aimed at assessing the safety of repurposing these RASi for the treatment of cancer, whereas phase II clinical trials are required to assess the efficacy of these treatments for cancer.

ACE Inhibitors and Angiotensin Receptor Blockers

A prospective study using combination therapy consisting of captopril, marimstat and fragmin on advanced cancer refractory to conventional treatment reports a partial reduction in tumor size in 2% of patients, and stable disease in 8% of individuals, as well as anti-angiogenic effects [121]. In hepatocellular cancer, combination therapy of perindopril with either branched chain amino acids [123] or vitamin K [122] results in a significant reduction in tumor recurrence and serum VEGF levels, suggesting that treatment may exert inhibitory actions on angiogenesis [122, 123]. Interestingly, monotherapy with perindopril alone does not result in significant inhibition of angiogenesis in either study [122, 123]. In prostate cancer, treatment with captopril [125] or candesartan [124] leads to a reduction of prostate specific antigens levels, although observations are not made on survival period, recurrence, or disease progression in these studies. In epidemiological studies, candesartan use is associated with longer progression-free survival and overall survival in patients with prostate cancer [34],

Table 15.3 Clinical trials on the effect of renin-angiotensin system inhibitors on cancer

Authors and year	Type of study	Cancer type(s)	Sample size	Treatment	Outcomes of treatment
Jones et al. [121]	Phase I clinical trial	All types	50	Combination therapy: captopril (50mg BD), marimastat (10mg BD), fragmin (200units/kg/day)	Possible anti-angiogenesis effects 2% partial response to treatment, 8% stable disease, and 80% progressive disease
Yoshiji et al. [122]	Randomized control trial	Hepatocellular cancer	100	ACEI only: perindopril (4mg/day) Vitamin K only: menatetrenone (45mg/day) Combination therapy: ACEI (perindopril) and vitamin K (menatetrenone)	Monotherapy with perindopril or menatetrenone: No difference in recurrence or serum VEGF levels or survival Combination therapy with perindopril and menatetrenone: Reduced recurrence and serum VEGF levels but no difference in survival
Yoshiji et al. [123]	Randomized control trial	Hepatocellular cancer	110	ACEI only: perindopril (4mg/day) Branched chain amino acid only: livact (12g/day) Combination therapy: perindopril and livact	Monotherapy with perindopril or livact: No difference in recurrence or serum VEGF levels Combination therapy with perindopril and livact: Reduced recurrence and serum VEGF levels
Uemura et al. [124]	Single-center prospective trial	Prostate cancer	23	ARB: candesartan (8mg OD)	26.1% immediate reduction in PSA levels, 8.7% stable PSA levels, and 52.2% improved performance status
Ronquist et al. [125]	Single-center prospective trial	Prostate cancer	62	ACEI: captopril (12.5mg BD)	Reduced PSA levels in all patients Time to elevation of PSA levels significantly reduced with captopril compared to controls
Nakai et al. [126]	Phase I clinical trial	Pancreatic cancer	14	ARB: candesartan (dose-escalation 4mg, 8mg, 16mg to maximum of 32mg OD)	16mg maximum tolerated dose 79% disease progression and 14% treatment discontinued due to symptomatic hypotension
Nakai et al. [127]	Phase II clinical trial	Pancreatic cancer	35	ARB: candesartan (8mg or 16mg OD)	0% complete response, 11.4% partial response, and 51.4% stable disease Increased progression-free survival with candesartan 16mg OD compared to 8mg OD

(continued)

Table 15.3 (continued)

Authors and year	Type of study	Cancer type(s)	Sample size	Treatment	Outcomes of treatment
De Giorgi et al. [128]	Non-randomized prospective trial	Malignant melanoma	53	β-blocker: propranolol (80mg OD)	Reduced risk of recurrence and increased disease-free survival
Gandhi et al. [129]	Phase I clinical trial	Malignant melanoma	9	Combination therapy: pembrolizumab (200mg IV every 3 weeks), propranolol (up to 30mg BD)	Propranolol treatment: At 10mg BD: 33.3% stable disease, 66.7% partial response At 20mg BD: 66.7% partial response, 33.3% progressive disease At 30mg BD: 100% partial response
Hiller et al. [130]	Randomized control trial	Breast cancer	60	β-blocker: propranolol (40mg BD for 3 days and increased to 80mg BD if tolerated) (short-term peri-operatively for 7 days pre-operatively, and discontinued 3 days post-operatively)	Up-regulated expression of epithelial-related genes Down-regulated expression of mesenchymal genes, EMT-related transcription factors (Snail/Slug), pro-inflammatory transcription factors (NF-κB/Rel and AP-1) Increased recruitment of inflammatory cells
Jang et al. [131]	Randomized control trial	Ovarian cancer	16	β-blocker: propranolol (40mg OD) (short-term peri-operatively commenced 2 days pre-operatively and discontinued 3 days post-operatively)	Reduced peri-operative CA125 levels compared to controls No difference in disease-free survival
Ramondetta et al. [132]	Single-center prospective trial	Ovarian cancer	26	Combination therapy: propranolol (up to 40mg BD), carboplatin and paclitaxel ($150\text{-}175 mg/m^2$ every 21 days or $60\text{-}80 mg/m^2$ weekly)	88.5% improved overall quality of life, anxiety and depression Decreased expression of pro-inflammatory transcription factors Reduced monocyte expression Increased CD8 + T lymphocytes
Haldar et al. [133]	Randomized control trial	Colorectal cancer	34	Combination COX-2 inhibitor and β-blocker: etodolac (400mg BD), propranolol (up to 80mg BD) (short-term peri-operative use of 20mg BD for 5 days prior to surgery, 80mg BD on surgery day, 40mg BD for 7 days post-operation, followed by 20mg BD for further 7 days)	Down-regulated expression of mesenchymal-related genes Up-regulated expression of genes with no polarization toward epithelial or mesenchymal phenotypes Reduced, but non-significant, risk of metastasis

(continued)

Table 15.3 (continued)

Authors and year	Type of study	Cancer type(s)	Sample size	Treatment	Outcomes of treatment
Knight et al. [134]	Randomized control trial	Multiple myeloma	25	Combination autologous hematopoietic cell transplant and β-blocker: propranolol (up to 40mg BD) (commenced 7 days prior to transplant, and discontinued 4 weeks following transplant)	Down-regulated expression of genes derived from differentiated CD33 + myeloid progenitors Up-regulated expression of genes derived from CD34 + stem cell / early progenitor cells Reduced activity of pro-inflammatory transcription factors
Kast et al. [135]	Treatment protocol	Glioblastoma	10	Combination therapy: aprepitant (80mg BD), artesunate (50mg BD), auranofin (3mg BD), captopril (50mg BD), celecoxib (400mg BD), disulfiram (250mg BD), itraconazole (200mg BD), ritonavir (400mg BD), sertraline (100mg BD)	Final results of clinical trial pending To date 3 of 10 patients remain progression-free at 63, 54 and 52 months following tumor recurrence
O'Rawe et al. [136]	Phase I clinical trial	Glioblastoma	17	Combination therapy: propranolol (up to 80mg BD), aliskerin (150mg OD), cilazapril (up to 5mg OD), celecoxib (200mg OD), curcumin (1000mg BD), aspirin (100mg OD), metformin (up to 500mg BD)	Increase in overall survival by 5.3 months although not statistically significant 5.9% reduced tumor volume 5.9% unchanged tumor volume 41.2% increased tumor volume

ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BD, twice daily; OD, once daily; PSA, prostate specific antigen

with phase I clinical trials confirming that candesartan can be safely used in individuals at a recommended dose of 16 mg/day, although there is no comparison of secondary outcomes including overall survival, progression-free survival and 1-year survival rates to those not taking candesartan [126]. A subsequent phase II clinical trial demonstrates that candesartan use does not significantly prolong overall survival or progression-free survival of advanced pancreatic cancer, and interestingly, those taking a higher dose of candesartan at 16 mg have significantly longer progression-free survival and median overall survival, compared to those taking 8 mg daily, although this is not statistically significant [127].

β-Blockers

The use of propranolol for the treatment of MM has been investigated in several clinical trials. A single-center, non-blinded, prospective trial on the use of propranolol in MM shows a significant reduction in tumor recurrence and longer disease-free survival, but no significant effect on overall survival [128]. In a phase I clinical trial of propranolol treatment at up to 30 mg twice daily in conjunction with immunotherapy pembrolizumab leads to stable disease, partial response and disease progression in 11.1%, 77.8% and 11.1% of patients, respectively, at a median follow-up of 15.6 months [129]. A triple blinded placebo-controlled randomized control trial of breast cancer investigating the short-term perioperative use of propranolol reports down-regulation of EMT-related and pro-inflammatory transcription factors, increased recruitment of inflammatory cells [130], and reduced CA125 levels, but no significant difference in disease-free survival at a median follow-up period of 17 months [131]. In ovarian cancer, propranolol administered up to 40 mg twice daily, in conjunction with standard chemotherapy, is associated with a significant improvement in overall quality of life, anxiety and depression as reported by 88.5% of patients, decreased expression of pro-inflammatory transcription factors including NF-κB, and increased expression of CD8+ T lymphocytes [132]. In colorectal cancer, perioperative treatment with a COX-2 inhibitor and propranolol, is associated with a down-regulation of mesenchymal-related genes [133], suggesting a possible role of COX-2 and β-blockers in the inhibition of EMT. While this study is not sufficiently powered to assess the effects of this drug on survival outcomes or recurrence, a non-significant reduction in metastasis is observed in the treatment group compared with the placebo-treated controls at 3-year follow-up [133]. Propranolol use in conjunction with autologous hematopoietic cell transplant for multiple myeloma is associated with up-regulation of genes associated with early stem cells, and down-regulation of genes derived from downstream CD133+ myeloid progenitor cells, with earlier engraftment [134].

Many of these studies are phase I clinical trials with limited sample size, exploring the safety of re-purposing RASi for cancer. While RASi show promise for the treatment of cancer, further robust studies, ideally in the form of randomized control trials, are warranted to explore the direct effect of RAS modulators in human cancers.

Multi-step Inhibition of the Renin-Angiotensin System

Blockade of the RAS using traditional RASi may be circumvented by the presence of redundancy and convergent signaling pathways, thus theoretically, effective inhibition of the RAS requires a multi-step blockade. This is an emerging concept, and at present there are limited studies exploring the concept of combined drug therapy to inhibit the RAS at multiple points.

A phase I clinical trial consisting 17 patients with recurrent glioblastoma following conventional treatment, treated with a combination of seven re-purposed drugs (propranolol, aliskerin, cilazapril, celecoxib, curcumin with piperine, aspirin and metformin) demonstrates a trend towards increased survival by 5.3 months [136]. The encouraging results of this trial warrant further clinical trials on this novel, well-tolerated and cost-effective potential therapeutic option for patients with recurrent glioblastoma, and potentially other cancer types. Treatment of glioblastoma using combination drug therapy has also been explored using the CUSP9* protocol, where nine repurposed drugs (aprepitant, artesunate, auranofin, captopril, celecoxib, disulfiram, itraconazole, ritonavir and sertraline) are used simultaneously. While the final results of this trial are pending, at present three of the 10 patients remain progression-free 63, 54 and 52 months following tumor recurrence [135].

The concept of combination drug therapy to inhibit CSCs via modulation of the RAS, components with redundant activities which constitute “bypass loops”, and convergent signaling pathways through the re-purposing of exisitng, low-cost and safe drugs warrants further investigation.

Conclusion

The hierarchical model of cancer attributes tumorigenesis to CSCs sitting atop the cellular hierarchy in cancer. These CSCs possess the capacity for self-renewal, pluripotency, and differentiation into differentiated cancer cells, giving rise to intratumoral heterogeneity. This hierarchy is dynamic due to cancer cell plasticity, by which differentiated cancer cells are capable of reverting to a pluripotent state. The phenotype and function of these CSCs are modulated by the surrounding TME which is influenced by cytokine signaling, the RAS, and the immune system, and work in concert to promote tumor recurrence, progression and metastasis, and treatment failure. CSCs have been identified in numerous cancer types, although they remain an elusive upstream therapeutic target in the treatment of cancer.

There is compelling evidence for the presence of a local paracrine RAS in numerous cancer types, with proposed CSCs co-expressing pluripotency markers and components of the RAS. Therefore, CSCs may be a potential novel therapeutic target by modulation of the RAS. Current RASi, including ACEIs, ARBs and β-blockers, are commonly used for the treatment of cardiovascular conditions. Epidemiological studies demonstrate a largely positive correlation between RASi use and reduced cancer risk and improved cancer outcomes. These findings are

overwhelmingly supported by investigations using in vitro and xenograft models of cancer. To date there are only a small number of clinical trials, mostly in phase I and with small sample sizes, using RASi in the treatment of cancer.

The effectiveness of traditional RASi on the paracrine RAS may be circumvented by the presence of "bypass loops" consisting of enzymes such as cathepsins B, D and G, which provide alternative pathways for ATII biosynthesis in addition to the classical RAS, and numerous converging signaling pathways such as the upstream Wnt/β-catenin, and downstream NOX, ROS, NF-κB and COX-2 signaling pathways. This may explain the absence of beneficial effects of RASi use on cancer outcomes in a small number of studies. Cathepsins B and D are co-expressed with phenotypic CSCs expressing pluripotency markers, and cathepsin G is expressed by phenotypic mast cells. Thus, effective modulation of these CSCs via inhibition of the RAS requires additional consideration of the components of the classical RAS, its "bypass loops" and convergent signaling pathways.

Instead of the longstanding pursuit of a 'silver-bullet' solution to cancer, future treatments of cancer should consider targeting CSCs, the proposed origin of cancer, by exploiting key factors that directly and indirectly influence CSCs including the TME, the RAS, its "bypass loops", and convergent signaling pathways. A novel approach to achieve this is by re-purposing an array of low-cost medications with high safety profiles, to inhibit the RAS at multiple steps. This concept warrants further attention and investigation.

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Chapter 16
Renin-Angiotensin System and Cancer: From Laboratory to Clinics

Jinxuan Su, Qiuming Zou, Sijia Li, and Qi Qi

Abstract Renin-Angiotensin System (RAS) is a vital system regulating blood pressure and maintaining sodium homeostasis in the human body. It consists of Angiotensin I (Ang I), Angiotensin II (Ang II), Angiotensin-converting enzyme (ACE), Angiotensin II type 1 receptor (AT1R), and angiotensin II type 2 receptor (AT2R), which functions in both normal and pathological conditions including cancer. Besides, the effectors of RAS are also included, such as Angiotensin-(1-7). This review focuses on the pre-clinical studies and clinical trials assessing the roles of RAS in regulating tumor progression as well as the underlying mechanisms.

Keywords Renin-angiotensin system · Cancer progression · Proliferation · Angiogenesis · Metastasis

Abbreviations

ACE	Angiotensin-converting enzyme
Ang II	Angiotensin II
AT1R	Angiotensin II type 1 receptor
AT2R	Angiotensin II type 2 receptor
Ang (1-7)	Angiotensin 1-7
MASR	MAS Receptor
ACE-2	Angiotensin-converting enzyme 2
VEGF	Vascular endothelial growth factor
EMT	Epithelial mesenchymal transition
MMPs	Matrix metalloproteinases
ECM	Extracellular matrix

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ACE-Is	ACE inhibitors
ARBs	AT1R blockers
EC	Endometrial cancer
PC	Prostate cancer
PTK	Protein tyrosine kinase
ROS	Reactive oxygen species
AMPK	AMP-activated protein kinase
mTOR	Mammalian target of rapamycin
EGFR	Epidermal growth factor receptor
MAPK/STAT	Mitogen-activated protein kinase/signal transducer and activator of transcription
PI3K/AKT	Phosphoinositide 3-kinase/Akt
RCC	Renal cell carcinoma
HCC	Hepatocellular carcinoma
CRC	Colorectal cancer
NSCLC	Non-small cell lung cancer

Introduction

Renin-Angiotensin System (RAS) is a complex systemic hormonal cascade of interacting peptides and enzymes, which plays a vital role in regulating blood pressure and maintaining normal sodium homeostasis of the human body [1]. It contains Angiotensin I (Ang I), Ang II, Ang-(1-7), MAS Receptor (MASR), Angiotensin-converting enzyme (ACE), ACE-2, Angiotensin II type 1 receptor (AT1R), and angiotensin II type 2 receptor (AT2R) [2]. Angiotensinogen (AGT), a precursor peptide related to the family of serine protease inhibitors (serpins), is the unique substrate for the protease renin (EC 3.4.23.15). The hydrolysis of AGT by renin is rate-limiting for the whole system and results in the production of des(Ang I)-AGT and of the vasoinactive peptide Ang I, which is converted to the vasoactive peptides Ang II and Ang III by ACE (EC 3.4.15.1) and aminopeptidase A (EC 3.4.11.7), respectively [3]. Angiotensin-(1-7), converted from Ang II by ACE and ACE2, has been reported to counteract the function of Ang II in many aspects, serving as a biologically active intermediate of the vasodilatory arm of the renin-angiotensin system [4]. RAS plays a pivotal role in the maintenance of normal physiological state in the human body, whose dysregulation has been reported to lead to the onset of various diseases, such as hypertension [5], diabetes [5], stroke [6], chronic obstructive pulmonary disease (COPD) [7], cancer [8], etc.

Cancer is one of the leading causes of death in the world and acquires the malignancy depending on several abilities: constant proliferation signals, sustained angiogenesis, tissue invasion and metastases, and evasion of apoptosis [9]. It has been reported that upregulation of the components of RAS is in close relationship with some types of cancers, such as colorectal cancer [10], prostate cancer [11], renal

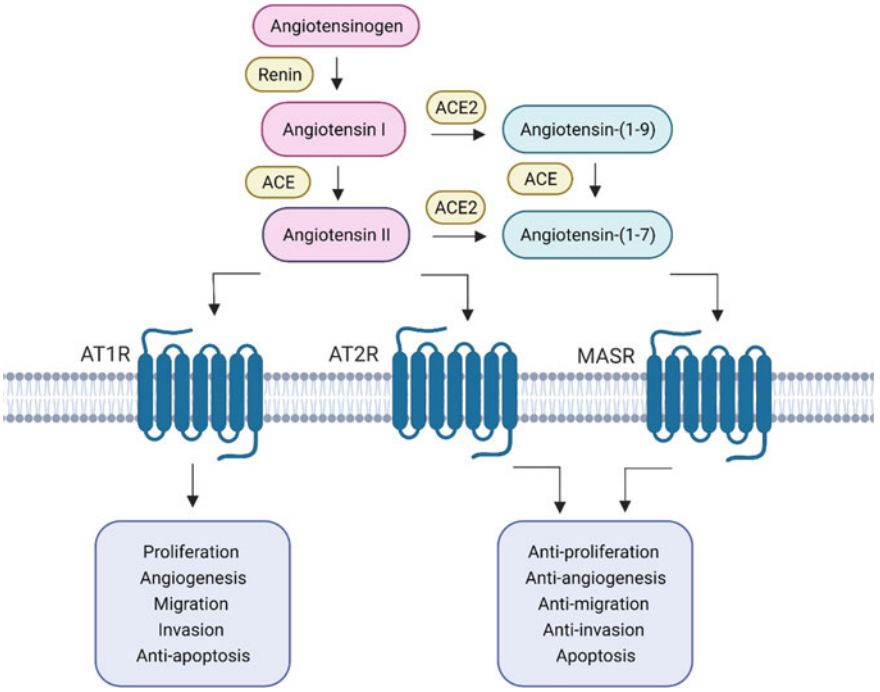

Fig. 16.1 The role of RAS in tumor progression. Main members of RAS include Ang I, Ang II, Ang-(1-7), MASR, ACE, ACE-2, AT1R, and AT2R. Binding of Ang II to AT1R increases angiogenesis, tumor proliferation, migration, invasion, and metastasis. On the contrary, binding of Ang II to AT2R inhibits angiogenesis, cell proliferation, migration, invasion, and metastasis. Binding of Ang-(1-7) with MASR exerts similar anti-tumor effects

clear cell carcinoma [12], and gliomas [13], etc. The importance of RAS and its components in cancer development merits attentions to cancer researchers. Here, this article reviews the role of RAS in tumor progression including cell proliferation, angiogenesis, metastasis, and the potential strategies for cancer treatment through modulation of RAS functions (Fig. 16.1).

RAS and Tumor Progression

RAS and Cell Proliferation

RAS has been first revealed as the regulator in proliferation of tumor cells. Among the members of RAS, Ang II and ACE have a close association with tumor cell proliferation, which are involved in multiple signaling pathways

including AT1R/PI3K/Akt/mTOR, AT1R/Raf/ERK1/2, and MAPK signaling pathways (Fig. 16.2). Insulin resistance induces the secretion of insulin-like growth factor (IGF)-1 in the liver, which promotes cell proliferation and inhibits cell apoptosis through Akt signaling pathway [14]. Besides, IGF-1 also triggers MAPK signaling and promotes mitosis in a variety of cancer cells [15, 16]. Ang II can activate the AT1R-mediated PI3K/Akt/mTOR pathway, promoting the occurrence, survival, and growth of cancer cells [17, 18], which can be blocked by antagonizing AT1R [19]. Ang II is able to upregulate CyclinD1, GSK3β, and downregulate p27 in hepatocellular carcinoma via AT1R-induced activation of PKC and MAPK signaling pathways [20].

The ACE inhibitor elicits outside-in signaling in endothelial cells, enhancing the activity of ACE-associated kinase CK2 and increasing the phosphorylation of the intracellular tail of ACE [21]. Researchers have found ACE activation might control the expression of diverse proteins besides ACE itself. Upon binding to ACE, Ang II internalizes with a faster onset compared to the binding of Ang II to its classical AT1 receptor [22]. Ang II and ACE can form a complex, which translocates to the nucleus through a clathrin-mediated process, and interacts with β3 isoform of PLC, triggering a nuclear Ca^{2+} signal resulting in induced cell proliferation [23, 24]. In melanoma TM-5 cells, Ang II induced cell proliferation through ACE activation, which was confirmed by ACE inhibitor (Lisinopril) or by the silencing of ACE [22].

Different RAS receptors either activate or inactivate various signaling pathways related to cancer development. AT1R activation through binding of Ang II leads to

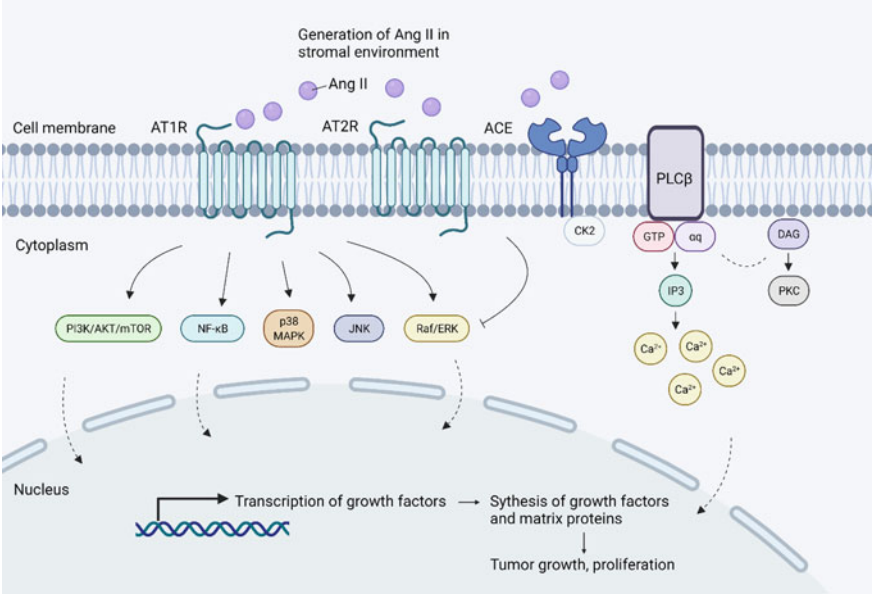

Fig. 16.2 The regulation of RAS in proliferation of tumor cells

activation of PI3K/AKT/mTOR, NF-κB, MAPK, JNK, Ras/ERK pathways. Transcription of growth factors and formation of matrix proteins are then triggered and lead to increased cell proliferation. Ang-(1-7) bind to MASR and is mediated through TGF-β, PAK1/NF-κB/Snail pathways.

RAS and Tumor Angiogenesis

Tumor angiogenesis produced by RAS is mainly induced by vascular endothelial growth factor (VEGF) and its related pathways. As opposed to the systemic RAS, recently, the concept of a localized RAS has been reported, such as in the central nervous system (CNS) [13] and female reproductive organs [25] (Fig. 16.3).

In CNS, the predominant cells expressing AGT are astrocytes [26], whereas renin, another key member of RAS system, is expressed in both astrocytes and neuron cells [27]. In glioblastoma, ACE activity cannot be detected in glioblastoma cells; however, ACE is highly expressed in the aberrant vasculature of human glioblastoma [28]. It was postulated that a complete angiotensin system existed in the brain, independent from the circulatory system and that its role in the regulation of vascular functions was crucial in tumors. In the vasculature, AGT, the unique and specific substrate of renin, is demonstrated to be antiangiogenic [29], while angiotensin peptides, in particular Ang II, have been shown to be proangiogenic and to be involved in vascular growth [3].

The mammalian VEGF family is comprised of five members: VEGFA, VEGFB, VEGFC, VEGFD, and placenta growth factor (PGF). VEGFA is the most functional

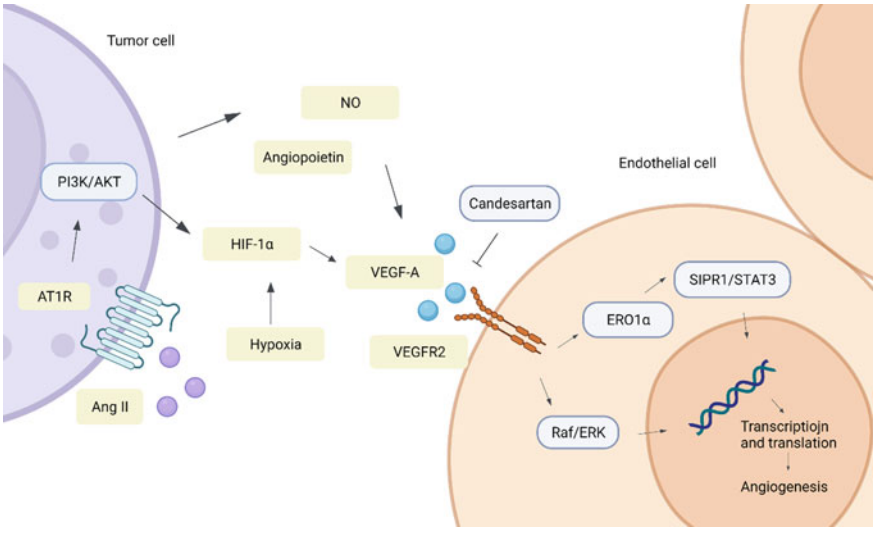

Fig. 16.3 The regulation of RAS in tumor angiogenesis

factor, which exerts angiogenic effects by activating VEGFR2 expressed in endothelial cells [30]. It has been revealed that ACE2 inhibits angiogenesis via suppressing the VEGFA/VEGFR2/ERK pathway in breast cancer [31]. Hypoxia leads to hypoxia-inducible factor 1α (HIF-1α) stabilization, increasing VEGF production in tumor cells. Besides, activation of the PI3K/AKT pathway also increases VEGF secretion in both HIF-1 dependent [32] and independent [33] manners. Moreover, the PI3K/AKT pathway also modulates the expression of other angiogenic factors such as nitric oxide [34] and angiopoietins [35, 36].

In the female reproductive system, activated RAS exists in the uterine endometrium, ovary, and placenta under both physiologic and pathological conditions [25, 37–39]. In ovarian carcinoma, VEGF is induced by Angiotensin II via AT1R, which is significantly correlated with poor prognosis [40]. AT1R upregulation in different cancers performs the function of promoting angiogenesis, contributing to cancer progression [25]. Angiotensin II also stimulated cell proliferation, invasion, and VEGF secretion via AT1R in cervical cancer [41, 42], endometrial cancer [43], choriocarcinoma [20], and ovarian cancer [44]. Besides, the host AT1R pathway supports tumor-associated macrophage infiltration, which results in enhanced VEGF levels [45]. Under neoplastic conditions, ACE inhibitors could inhibit the function of Ang II in angiogenesis. Serving as the most important cytokine affecting angiogenesis, VEGF is in a positive relationship with ERO1α, which promotes angiogenesis through the S1PR1/STAT3/VEGF pathway [46, 47]. Candesartan, an AT1R antagonist, has been reported to inhibit angiogenesis by downregulating AT1R/VEGF pathway [17, 48].

Angiogenesis generated by RAS system is mainly due to the binding of Ang II and AT1R. Activation of the PI3K/AKT pathway increases VEGF secretion in both HIF-1 dependent and independent manners. VEGFA exerts angiogenic effects by activating VEGFR2 expressed in endothelial cells through SIPR1/STAT3 and Raf/ERK pathways.

RAS and Tumor Metastasis

Metastasis is a multi-step process that accelerates tumor spread in the whole body, which starts with proliferation and acquires the escape capability from the primary tumor, then enters into the body circulation system, seeds into adjacent tissue cavities [49]. Growing evidence indicates that components of RAS are involved in tumor metastasis, especially Ang II [49]. Clinical research has also found that the use of renin-angiotensin system inhibitors (RASIs) can reduce cancer metastasis in several different cancer types, including hepatocellular carcinoma (HCC) [50] and bladder cancer [51].

Preliminary research in one murine renal cancer model has found that the blockade of Ang II with ACEI or ARB alone or in combination reduced tumor growth and the number of lung metastases [52]. NF-κB, a multi-regulatory transcription factor,

can upregulate the expression of VEGF and matrix metalloproteinase-9 (MMP-9) genes, and accelerate the occurrence, invasion, and metastasis of liver tumors [53]. RAS inhibitors, such as perindopril, fosinopril, and losartan, could inhibit the activation of NF-κB and further downregulate the levels of VEGF and MMP, thereby inhibiting metastasis [17, 54]. ACE2/Ang-(1-7)/MAS axis is silenced in human breast cancer; its downregulated expression critically promotes breast cancer metastasis to the lungs through activating PAK1/NF-κB/Snail signaling pathway by increasing SOCE-mediated Ca^{2+} influx, leading to decreased E-cadherin expression [55]. MAS receptor antagonist A-779 blocks the ACE2/Ang-(1-7)/Mas axis, leading to anti-metastatic effects [55].

Epithelial-mesenchymal transition (EMT) is one major process in metastasis, which can be indicated by downregulation of E-cadherin and upregulation of Vimentin. In A549 lung cancer cells, overexpression of ACE suppressed metastasis in vivo with increased level of E-cadherin and decreased level of Vimentin both in vitro and in vivo [56]. Dysregulation of TGF-β may contribute to the metastasis and invasiveness of cancerous pancreatic cells in advanced cancers [57]. ACE2 attenuated TGF-β1-mediated EMT in A549 cells. DX600, an inhibitor of ACE2, could reverse the sensitivity to TGF-β1 [56]. Ang II-treatment exacerbated hematogenous cancer metastasis by promoting E-selectin-mediated adhesion of cancer cells to vascular endothelial cells [58]. 20(S)-protopanaxadiol (PPD), the final metabolite of protopanaxadiol-type ginsenosides, effectively prevented Ang II-induced EMT via upregulation of the class III deacetylase sirtuin 1 (SIRT1). Downregulation of SIRT1 was involved in the suppression of Ang II-induced EMT by PPD [59].

Inhibition of the AT1R via angiotensin-converting enzyme inhibitors (ACE-Is) has demonstrated a decrease in solid tumor development and metastasis. In colorectal cancer (CRC) liver metastases, decreased Ang II and increased Ang-(1-7) were detected [60]. Captopril not only inhibits ACE, but also reduces the level of AGT in the host liver, leading to a distinct anti-metastatic effect [60, 61]. A study demonstrated an inverse relationship between the history of hypertension with the prescription of ACE-Is and the risk of distant metastasis in stage 2 CRC patients, implicating ACE-Is as a potential chemo-preventative option [61] (Fig. 16.4).

Angiotensin-(1-7), converted from Ang II by ACE2, binds to MasR and activates TGF-β and PAK1/NF-κB/Snail pathways. Dysregulation of these signalling pathways contributes to the transcription of targeted genes, including E-cadherin, Vimentin, and SIRT1, etc., which regulates tumor metastasis. RAS inhibitors, such as perindopril, fosinopril, and losartan, inhibit the activation of NF-κB. A-779 blocks the ACE2/Ang-(1-7)/Mas axis.

RAS and Cell Apoptosis

Research investigating the relationship between RAS and apoptosis has been focused on the AT2R which plays an opposite role of AT1R, protecting the normal function of RAS. It is reported that AT2R could stimulate apoptosis in various cancer cell

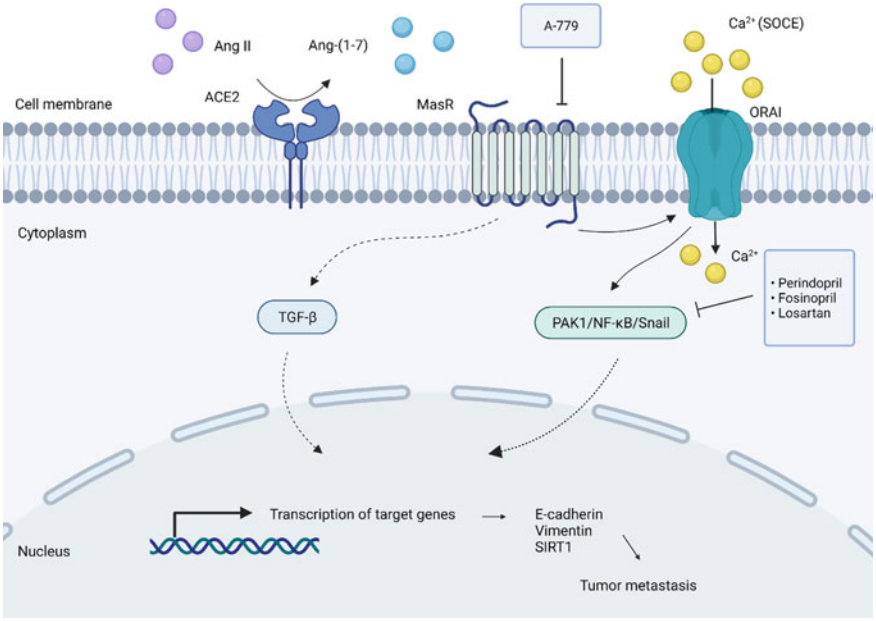

Fig. 16.4 The regulation of RAS in tumor metastasis

lines, including vascular smooth muscle cells, such as cardiomyocytes, endothelial cells, prostate cancer cells, and lung cancer cells [62–67]. Depending on the cell type, AT2R-mediated apoptosis involves distinct biological processes. In INS-1O rat insulinoma cells, overexpression of AT2R induced cleavage of caspase-8, caspase-9, and caspase-3, and decreased Bcl-2, p-AKT, and p-ERK levels [68]. In intestinal epithelial cells, Ang II signals upregulate GATA-6 expression through AT2R, which in turn upregulates the expression of Bax and eventually leads to apoptosis in these cells [63]. Increased apoptosis appears to be caused by INOs upregulation following enhanced AT2R expression in HL-1 cardiomyocytes [64]. Moreover, AT2R signaling stimulates the MAPK tyrosine phosphatase, which inhibits MAPK activation and consequently inactivates Bcl-2 and induces apoptosis in proximal tubular cells [69]. AT2R-mediated apoptosis was mediated by p38 MAPK and downregulation of Gadd45a, TRAIL-R2, and harakiri Bcl-2-interacting protein (HRK) in prostate cancer cells [65, 70]. In HCC cells, researchers found that apoptosis caused by AT2R overexpression is due to the activation of p38 MAPK, phosphorylated c-Jun N-terminal kinase (p-JNK), caspase-8, and caspase-3, and inactivation of pp42/44 MAPK (ERK1/2) [66]. Similarly, AT2R overexpression-induced apoptosis in BCA cells is mediated via an extrinsic cell death signaling pathway that is dependent on activation of p38 MAPK, caspase-8, and caspase-3 and downregulation of ERK/MAPK [71]. AT2R overexpression also leads to upregulation of 2 apoptosis-related genes (BCL2A1, TNFSF25) and downregulation of 8 apoptosis-related genes

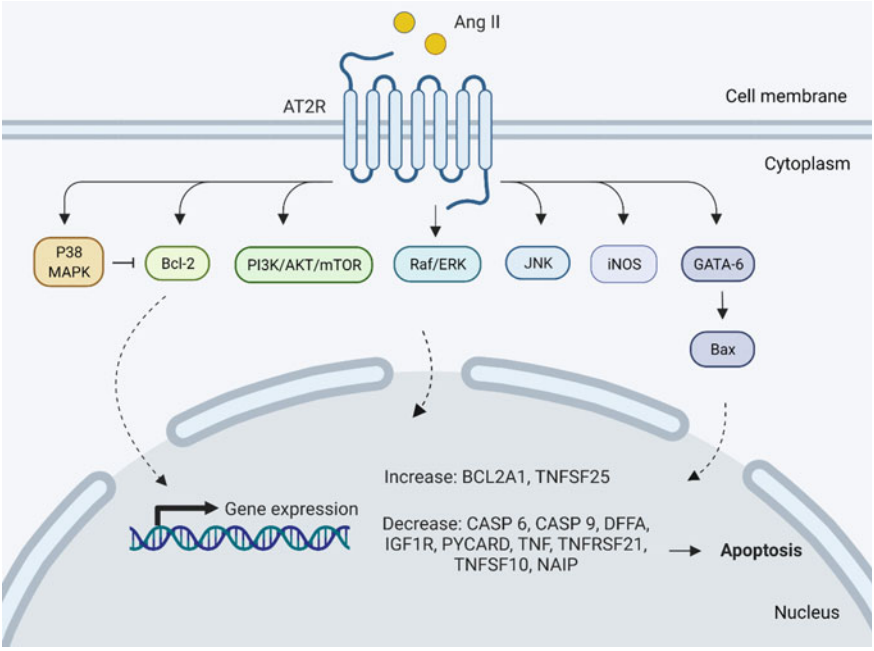

Fig. 16.5 The regulation of RAS in cell apoptosis

(CASP 6, CASP 9, DFFA, IGF1R, PYCARD, TNF, TNFRSF21, TNFSF10, NAIP) in transduced EJ cells [71] (Fig. 16.5).

Ang II binds to the AT2R, which activates P38 MAPK, GATA-6, INOS, PI3K/AKT/mTOR, and Ras/ERK pathways. Signals in the cytoplasm are then transported into the nucleus to upregulate anti-apoptotic genes and downregulate pro-apoptotic genes, leading to apoptosis.

Angiotensin-(1-7) and Tumor Progression

In addition to RAS system itself, the main effector peptide of RAS, Ang-(1-7), has been identified as a biologically active mediator of the RAS [72]. Ang-(1-7), converted from Ang II by ACE and ACE2, has been reported to counteract the function of Ang II in many aspects, which has attracted a lot of attention in recent years. Ang-(1-7), acting mainly through the MAS receptor, abolishes Ang II-induced migration, invasion, VEGF expression, and MMP-9 activity in breast cancer cells [72]. Moreover, Ang-(1-7) has been reported to completely block Ang II-induced EMT in breast cancer [72].

The angiotensin II receptor family members include AGTR1 and AGTR2, which belong to the G-protein-coupled receptor superfamily. AGTR1, overexpressed in

many primary and metastatic tumors [73, 74], enhances tumor growth and angiogenesis in breast cancer cells, as evidenced by upregulation of nuclear accumulation of phospho-Smad3, Snail, increased Smad4 and N-cadherin levels [75]. By enhancing adhesion of epithelial to the extracellular matrix, Ang-(1-7) can partially prevent the EMT process [72]. Besides, Ang II promotes survival and proliferation in breast cancer cells through activating PI3K/Akt pathway [72]. Ang II induces AKT phosphorylation in mammary epithelial cells at a very early time point (1 min). In contrast, Ang-(1-7) can blunt the AKT phosphorylation by the Ang II at a later time point (15 min) [72]. Recent studies have reported that AT1 stimulation by Ang II induces EMT via the Smad signaling pathway in renal epithelial cells and vascular smooth muscle cells in vitro [76, 77]. Ang-(1-7) reduced the cell migratory and invasive abilities by reducing the expression and activity of MMP-2 and MMP-9 mediated through inactivation of the PI3K/Akt, P38, and JNK signal pathways [78, 79].

Ang-(1-7) hampers the angiogenesis caused by the activation of Ang II [80]. In MDA-MB-231 cells, knockdown of MMP-2 and MMP-9 by siRNA significantly suppressed Ang II-induced cell migration [80, 81]. Enhanced expression and enzymatic activity of MMP-9 by Ang II are significantly abolished by Ang-(1-7). Besides, Ang-(1-7) suppresses Ang II-induced VEGF expression in breast cancer cells, which is consistent with prior research showing considerable VEGF reduction in nasopharyngeal carcinoma cells overexpressing Ang-(1-7) [82]. It has been discovered that Ang-(1-7) inhibits cancer cell invasion. Induced activation of ACE2/Ang-(1-7)/Mas axis attenuates breast cancer cell invasion by upregulating E-cadherin expression through downregulating SOCE-induced NF-κB and PAK signal pathways [55].

However, in contrast to the anti-cancer properties, there are also reports showing Ang-(1-7) exerted a growth-stimulatory effect on glioma cells [83]. Ang-(1-7)/Mas axis has also been shown to mediate Ang II-stimulated epithelial-to-mesenchymal transformation (EMT) in tubule cells [84]. Ang-(1-7) is also reported to promote the migration and invasion of human renal cell carcinoma cells via MAS-mediated AKT signaling pathway [85]. The reasons for the different functions of Ang-(1-7) in different cancer types are unknown, which are possibly due to experimental conditions or cell-specific signaling.

Regulation of RAS in Clinical Utilization

Currently, two types of regulators of RAS are being studied for clinical utilization. One is ACEIs that suppress signaling of Ang II receptors by reducing Ang II synthesis; the other is ARB, blockers of AT1R signaling, which have been tested in clinical studies (Table 16.1).

Table 16.1 The effect of ACEIs and ARBs in clinical cancer treatments

Types of cancer	Study characteristics			Treatment outcomes	Year	References
	Types of studies	Types of drugs	Number of patients			
Gastric cancer	Clinical study	ACEIs and ARB	63 patients	Disease control rate:77.8% Response rate:25.4% Patient characteristic: no different MPFS and OS: elevation of survival in user groups compared to non-user group	2001–2009	[102]
Pancreatic cancer	Clinical study	Enalapril, lisinopril, temocapril, candesartan, losartan, Olmesartan, valsartan	155 patients	PFS and OS: elevation of survival in user groups compared to non-user group Patient characteristic: no different except age and HT medication	2003–2011	[103]
Nonmetastatic Pancreatic Ductal Adenocarcinoma	Cohort study	Angiotensin system inhibitors (ASI)	794 patients	OS: chronic ASI use is associated with longer OS	2006–2010	[100]

(continued)

Table 16.1 (continued)

Types of cancer	Study characteristics			Treatment outcomes	Year	References
	Types of studies	Types of drugs	Number of patients			
Renal cancer	Cohort study	ACEIs and ARBs	127 patients	Objective response:86% Progressive disease:14% MPFS and OS: elevation of survival in user groups compared to non-user group	1995–2002	[104]
	Clinical study	ACEIs and ARBs	4736 patients	OS: elevation of survival in RASIs groups compared to other hypertensive drug and non-user groups Reduction of cell viability	2004–2010	[105]
	Clinical study	ACEIs and ARBs	213 patients	PFS and OS: elevation of survival in hypertensive patients compared to non-hypertensive elevation of survival in user groups compared to non-user group	2003–2013	[106]

(continued)

Table 16.1 (continued)

Types of cancer	Study characteristics			Treatment outcomes	Year	References
	Types of studies	Types of drugs	Number of patients			
Lung cancer	Observational study	Angiotensin system inhibitors (ASI)	117 patients	OS: elevation of survival in user groups compared to non-user group Follow-up period: it was longer in user groups	2004–2013	[107]
Lung cancer	Retrospective analysis	Long-term medication with ACEIs and ARBs	287 patients	MPFS: patients receiving either ACEI or ARB had a 3.1 month longer MPFS	1996–2007	[108]
Lung cancer (NSCLC)	Retrospective analysis	Chemotherapy or Erlotinib with ACEIs and ARBs	37 patients	OS: The use of ARBs during erlotinib treatment may prolong OS of patients with metastatic NSCLC	2003–2011	[103]

(continued)

Table 16.1 (continued)

Types of cancer	Study characteristics			Treatment outcomes	Year	References
	Types of studies	Types of drugs	Number of patients			
Lung cancer NSCLC (stage IIIb or IV) or early-stage disease (stage I-IIIa)	Retrospective analysis	Platinum-based chemotherapy ACEIs and ARBs	228 advanced NSCLC patients 73 early-stage disease patients	PFS: elevation in the ACEI/ARB group than the non-ACEI/ARB group OS: in combination with standard chemotherapy or TKIs, elevation in the ACEI/ARB group than the non-ACEI/ARB group both in the early or advanced stage	2000–2014	[109]
Lung cancer	Retrospective analysis	RAS-Is combined with paclitaxel (CP) or bevacizumab (CPB)	1813 patients 273 of 1,465 CP + ACEI/ARB 78 of 348 had CPB + ACEI/ARB	OS: elevation in the ACEI/ARB group than the non-ACEI/ARB group combined with CP No significant difference combined with CPB	2005–2011	[110]

(continued)

Table 16.1 (continued)

Types of cancer	Study characteristics			Treatment outcomes	Year	References
	Types of studies	Types of drugs	Number of patients			
Lung cancer	Retrospective cohort study	ACEIs and ARBs	678 patients	PFS: elevation in the ACEI/ARB group than the non-ACEI/ARB group OS: no significant difference between ACEI/ARB group than the non-ACEI/ARB group	2016–2018	[111]
Glioblastoma	Clinical study	Propranolol, aliskiren, cilazapril, celecoxib, curcumin with piperine, aspirin, and metformin	17 patients	Increased survival by 5.3 months but not statistically significant	2022	[112]
Infantile hemangioma (IH)	Randomized controlled trial	Captopril, propranolol	30 patients	Clinical improvement was significantly better and faster in the patients treated with propranolol	2016	[113]

(continued)

Table 16.1 (continued)

Types of cancer	Study characteristics			Treatment outcomes	Year	References
	Types of studies	Types of drugs	Number of patients			
Colorectal cancer	Nested case–control study	ACEIs and ARBs	2847 patients	Long-term/high dose exposure to ACE-Is/ARBs may be associated with a decreased incidence of CRC	1987–2002	[114]

Angiotensin-Converting Enzyme Inhibitors (ACEIs)

ACEIs have been shown to block solid tumor development and metastasis. Captopril, a classic ACEI, not only inhibits ACE, but also lowers ACE levels and angiotensinogen expression in the host liver, leading to inhibition of metastasis and further inhibitory mechanisms [60]. In lung cancer, Captopril also exerted anti-metastatic and growth inhibitive effects [86]. However, in renal carcinoma, the effects of Captopril are controversial. One group reported that Captopril inhibited tumor growth [87], while another group showed the induction of tumor by Captopril [88]. Independently of VEGF expression, ACE inhibition promoted neovascularization through activation of bradykinin B2 receptor signaling, whereas it reduced blood vessel growth through inhibition of the Ang II pathway [89, 90]. Besides, Perindopril was tested targeting breast cancer and found that Perindopril reduced tumor volume and downregulated the level of VEGF [91], leading to blockage of angiogenesis and metastasis in hepatocellular carcinoma [92].

AT1R Blockers (ARB)

ARBs are drugs that target the AT1R receptor. The effects of ARBs on cancer are not consistent currently. ARBs may suppress the promotional effect on proliferation by antagonizing AT1R caused by Ang II [19]. The blockade of Ang II with ACEI and ARB alone or in combination reduced tumor proliferation and metastatic capacity of RCC [52]. Losartan, the most commonly used ARB, was found to inhibit tumor growth and promote apoptosis in glioma [68]. However, Losartan was revealed to induce cancer progression and angiogenesis in lung cancer [93, 94]. Besides, it was also found to promote cell proliferation in human melanoma [95]. One research found that Losartan slowed pancreatic tumor progression by abrogating aberrant TGF-β activation [96].

Clinical Study of ACEI/ARB

In clinical studies, the efficacy of ACEI/ARB in the treatment of cancer patients is also controversial currently. It has been revealed that in 13 projects with breast cancer, only two studies showed beneficial effects of ACEI/ARB, whereas three studies reported poor outcomes [57]. Evaluating the tumor subtype information may be helpful to understand the different responsiveness of patients to the ACEI/ARB treatment [74]. Similarly, population-based studies failed to show any association or risk reduction, in patients receiving ACEI/ARB treatment [97–99]. However, a retrospective study in stage I-II colorectal cancer (CRC) patients showed that ACEI/ARB treatment reduced tumor recurrence in left-sided CRC and early-stage CRC [99]. Improved survival

was also observed in non-metastatic pancreatic ductal adenocarcinoma (PDAC) in a large-scale study [100]. Hence, multiple strategies and strict criteria should be applied to identify and include the studies to reveal the factors that may influence the association between RAS inhibitors and cancer progression [101]. Response to ACEI/ARB treatment may not only vary with tumor types but also depend on certain tumor characteristics, cancer treatment, and RASI type and dosing [50].

Conclusion

RAS participates in tumor progression in many cancer types and its dysregulation leads to increased malignancy of cancer. Although the role of RAS and the underlying mechanisms have been well studied, there are also controversies in its functions in cancer due to different environments and tissue conditions. ACE inhibitors and ARB have been investigated in preclinical research, and their roles in restricting the development of cancer is promising. However, limitations of the in vivo experiments with animal models indicate that there is still a possibility that the mechanism will be different in the human body. How to accelerate the transformation from experimental animals to clinics still needs further exploration.

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Chapter 17
Role of Renin-Angiotensin System in Cancer Cachexia

Vivek Bora and Bhoomika M. Patel

Abstract The diverse role of Renin-angiotensin system (RAS) in chronic disorders such as chronic kidney disease (CKD), chronic heart failure and chronic obstructive pulmonary disease (COPD) has been reported till now. Maintenance of cardiovascular homeostasis is the main role of RAS, but RAS is expressed by many tissues in body and RAS is involved in functions of cells such as metabolism and growth. Some studies reveled the role of RAS in progression of cancer. However, the role of RAS in cancer cachexia is yet to be explored, so we thought it worthwhile to explore the RAS and its involvement in cancer cachexia development. RAS causes cancer cachexia through a variety of mechanisms such as increased protein breakdown or muscle wasting via increased cytokine signalling and reduced IGF-1, activation of NADPH oxidase resulting in increased oxidative stress, inhibition of AMPK resulting in impaired energy balance and variation in hypothalamic neuropeptide (orexigenic/anorexigenic) expression resulting in reduced appetite. The inhibitors of RAS can be potential candidates for the management of cancer cachexia.

Keywords Renin-angiotensin system · Cancer cachexia · Cancer · Muscle wasting · Cardiovascular homeostasis

Introduction

Cancer cachexia is progression is a bad sign of prognosis. It accounts for nearly 25–30% of mortality in cancer patients [1]. The prevalence of cachexia in patients with solid tumours is high and about 87% gastric and pancreatic cancer patients suffer from cachexia, about 61% lung, colon and prostate cancer suffer from cachexia and about 40% patients with sarcoma, breast cancer and leukemia suffer from cachexia

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[2]. The cancer cachexia develops in cancer patients as a secondary syndrome and alters the energy balance and carbohydrate metabolism, protein metabolism and fat metabolism, it stimulates systemic inflammation, loss of body mass and lean body mass [3]. Cancer has a negative impact on the patients response to chemotherapy, radiation and surgery and increases suffering of the patient by worsening the quality of life of cancer patient [1, 4]. Depletion of fat and skeletal muscles is observed in cancer cachexia patients and it is also accompanied by atrophy of heart i.e. wasting of cardiac muscles [2]. The drastic effect of cancer cachexia is observed in clinical setup i.e. the effect on physiological systems such as skeletal system, cardiovascular system, neurological system, renal system, gastrointestinal system and haematological system [5–10]. With the incidence of cancer increasing drastically the development of cancer cachexia is also at alarming rate.

Not many clinical trials are reported till now with specific focus on cancer cachexia, but still limited research work reported till now has been able to explore various mechanisms involved in wasting of skeletal muscles, fat and carbohydrates, systemic inflammation in cancer cachexia [1, 11–13]. RAS is very crucial in maintenance of fluid balance and electrolyte balance and thus, cardiovascular homeostasis. Apart from maintenance of cardiovascular homeostasis the RAS is expressed by many tissues in body and RAS is involved in functions of cells such as metabolism and growth [14, 15]. Pathophysiology of some of the diseases related to fibrosis and inflammation [16], in cancer pathophysiology the impairment RAS system facilitates progression of cancer and spreading [17, 18]. Cachexia is associated with many diseases and disorders like cancer, chronic kidney disease (CKD), chronic heart failure (CHF), diabetes and chronic obstructive pulmonary disorder (COPD). The cachexia associated with several diseases often goes with alterations in the RAS. Thus there is need to study the role of RAS in the cancer cachexia development [19].

One of the pathways involved in cancer cachexia is the Renin-Angiotensin system (RAS), it causes increased hypothalamic mediated anorexia, inflammation mediated muscle wasting and reduced differentiation of satellite cells leading to reduced muscle regeneration thus progression of cancer cachexia (Fig. 17.1). Previously the RAS was known to be involved in regulation of blood pressure as well as fluid balance. But recent findings support that RAS system is involved in various diseases of heart, kidney, adipose tissue, brain and skeletal muscle [15]. RAS blockage leads to reduced progression of cancer. RAS system also reduces the side effects related to anti-cancer treatment [20]. The side effects of anti-cancer treatment interfere with the quality of life of cancer patients and come with negative consequences as dose adjustment mostly reduction of doses, changes in treatment plan due to side effects and even change or termination of anti-cancer treatment [21]. A study proposed that the renin-angiotensin inhibitors (RASi) may be helpful in the reduction of side effects associated with anti-cancer treatment and reduction of doses of anti-cancer agents with nearly same anti-cancer effect [18].

A recent study demonstrated the significant role of angiotensin-converting enzyme inhibitors (ACEi) and angiotensin receptor blockers (ARB) in restricting cancer proliferation and increases survival of cancer patients [22]. Angiotensin II (AngII) is involved in the restriction of myotube protein synthesis, upregulation of the ubiquitin

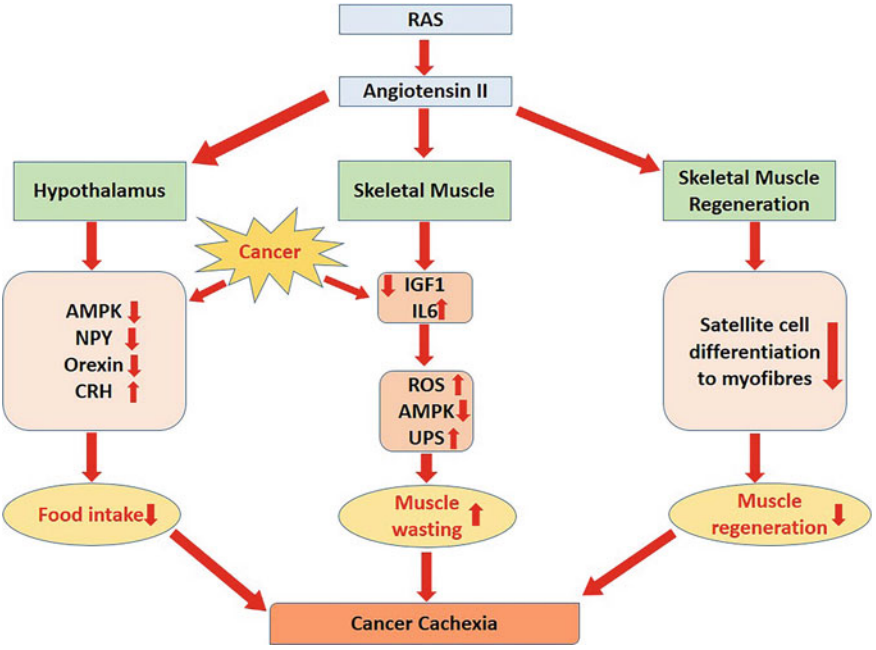

Fig. 17.1 Renin-angiotensin system (RAS), it causes increased hypothalamic mediated anorexia, inflammation mediated muscle wasting and reduced differentiation of satellite cells leading to reduced muscle regeneration. Presence of cancer causes inflammation mediated by IL6 and decreases food intake, thus leading to Cancer cachexia progression. RAS, Renin angiotensin system; IGF1, Insulin like growth factor 1; IL6, Interleukin 6; AMPK, AMP activated protein kinase; UPS, Ubiquitin proteasome pathway; NPY, Neuropeptide Y; CRH, corticotrophin-releasing hormones

proteasome pathway (UPS) and apoptosis induction in myocytes leading to wasting of muscles and thus promotes cancer cachexia development [23–25]. A study in women with older hypertension was conducted and the results of the study revealed the long term use of ACEi was associated with reduced muscle strength [26]. So we thought it to be worthy to study the importance of RAS in cancer cachexia.

Pathophysiology of Cancer Cachexia

The occurrence of cancer cachexia is caused by a variety of mechanisms; tumours can cause changes that lead to malnutrition, and these changes are particular to the type of tumour. Cachexia is caused by a number of reasons, including humoral factors, systemic inflammation, tumoral factors, and changes in carbohydrate, protein, and lipid metabolism [2, 3]. Tissue necrosis factor (TNF-α), interlukin-6 and 1 (IL-6, IL-1) and interferon-γ (IFN-γ) are examples of host tumour factors that play important role in cancer cachexia [27]. TNF-α is a cell signalling protein that causes anorexia,

weight loss, cachexia, inflammation, and skeletal muscles. Food intake is reduced in cachexic patients because TNF-α reduces the sensitivity of neurons involved in uptake of glucose [28]. TNF-α stimulates the production of corticotrophin-releasing hormones (CRH), which suppresses appetite [29]. In individuals with cancer cachexia, systemic inflammation is a common symptom. The release of specific proteins known as acute phase proteins (APP) mediates systemic inflammation. C-reactive proteins (CRP) and fibrinogen are examples of APPs [30]. Tumor factors have a critical role in cancer cachexia progression. Lipid mobilising factor (LMF) and proteolysis inducing factor (PIF), as well as myostatin and angiotensin II, are tumoral factors and their levels are elevated in cancer cachexia patients [29]. In smooth muscles, a protein known as myostatin is extensively produced. Myostatin is a TGF-family member. Myostatin is known to play a role in muscle and fat loss when its expression is increased, as well as skeletal muscle hypertrophy when its levels are decreased [31]. Another component that contributes to muscle protein catabolism and results in a loss of lean body mass and weight loss is angiotensin II (ANG II). ANG II inhibits NF-B activation via stimulating the ubiquitin-proteasome pathway and reducing insulin-like growth factor 1 (IGF-1) in skeletal muscle, resulting in dephosphorylation of double stranded RNA-dependent protein kinase (PKR) and restricts NF-κB stimulation [29]. PIF and LMF are also produced by tumours, which initiate the wasting process in cancer cachexia. LMF is a zinc 2-glycoprotein (ZAG) that leads to lipid depletion in cancer cachexia. PIF promotes protein breakdown while inhibiting protein synthesis and causing skeletal muscular atrophy [31]. Lactate and inflammatory cytokines are released by tumor. Lactate produced by tumour cells is used by gluconeogenic enzymes in the synthesis of hepatic glucose [13]. As a result, higher glucose production and energy expenditure are supported by the continuous Cori cycle between the liver and tumor. Increased insulin production occurs when blood glucose levels are high. Insulin resistance develops when the PI3K/Akt/mTOR pathway is inactivated as a result of excessive insulin synthesis. Insulin plays a role in sustaining organ viability and regulating glucose, protein, and lipid metabolism. Insulin sensitivity reduces glucose uptake in the organs, resulting in skeletal muscle and adipose tissue loss [13, 32]. Muscle tiredness and weakness are caused by the loss of myofibrillar proteins in muscle cells. Cancer cachexia is characterised by changes in protein synthesis and production, as well as increased apoptosis which culminates in muscle atrophy. Muscle wasting is triggered by pro-inflammatory cytokines and tumoral factors. As previously mentioned, these include proteolysis inducing factor (PIF), TNF-, myostatin, and insulin-like growth factor-1 (IGF-1). Protein degradation occurs via the lysosomal pathway, calpain-mediated degradation of protein, and the ubiquitin proteasome pathway (UPS) [31]. Cancer cachexia results in a significant reduction of body weight due to adipose tissue loss. Wasting of both the white adipose tissue (WAT) and brown adipose tissue (BAT) is observed in patients with cancer cachexia. WAT loss occurs, as does an increase in lipolytic activity, resulting in higher levels of glycerol and fatty acids due to LMF, as well as pro-inflammatory cytokines [33]. Mitochondrial function refers to the ability of mitochondria to produce ATP through oxidative phosphorylation and beta-oxidation, whereas mitochondrial dysfunction refers to the inability of mitochondria

to produce ATP observed during cancer cachexia. Muscle atrophy is also linked to the loss of mitochondria and disrupted fission and fusion events. With the progression of cachexia in cancer patients, changes in calcium levels and mitochondrial membrane potential develop as a result of impairment of mitochondrial function [12].

Management of cancer cachexia must stick to a complete, customised, structured, and ongoing treatment paradigm so as to meet the comprehensive aims of enhancing muscle weight, boosting the status of the body, and raising the endurance of anti-tumor medicines [2]. Palliative treatment tactics are superior to other treatment options for end-stage cachexia. It is critical to build a intervention system centred on pharmacological therapy and supported with exercise, nutrition, and measures to improve psychological status in order to maximise patients' quality of life [2].

Drug intervention lowers inflammation mediated by tumor, promotes constructive metabolism, lessen catabolism, revives appetite, stimulates weight gain along with muscle gain, develops physical fitness scores, and increases the chances of survival in cancer cachexia patients. For the treatment of cachexia till now a limited number of agents are recognized or approved by the FDA such as megestrol acetate (MA) [2, 34]. Medroxyprogesterone acetate (MPA) is a typical medicine, which enhances hypothalamic neuropeptide Y release to attenuate cytokines and revives appetite, resulting in better nutritional intake, weight gain, and enhances quality of life [2]. Cannabinoids engage with endorphin receptors, suppress prostaglandin synthesis, disrupt the synthesis of IL-1, and stimulate cannabinoid receptors in the leptin neural circuit. They can enhance the nitrogen balance and promote energy storage, but they can also cause major central nervous system side effects such hallucinations, disorientation, and psychosis [35]. A 5-HT inhibitor Cyproheptadine, is a type of tissue serotonin that causes stimulation of appetite along with sedation. Mixed evidence is available from recent clinical trials involving this drug, caution is to be taken with use of cyproheptadine use in clinical settings [36, 37]. COX-2 inhibitors like celecoxib, ibuprofen and indomethacin are examples of non-steroid anti-inflammatory drugs (NSAIDs). They supress inflammation related to tumor and reduce TNF levels, boost grip strength and increase lean mass, and enhance mGPS score [38, 39]. Enobosarm, a selective androgen receptor modulator targets androgen receptors in musculoskeletal system, reducing irritation in tissues/organs like the skin, prostate, and liver. Androgen receptor modulators can help cachexia patients gain muscle mass and raise your exercise tolerance [2, 40].

These medications have a better chance of treating cancer cachexia. When complicated inflammatory cascades are activated, however, there are various interactions between diverse mediators, implying that a single target medicine cannot be utilised as a therapy. To maximise the influence on the syndrome, most patients suffering from cancer-related cachexia are advised focused or tailored intervention point [2]. Cancer protective action of Telmisartan and cisplatin combination was reported, confirmed by lower cachexia markers and tumour markers in a recent study. Telmisartan's mediated its effect via reduction in IL-6 levels [41].

Renin-Angiotensin System (RAS)

The renin-angiotensin system or the renin-angiotensin-aldosterone system (RAS) is a very important regulator of not only the blood volume but also the vascular resistance in the body [42]. The baroreceptor reflex responds to homeostatic changes and lower arterial pressure, the RAS is responsible for fluid balance, blood pressure and related long-term changes. Renin, angiotensin II, and aldosterone make the RAS. Decrease renal blood pressure, decreased transport of the salts to the distal convoluted tubule (DCT), and/or beta-agonism all give rise to arterial pressure through renin, angiotensin II and aldosterone. The body can maintain elevation in blood pressure for extended period through these physiological processes [42, 43]. Long-term elevation in blood volume and increased arterial tone is due to the activation of RAS. It achieves this by enhancing reabsorption of water along with sodium and also the vascular tone [42].

Kidneys afferent arterioles have juxtaglomerular cells which release prorenin. The renin is present in its inactive form prorenin, but the prorenin is cleaved to form renin after the stimulation of juxtaglomerular cells. These cells are stimulated due to lower sodium load in DCT caused by stimulation of macula densa cells as a consequence of reduction in blood pressure. Thus renin is secreted into the blood stream and works on angiotensinogen [42]. Liver secretes the angiotensinogen protein and is released into the blood stream. The renin in blood stream cleaves angiotensinogen to angiotensin I. Inactive form of Angiotensin II, Angiotensin I is inactive and does not have effect on physiology. Angiotensin converting enzyme (ACE) converts the inactive angiotensin I to the active angiotensin II. Angiotensin II levels are higher in the vascular endothelium of both the kidneys and lungs. Angiotensin I binds with the receptors angiotensin II receptors type I (AT) and type II (AT), this binding leads to release of angiotensin II. Angiotensin II exerts its effects on the kidneys, brain, the arterioles and the adrenal cortex. AT receptors role is not established until now. AT are responsible for inducing vasodilation by increasing the production of nitric oxide. The plasma half-life of Angiotensin II is very short i.e. 1–2 min, degradation of Angiotensin II leads to formation of angiotensin III and IV. The 100% triggering of aldosterone is exerted by the angiotensin III but it exerts smaller pressor effect (40%). Angiotensin IV has a negligible systemic effect [42, 44].

The renin-angiotensin system (RAS) is a circulatory or hormonal system that regulates blood pressure, electrolyte balance, and fluid balance. This action of RAS are due to significant effects on vascular smooth muscle, fluid retention, renal re-absorption of electrolytes, and activation of aldosterone as well as vasopressin [45]. Angiotensinogen the hepatic precursor, renin and ACE, physiological activation of peptide angiotensin II, and the AT receptors are the main components of RAS. In addition a variety of bioactive peptides are produced from angiotensin I and/or angiotensin II, these bioactive peptides include angiotensin III, angiotensin IV, and angiotensin (1-7). Chymase, tonin, chymotrypsin, ACE2 (a homolog of ACE), and aminopeptidase A, as well as aminopeptidase B/N, are examples of angiotensin-processing

peptidases. Angiotensin II, along with various bioactive peptides, exerts their unique actions via target tissue organ cellular receptors [45, 46].

Muscle atrophy can be a consequence of diverse pathophysiological conditions such as cachexia, sarcopenia, hunger and denervation. Till now the role of satellite cells in muscle atrophy is unknown. It has been proposed that in cancer cachexia animal models, decreased regeneration and maybe a blockage of satellite cell function is observed [47, 48]. Even though the mechanisms involving RAS-mediated complex control of satellite cell function are not understood, some studies suggest that the angiotensin and their receptors are involved in the regulation of satellite cells leading to muscle atrophy, and that changes in RAS impacts involved in the pathogenesis states may result to a poor satellite cell function [19, 47, 48].

Angiotensin II

Angiotensin II is involved in increasing the sodium reabsorption by enhancing the exchange of Na–H in the proximal convoluted tubule (PCT) of the kidney. Rise in the levels of sodium in body increased the osmolarity of blood and thereby increasing the blood volume due to transfer of fluid and extracellular space (ECF). This increase in osmolarity leads to rise in arterial pressure. Vasoconstriction takes place in the systemic arterioles due to action of angiotensin II. Angiotensin II bids with the G-coupled protein receptors in the systemic arterioles and initiate a secondary messenger cascade ultimately leading to vasoconstriction of arterioles and thus elevation of blood pressure. Blood pressure is increased due to increase in the total peripheral resistance. The Angiotensin II exerts its effects on the brain, it initially binds to the hypothalamus thereby triggering thirst leading to increase in water consumption. Then, antidiuretic hormone (ADH) is released by the posterior pituitary gland. ADH also called as vasopressin facilitates the opening of aquaporin channels located on the collecting duct of kidneys thus increasing the reabsorption of water. Lastly, the sensitivity of baroreceptors is decreased by Angiotensin II. Decreased sensitivity of baroreceptors is in countering the action of baroreceptor to rise in blood pressure thus maintaining activity of RAS system. All the actions of angiotensin II mentioned above lead to increase in sodium reabsorption along with fluid retention and vascular tone [42, 44]. Elevated plasma concentrations of Angiotensin II in cancer cachectic patients play a role in cardiac dysfunction by lowering IGF1 and MMP activation in the blood [49].

Anorexia is typically, though not always, linked to wasting, and interestingly anorexia and body fat loss are strong predictors of mortality in cancer cachexia patients. Pair-feeding studies were conducted, in which the same quantity of food was given to animals with or without Ang II, and it was discovered that reduced food intake accounts for roughly 80% of the body weight loss in Ang II-infused animals. Complex systems control food intake, including the activities of hypothalamic neuropeptides (orexigenic/anorexigenic) and circulating substances released by peripheral such as adipose tissues as well as gastrointestinal tract [19]. A study discovered that Ang

II promotes wasting via two mechanisms: accelerated catabolism of proteins in skeletal muscle and a reduction in food intake [50]. Multiple studies have found that intra-cerebroventricular infusions of Ang II reduced food intake and altered orexigenic/anorexigenic neuropeptides like neuropeptide-Y (NPY), thyrotropin-releasing hormone, agouti-related protein (AgRP), orexin, corticotropin-releasing hormone and proopiomelanocortin (POMC) implying that systemically increased Ang II can negatively act on neurons of hypothalamus in chronic diseases to modulate food intake by regulating expressions of orexigenic/anorexigenic neuropeptide [19, 51, 52].

In diverse cell types, reactive oxygen species (ROS) play a significant role in Angiotensin II-induced signalling, leading to endothelial dysfunction, insulin resistance, cardiac myocytes hypertrophy, hypertension and vascular smooth muscle cell hypertrophy. Angiotensin II has been demonstrated to cause the production of reactive oxygen species (ROS) in skeletal muscle, which adds to disuse muscle atrophy [19]. In atrophied skeletal muscles, NAPDH oxidase and mitochondria are important producers of reactive oxygen species (ROS) [53, 54]. Proteolysis and atrophy of skeletal muscles are caused by oxidative stress [24]. Muscle atrophy and 20S proteasome activity were both induced by Angiotensin II [55]. Angiotensin II also boosts mitochondrial ROS production, and it's been hypothesised that NADPH oxidase-induced ROS could excite mitochondria directly [56]. The mitochondria-targeted antioxidant Mito-TEMPO, on the other hand, was unable to attenuate Angiotensin II-initiated muscle loss, implying indirect implication of mitochondrial ROS [57]. These findings imply that targeting ROS and NADPH oxidase specifically could be a promising new treatment for Angiotensin II-induced wasting [19].

The set of genes essential for adenosine triphosphate (ATP) generation and in later steps of glycolysis are often downregulated, according to DNA microarray study in diverse atrophying circumstances. These modifications would diminish muscle energy generation by suppressing the muscle's ability to utilise glucose [58]. Decreased glucose utilisation is evident in the context of cancer and also renal failure, suggesting that metabolic imbalance is crucial mechanisms behind cachexia progression [19]. A study investigated metabolic alterations in wasting condition induced by Ang II and discovered that Ang II promotes muscle wasting by depleting skeletal muscle ATP content and inducing mitochondrial dysfunction [57]. Reduced activity cellular energy sensor 5′ adenosine monophosphate (AMP)-activated protein kinase (AMPK), causes the drop in ATP. Low cellular energy (high AMP:ATP ratio) promotes ATP synthesis by AMPK, and findings suggest that muscle atrophy is initiated by Ang II in part by inhibiting homeostatic capacity of skeletal muscle and helps in energy balance maintenance. The AMPK activator 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) restored AMPK inhibition by Ang II, restoring ATP levels and preventing muscle atrophy induced by Ang II. AICAR also inhibited the expression of the E3 ubiquitin ligases atrogin-1 and MuRF-1 stimulated by Ang II [59]. Interestingly, the authors discovered that the overexpression of the upstream phosphatase PP2Cα mediates the reduction in AMPK activity induced by Ang II, and that knocking down PP2Cα reviving mitochondrial function and wasting of muscle mass in Ang II-infused rats. Exact mechanism by which Ang II inhibits AMPK by

upregulating PP2Cα is unknown, but recent findings imply that targeting PP2Cα in disorders of chronic wasting with high Ang II levels could be beneficial [60].

Angiotensin Converting Enzyme 2 (ACE2)

ACE2 is involved in the progression of cancer. Inflammation, cytokine buildup, increased vasoconstriction, and increase in permeability by the vascular endothelium are all frequent in cancer patients. In some malignancies, such as pancreatic, lung, cervical, and renal carcinomas, ACE2 expression is higher, while it is lower in breast, prostate, and liver cancers [61, 62]. The prognosis is mostly determined by the type of tumour and cancer stage. RAS is involved in cell proliferation, angiogenesis, cancer-induced inflammation, cancer-induced immunomodulation, and the upregulation transcription factors along with cytokines and growth factors thus development of cachexia in advanced stages [63]. ACE2 is involved in the renin-angiotensin system's role in maintenance of normal blood pressure. Heart and lung cells have more concentration of ACE2 receptors, allowing SARS-CoV-2 to enter the respiratory system more quickly and causing ACE2 levels to rise. As a result, ACE2 was linked to COVID-19 pandemic. Binding of SARS-CoV-2 to the ACE2 receptor on the other organs such as stomach, kidneys, bladder, liver, and intestine in a secondary infection following the lungs [62, 64]. It is more prone to diabetes, heart disease, and infection of other body organs. Thus leading to collapse of multiple organs and mortality in COVID-19 patients [63].

Aldosterone

Aldosterone is another RAS component that was discovered 50 years ago. It is produced in the adrenal cortex's zona glomerulosa as a result of enzymatic processes specific to sequence of locus and orientation. Furthermore, sources of aldosterone synthesis other than adrenal cortex occurs in brain, myocardium and the vascular tissue [42, 44].

Sodium reabsorption and excretion of potassium in the DCT and collecting duct is promoted by aldosterone. Aldosterone also stimulates insertion of luminal Na channels and basolateral Na–K ATPase proteins. Thus increasing salt reabsorption thereby increasing osmolarity, which causes blood volume and ECF volume to rise. Additionally, the role of aldosterone in limiting salt and water loss in conditions specific to dietary sodium deficiency. Mineralocorticoid receptors in the brain, heart and vascular tissue are activated by aldosterone [45]. Unlike angiotensin II, aldosterone is a steroid hormone. Aldosterone binds to nuclear receptors and initiates gene transcription leading to slow effect of aldosterone as compared to Angiotensin II [44].

Conclusion

The role of RAS in a variety chronic disorders such as chronic kidney disease (CKD), chronic obstructive pulmonary disease (COPD), and chronic heart failure has been reported till now. The role of RAS in the development cancer cachexia has been elucidated in the present work. RAS causes cancer cachexia through a variety of mechanisms such as increased protein breakdown or muscle wasting via increased cytokine signalling and reduced IGF-1, activation of NADPH oxidase resulting in increased oxidative stress, inhibition of AMPK resulting in impaired energy balance and alteration in hypothalamic neuropeptide (orexigenic/anorexigenic) expression resulting in reduced appetite.

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Chapter 18
Renin Angiotensin System Activity in Different Cancers: Mechanistic Insight and Preclinical Studies

Atamjit Singh and Preet Mohinder Singh Bedi

Abstract The Renin Angiotensin System (RAS) is maintain arterial blood pressure as well as fluid and electrolyte balance in human body. In recent there decades, it has been emerged as an important hallmark of cancer due to its local expression in all body tissues. This chapter will shed light on the impact of RAS in various type of cancers including breast, gynaecological, gastrointestinal, lung and skin cancer. Mechanistic insights of RAS modulating cancer activity has been discussed in each cancer type individually. Additionally, effect of angiotensin receptor blockers and angiotensin converting enzyme inhibitors on various types of cancers either alone or in combination with different anticancer agents has been also discussed.

Keywords Cancer · Renin angiotensin system · Angiotensin-II · Angiotensin receptor blockers · Angiotensin converting enzyme inhibitors

Introduction

Human physiology is full of certain homeostatic systems that maintain balance between various ongoing physiological processes. Renin-angiotensin system (RAS) is one among them that maintain arterial blood pressure as well as fluid and electrolyte balance. Various in vitro, animal and clinical studies pointed toward the deregulation of RAS in malignancy resulting poor clinical outcomes. Interruption of RAS has been reported to suppress tumour growth, metastasis and angiogenesis while long-term use of inhibitors of angiotensin-converting enzyme (ACE) has been reported to provide protection in cancer, reported in retrospective studies in humans. This chapter will summarize the relationship between RAS and common type of cancers along with present scenario of drug developments and future perspectives.

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Overview of RAS

In classical/general view composition of RAS include renin, angiotensinogen (AGT), Angiotensin I (Ang-I), angiotensin converting enzyme (ACE), angiotensin II (Ang-II), type 1 angiotensin receptor (AT_1R) and type 2 angiotensin receptor (AT_2R). In contemporary view, RAS chiefly consist of angiotensin 1–7 (Ang 1–7), angiotensin converting enzyme 2 (ACE-2 and MAS receptor (MASR) [1]. On indication of low blood pressure or plasma sodium, pro-renin is released in blood from kidneys where it is converted into renin (active form). Renin converts AGT to Ang I, which further converted into either Ang-II by ACE which is expressed in the on luminal side of endothelial cells of lungs or Ang-1–7 by neprilysin respectively. Additionally, ACE-2 is also able to convert Ang-II to Ang 1–7 [2–4]. Ang-II which is an active hormone of RAS results in increase of blood pressure from lower to normal level. Ang-II implement this process viaG protein coupled receptors i.e., AT_1R mainly expressed in liver, brain and kidney tissues (Ang-II primarily ACT through this receptor) and AT_2R primarily found in foetal tissues, ovary and uterus. Ang-II execute actions like promoting vasoconstriction, increasing plasma aldosterone, retention of sodium and water and enhancement of thirst and salt appetite via AT_1R. These actions together results in increase of blood pressure as well as fluid and electrolyte homeostasis [2].

RAS and Cancer

In the patients suffering from hypertension, RAS is over-activated, which enhance the incidence of cancer development along with increased risk of cancer progression as well as mortality in patients already suffering firm cancer [2]. Apart from liver, kidney and lung, RAS is also expressed adequately in adipose, breast, brain, ovaries pancreas and heart tissues, where it is involved in tissue remodelling and endothelial dysfunction like processes. RAS dysregulation contributes to metastasis, adhesion, invasion, angiogenesis, proliferation and Epithelial Mesenchymal Transition (EMT) like processes in cancer development and progression [1].

Progression of cancer starts from the proliferation of cells at the site of origin and metastasis to far sites from origin [5]. Metastasis is recognised as major cause of cancer related deaths and critical process in development of cancer. Overstimulation of AT_1R enhance vascular remodelling which upkeep the tumour cell movement during metastasis [6, 7]. NF-kB is expressed is almost all cell types and has a dominant role in cancer progression as well as in metastasis. It promotes cancer survival by preventing apoptosis. NF-kB is activated though Ang-II/AT_1R signalling pathway. NF-kB promote metastasis by inducing E-selection like adhesion molecules that spread cancer cells to ease their adherence to vascular endothelial cells. NF-kB also enhance Epithelial Mesenchymal Transition (EMT) process, in which cancer cells lose their adhesion, polarity and invade adjacent tissues [8–11]. Apart from that, NF-kB promote angiogenesis during cancer progression by up

regulating Vascular Endothelial Growth Factor (VEGF) which is accountable for neo-angiogenesis during invasion and migration of tumour cells. ACE inhibitors (ACEIs) and AT_1R blockers (ARBs) are suggested to reduce NF-kB activity [2, 10, 12].

ACEIs and ARBs are widely prescribed anti-hypertensive agents in clinical practice. Both classes are in popular culture for their utilization as adjuvant therapy for cancer patients, but their effect as antitumor agents is still under suspicion sue to conflicting effects on cancer. Large portion studies testimonies their efficacy in tumour regression while limited studies suggest the efficacy of losartan in increasing risk of lung cancer along with inducing cancer progression and angiogenesis [2, 13–15]. Associated mechanisms of these drugs may be the possible reasons behind this confliction. Telmisartan as ARB for example, activate Peroxisome Proliferator-Activated Receptor-gamma's (PPAR-γ) signalling which promote apoptosis and inhibit proliferation of cancer. Apart from that wide variety of reasons are there, like study design, type of cancer involved in investigation, levels of Ang-II in specific cancer types etc., which may be possible causes of conflictions [2, 34]. In this chapter we summarise the effect of RAS on common types of cancer including preclinical studies with inhibitors of RAS components.

Role of RAS in Different Cancers

RAS and Breast Cancer

Breast cancer is most common type of cancer among women having significant morbidity and mortality affecting both older as well as younger women. RAS component is locally expressed in breast tissues and acts differently in normal and cancer breast tissue [16]. In normal breast tissue it follows alternative RAS pathway (ACE-2/Ang 1–7/MASR) while in cancerous breast tissue it relies on classical RAS pathway (ACE/Ang-II/AT_1R) [17, 18]. That makes Ang-II a specific therapeutic target of breast cancer. In breast cancer, Ang-II/AT_1R axis has four major functions:

A. Ang-II/AT_1R axis promote tumour growth via AKT and ERK1/2 signalling and activation of CARMA-Bcl10-MALT1 (CBM) signalling pathway which promotes the activation of NF-kB. Activity of AKT and ERK1/2 was reduced on blockage of AT_1R suggesting the proliferative effect of Ang-II via AT_1R [9, 19, 20]. In addition, higher levels of Ang-II was detected in deaths related to breast cancer as compared to non-breast cancer related deaths, establishing role of Ang-II in breast cancer mortality. Ang-II is also reported to induce angiogenesis, cell proliferation and migration in breast cancer by stimulation of PI3K/AKT/NF-kB pathway through AT_1R. AT_1R is observed to be exceptionally overexpressed in breast cancer especially at high clinical stage [19, 21]. Expression of AT_1R was inversely correlated with metastasis. MCF-7 has been observed to have lower AT_1R level and higher metastasis potential while MDA-MB-231 has higher AT_1R level and lower metastasis potential respectively [11, 22].CBM dependent

NF-kB activation affect tumour microenvironment i.e. stimulates secretion of VEGF, Interleukin-6 (IL-6), Interleukin-8 (IL-8), and Interleukin-1B (IL-1B). VEGF promote angiogenesis while IL-1B allow cancer metastasis by modulating immune tolerance [20, 23].

B. Ang-II/AT_1R axis induce EMT via phosphorylation and TGF-β/Smad signalling pathway, in which Snail/Smad3/4 complex reduce E-cadherin [11].

C. Ang-II/AT_1R axis promote invasion and angiogenesis by increasing the expression of MMPs (MMP-2 and -9) and VEGF, that further modulate ECM. Lower levels of Ang 1–7 and MASR expression has been observed in breast cancer. Ang 1–7 is reported to exert anticancer effects via MASR by inhibition of fibrosis, reduce tumour volume and weight, and prevent angiogenesis and metastasis by reducing the expression of VEGF and MMP-9.Higher levels of ACE expression has been observed to reduce the overall survival of breast cancer patients [24].

D. Ang-II/AT_1R axis stimulates cell migration and lymph node metastasis via CXCR4/Sdf-1α and focal adhesion kinase (FAK)/Ras homology gene family member A (RhoA) signalling pathways. CXCR4/Sdf-1α pathway help tumour cells to reach lymph nodes while FAK/RhoA pathway direct the movement of cells. Losartan has been observed to reduce CXCR4 expression on the cell membrane of triple negative breast cancer cell line MDA-MB-231 and expression of Sdf-1α in lymph nodes that reduce the metastasis in breast cancer [25].

Majority of studies pointed toward the non-association between ARBs or ACEIs and breast cancer risk, reoccurrence and survival. However, some studies placed hypertensive patients taking ACEIs in low risk zone for breast cancer [26, 27]. Olmesartan was reported to reduce breast cancer growth by blocking AT_1R. Increased anticancer effect was observed when olmesartan was used in combination with Bay 11–7082 which potentiate cell apoptosis via inhibition of NF-kB [23]. Tamoxifen(TAM) is a highly prescribed drug in all stages of Estrogen Receptor (ER) positive breast cancer cases but suffer from resistance issue on long term use. It is observed that AT_1R is highly expressed in tamoxifen resistance ER positive breast cancer cells (MCF-7). Using losartan with tamoxifen restored the ER sensitivity and decreased cell proliferation which suggest that tamoxifen may potentiate AT_1R signalling pathway and promote tumour growth and proliferation [28]. Both studies pointing toward the establishment of ARBs for adjuvant therapy in breast cancer.

Genetic components has significant impact on breast cancer and RAS relationship. Genetic variationsin ACE and AT_1R modulate their expression in breast tissue, and ultimately in cancer activity. DD homozygote polymorphism of ACE gene is observed to be associated with higher levels of ACE and AngII. The C allele of AT_1R gene is associated with higher sensitivity to Ang-II in north indian women [29, 30]. Interactions between AT_1R gene polymorphism and ACE gene polymorphism increased risk of breast cancer in Iranian women but at the same time, similar interaction has no relation to breast cancer in Chinese women [29]. In other study, AT_1R gene polymorphism showed no association with breast cancer in Brazilian and Iranian women but showed significant association in Caucasian women. All reported

studies testifies the ACE and AT_1R genes polymorphism as a risk factor of breast cancer but one should consider racial disparity before taking lead [30].

RAS and Lung Cancer

Lung cancer is among most complicated cancer types with early asymptomatic nature and high mortality rate. It has numerous subcategories including adenocarcinomas, small lung cancer and non-small cell lung cancer (NSCLC) etc. [31]. Cancer stem cells (CSC) in tumour microenvironment are thought to be responsible for resistance to conventional therapy sue to their involvement in cancer metastasis and aggressiveness. CD24, CD44 and CD133 on CSC surface are considered as markers for diagnosis. In lung cancer cells, Ang-II has been found to increase cell proliferation and CSC-like phenotypes via elevation of CD133 expression [32, 33]. Ang-II/AT_1R signalling as found to supress tumour immunity by increasing the expression of programmed death lgand-1 that supress T-cell activity n NSCLC cell [33]. Ang-II has been observed to promote progression of lung cancer via transactivation of Epidermal Growth Factor Receptor (EGFR) which stimulate MEK/ERK pathway. EGFR also promote cancer progression via activation of Mitogen-Activated Protein Kinase/Signal Transducer and Activator of Transcription (MAPK/STAT) pathway. Ang-II also upregulates an oncogene, micro RNA-21 (miRNA-21) that stimulate PI3K/AKT pathway. The miRNA-21 is also linked with the short survival of lung cancer patients. Telmisartan has been observed to inhibit tumour growth, migration and invasion along with activating apoptosis in NSCLC via PI3K/AKT pathway [34, 35].

AT_1R, ACE and VEGF were found to be upregulated in platinum-resistant NSCLC cells while AT_2R was observed to be downregulated. Stimulation of ACE2 expression resulted in decreased production of AT_1R and VEGF thus inhibit tumour growth in NSCLC that makes it's a viable therapeutic strategy in NSCLC [36]. Tumour Necrosis Factor-Related Apoptosis-Inducing Ligand (TRAIL) is suggested to induce apoptosis in NSCLC. Resistance of TRAIL increase the survival of cancer cells under chemotherapy. Candesartan in combination with TRAIL therapy has been observed to re-sensitize TRAIL and promote programmed cell death [37]. Stimulation of AT_2R has been suggested to promote apoptosis and reduce lung cancer cell growth. Combination of candesartan and AGTR2 gene has been observed to inhibit tumour growth and formation of new blood vessels [38–40].

Positive correlation has been observed between risk of lung cancer and higher doses of ACEIs among Danish population. Long term use of higher doses of ACEIs and ARBs has been suggested to associate with adrenocarcinoma sparing small cell lung carcinoma. ACEIs induce accumulation of bradykinin thus promote cancer migration and proliferation through B2 receptors [41, 42].

RAS and Skin Cancer

Skin cancer is mainly classified into two types i.e., melanoma and non-melanoma. Non-melanoma is more common and further divided into two types including squamous cell carcinoma and basal cell carcinoma. Besides being less common, melanoma skin cancer is more aggressive than non-melanoma cells and metastasizes positively in lungs [43]. AT_1R is found to express in melanoma and Myeloid Derived Suppressor Cells (MDSC) of pulmonary metastasis. MDSC cells are of immunosuppressive nature in tumour microenvironment. Suppression of AT_1R was concluded to inhibit the growth and metastasis of melanoma cells [44, 45]. Ang-II has been found to increase melanoma cell proliferation as well as invasion by increasing the production of MMPs via AT_2R. N^+/H^+ exchanger isoform1 (NEH1) activity promotes cell migration in skin cancer. Ang-II has been observed to promote NEH1 activity and cell proliferation through Ca^{+2}/calmodulin signalling pathway, losartan has been identified to block this effect [46]. Pulmonary metastasis has been observed to be increased in melanoma cells by Ang-II/AT_1R via increasing E-selection expression that helps melanoma cells in adherence during adhesion stage of metastasis. In AGTR1 gene knockout melanoma mice model, treatment of Ang-II didn't showed any effect on cell proliferation while pulmonary metastasis was supressed [47]. Blocking AT_1R have anti-migratory effect on melanoma cells with Ang-II independent manner while AT_2R promotes cancer growth with Ang-II dependent manner respectively [46]. Suppression of AT_1R by losartan resulted in increased growth of AT_1R expressing melanoma cells. In AGTR1 lacking melanoma cells Ang-II treatment potentiate cell growth which was missing in melanoma cells lacking both AGTR1 and AGTR2 genes, that suggest Ang-II-dependent proliferation of melanoma cells by AGTR2. In melanoma cells, agonists of AT_2R induce cell proliferation and its antagonists decreases the process [48]. In case of non-melanoma skin cancer cells for example in head and neck squamous cell carcinoma (HNSCC), motility and invasion was stimulated by Ang-II/AT_1R signalling while Ang 1–7 block that effects. AT_1R blockers as well as ACEIs inhibit the motility and invasion of HNSCC [49]. Genetically, there is no link between utilization of ARBs and ACEIs and skin cancer among patients older than 65 years. However, low gene expressions of ACE and AGT are observed to be associated with risk of basal cell carcinoma [50, 51].

RAS and Prostate Cancer

Prolong exposure of Ang-II has been observed to increase cell proliferation and survival via up regulation of BCL2/BAX ratios, promotes ECM degradation by increasing the production of MMP-2 and MMP-9 in normal prostate cells, thus increasing risk of prostate cancer (PC) [52]. Increased production of MMPs by Ang-II has been observed to promote PC progression and invasiveness [53]. Positive correlation between metastasis of PC cells and AT_1R expression has been observed

while overexpression of AT_2R is observed to inhibit tumour growth that makes AT_1R inhibition and AT_2R stimulation a reasonable therapeutic approach for PC treatment. Long-term use of ARBs and ACEIs has been observed to reduce risk of PC. Fimasartan has been observed to reduce PC migration that makes it a viable adjuvant in PC therapy [54–56].

PC is common type of cancer among males and of two types i.e., androgen-dependant and androgen-independent PC [57]. During development of PC, Ang-II has significant impact on the expression of NF-kB and androgen receptors. The expression of NF-kB increases the risk of metastasis and drug resistance in PC. In advanced stages expression of androgen receptor is inversely correlated with PC while in later stages PC become independent from androgen receptor. Protein Tyrosinase Kinase (PTK) has been observed to be upregulated in advanced stages and promote PC progression. AngII/AT_1R and Ang 1–7 has been observed to reduce PTK activity in androgen independent PC [58, 59]. Thus careful utilization of ARBs/ACEIs should be needed in PC therapy.

RAS and Renal Cancer

Renal cell carcinoma (RCC) is a kidney cancer and among the late detection cancer types, less responsive to conventional chemotherapies that ultimately ended up in nephrectomy [60, 61]. Elevated levels of Ang-II has been observed to increase oxidative stress, DNA damage and mutation thus increase vulnerability for development of kidney cancer. Overexpressed Ang-II/AT_1R in RCC promote cell proliferation and metastasis via up regulation of VEGF and stimulation of CD43. In is worth to note that, Ang 1–7/MASR signalling is found to stimulate RCC by promoting migration in renal cell adenocarcinoma while it is well known for anti-tumour and protective effects in numerous tumours [61]. Bladder cancer is also less responsive to conventional chemotherapies that ultimately ended up in cystectomy and reoccur in 75% of cases. Elevated levels of AT_1R and MASR while down regulation of AT_2R has been observed in bladder cancer. Stimulation of AT_2R impart apoptotic pathways i.e., P38 MAPK, caspase-3 and -8 while reduced production of VEGF and inactivation of ERK pathway by AT_1R and MASR inhibit tumour growth and angiogenesis in bladder cancer. Particularly, AT_2R expression increase the survival rate of bladder cancer patients [61–64].

Combined use of ARBs and ACEIs proven to be tumorigenic in nature because simultaneous inhibition of AT_1R and Ang-II elevate bradykinin production that promote cancer growth, allow renin production that promotes Ang-II independent pathways. Some studies advocates about the link of prolong use of ARBs and ACEIs with the risk of kidney cancer while some suggesting non association. Combined therapy of sunitinib and ACEI was capable to reduce cell viability which was failed to achieve when used individually [61].

RAS and Gynaecologic Cancers

Ovarian Cancer

Ovarian cancer possess highest mortality rate among women due to indistinct and unspecific symptoms. Short survival and lack of diagnostic markets make its treatment difficult [65, 66]. Formation of multicellular spheroids (MCS) promote cell aggregation that prevent cell death and enhance cancer metastasis. RAS components Ang-II and AT_1R are correlated with development of ovarian cancer. Elevated levels of AT_1R are observed in ovarian cancer that stimulate EMT and metastasis to peritoneal cavity. Cancer cells avoid apoptosis by maintaining lipid haemostasis and reducing endoplasmic reticulum (ERC) stress by activating lipid desaturation process. Ang-II/AT_1R axis activates ERK and PI3K/AKT pathways that induce lipogenesis via activation of Sterole Regulatory Element-Binding Protein (SREBP) pathway. SREBP pathway stimulates key enzymes responsible for lipogenesis including Stearoyl-CoA Desaturase-1 (SCD-1) and ERC enzymes which are responsible for lipid desaturation. In the presence of losartan (AT_1R blocker), expression of SCD-1 was found to be reduced. Ang-II is also converted into Ang 1–7 when AT_1R is blocked and acts through MASR to reduce cancer metastasis [67]. AT_2R Interacting Proteins (ATIPs) has been suggested to have tumour supressing effects in ovarian cancer cells. AT-hook 2 (HMGA2) promotes tumour growth and metastasis via ERK signalling pathway and EMT phenotype, activation. ATIPs are found to be down regulated in ovarian cancer cells. It was observed that induction of ATIP3a downregulates HMGA2 mediated tumour growth, migration and invasion that makes ATIP3a, a viable target for ovarian cancer therapy [68, 69].

Uterine Cancer

Ang-II is required to induce the production of VEGF in menstrual cycle, for the formation of follicles, new endometrium and oocytes. VEGF, which controls the regulation of blood vessel formation is found to be overexpressed in Endometrial Cancer (EC) cells [70]. Ang-II promotes cancer progression by promoting cell proliferation, EMT phenotype, survival and migration via VEGF production. Ang-II/AT_1R enhance migration and invasion of EC cells by up regulation of EMT-related genes and down regulating E-cadherin. TGF-β has tumour suppressor effect is early stages and promoter effect in advanced stages of cancer. TGF-β is observed to have low expression in early stages while higher in advanced stages of EC which is regulated by Ang-II [71, 72]. Genetic variations also affect EC progression. G allele of AGT gene is being linked with higher expression of AGT while C allele of AT_1R gene is linked to increased AT_1R levels. AGT overexpression is associated with anti-angiogenesis effect while elevated levels of AT_1R is linked with growth and progression of EC. C allele of AT_1R gene is dominant in Australian women while G allele of AGT is found to be non-dominant that establish the role of gene polymorphism in EC [73].

Gastrointestinal Cancers

Liver Cancer

Liver cancer has high recurrence rate and second leading cause among cancer related deaths around the globe. Hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma are most common among liver cancer types [74, 75]. During development of HCC, Ang-II, Ang 1–7, CD34 and VEGF are found to be up regulated while ACE-2 was down regulated, showcasing important role of RAS in HCC progression. In HCC, Ang-II enhance AT_1R and stimulate cell proliferation by elevating the levels of pro-inflammatory cytokines and nicotinamide adenine dinucleotide phosphate oxidase activity that increase the levels of reactive oxygen species (ROS). Ang-II also found to promote EMT, migration as well as invasion via TGF-β pathway in HCC [76–78]. Overexpression of AT_1R in liver cancer promote angiogenesis and fibrosis by up regulating TGF-β, VEGF-A, and increasing microvascular density [79–81]. Ang 1–7 was found to inhibit cell proliferation and angiogenesis and activate cell apoptosis in HCC mouse models [81]. This response is justified via low expression of AT_1R and higher expressions of AT_2R and MASR on treatment with Ang 1–7. Overexpression of AT_2R has been suggested to promote apoptosis by increasing activity of caspase-3 and p38-MAPK activity. Ang 1–7 also down regulate the levels of cyclooxygenase-2 (Cox-2), hypoxia inducible factor-1α (HIF-1α) as well as VEGF-A expression via inhibiting ERK signalling [80, 81].

Candesartan was observed to reduce AngII/AT_1R/VEGF mediated angiogenesis, metastasis as well as tumour growth in liver cancer. Similarly telmisartan reduce cell proliferation via AMP-activated protein kinase (AMPK) activation and inhibiting pathway of mammalian Target of Rapamycin (mTOR) signalling that arrest cell cycle by blocking regulatory proteins in cell cycle i.e., cyclin D1 and cyclin E [80, 82]. Irbesartan found to inhibit VCAM-1 mediated adhesion of HCC cells by interrupting Ang-II/AT_1R-activated P38.MAPK pathway. Losartan potentiated the anticancer efficacy of Lenvatinib by inhibiting Ang-II mediated growth and increasing apoptosis in cancer cells along with antiangiogenic effect by attenuating production of VEGF-A. Azilsartan in combination with NF-kB antagonist Bay11-7082 is found to induce apoptosis pathways by elevating ROS in HCC [83].

Pancreatic Cancer

Pancreatic endocrine tumours and Pancreatic Ductal Adrenocarcinoma (PDAC) are two major types of pancreatic cancer. Absence of diagnostic markers, recurring nature makes its management difficult [84, 85]. Elevated levels of AT_1R and lower levels of AT_2R are observed in pancreatic cancer that makes them potential targets in pancreatic cancer therapy. MicroRNA 410 (miR-410) has been found to inhibit cell proliferation, angiogenesis and invasion in cancer cells. Overexpressed AT_1R promote progression of pancreatic cancer by interrupting MicroRNA 410 (miR-410).

Losartan has been observed to arrest cancer progression and prolong the survival of pancreatic cancer patients [86–88].

Esophagus Cancer

Esophageal adenocarcinoma (EAC) and esphageal squamous cell carcinoma (ESCC) are two most common esophagus cancer types [89, 90]. Ang-II through AT_1R is suggested to be involved in development of EAC [91]. Ang-II/AT_1R signalling has been observed to promote ESCC progression via mTOR activation. Inhibition of AT_1R reduce cell proliferation in ESCC [106]. Captopril, telmisartan, losartan and irbesartan inhibit cell proliferation and VEGF production in ESCC [89]. Telmisartan has been observed to inhibit cell proliferation via reducing the expression of cyclin A2 and cyclin-dependent kinase-2. Telmisartan arrest cell cycle at G_0/G_1 transition by supressing cell cycle regulatory proteins viaAMPK/mTOR pathway and reducing cyclin D1 and cyclin E [90, 92].

Colorectal Cancer

Both men and women are equally affected by colorectal cancer (CRC), thought to be originated from various risk factors including Age, sex, genetics, nutrition and obesity [93]. In normal colon, Ang-II stimulates absorption of sodium and water thus have important role in colon physiology. CRC usually metastasizes to liver and during this process ACE, Ang-II and MASR were found to be up regulated while AT_1R was down regulated. AT_1R knockout study in CRC mice suggest that Kupffer cells promote liver metastasis by stimulating TGF-β production via AT_1R signalling [94, 95]. Activating protein-1 (AP-1) complex has been identified to initiate CRC. Irbesartan inhibit AP-1 pathway via decreasing JUN gene expression, an encoding gene for AP-1 [96]. Losartan inhibit CRC by downregulation of VEGF, reducing tumour number and blocking blood supply changes during neoplastic angiogenic transformation process [97]. Captopril inhibit the production of Ang-II and promote production of Ang 1–7 by down regulating ACE expression and show antiproliferative effects via MASR in liver metastasis of CRC. Captopril and irbesartan in combination support antitumor effects of Kupffer cells in liver metastasis of CRC [94].

Conclusion

RAS expressed locally in both normal as well as cancerous tissue and plays crucial role in common type of cancers. Wide array of effects are observed by RAS in different cancer types. Ang-II was observed to promote caner progression, metastasis, angiogenesis and EMT mainly via AT_1R while growth, angiogenesis, cell differentiation and apoptosis through AT_2R respectively. Ang 1–7 also found to inhibit cancer

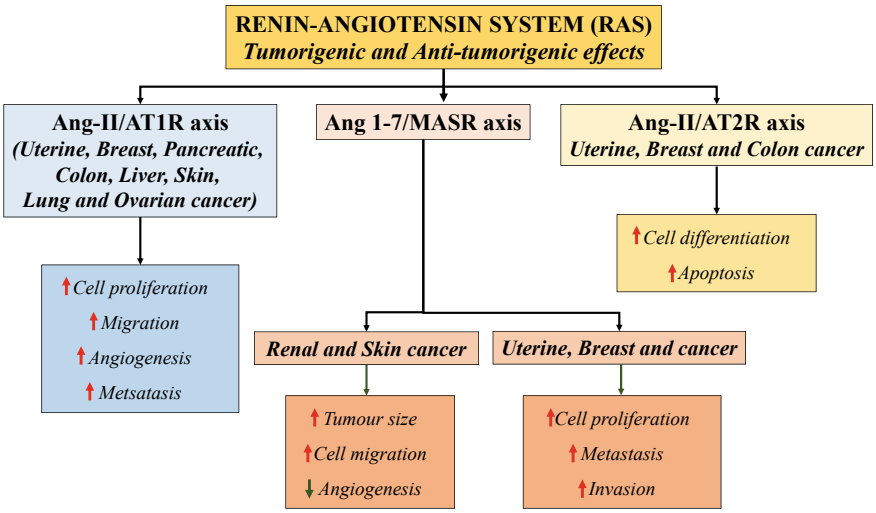

Fig. 18.1 Effect of RAS components on different type of cancers

progression, cell growth, metastasis, motility, angiogenesis, invasion reduce tumour weight and size. Some studies reported anti-tumorigenic efficacy of Ang-II/AT_1R pathway while tumorigenic nature of Ang-II/AT_2R and Ang 1–7/MASR has been observed (Fig. 18.1). However, dual nature of AT_1R in different type of cancers has come to known. Different preclinical studies of ARBs and ACEIs has been justified their anticancer efficacy either alone or in combination with other anticancer agents. Overall analysis suggest the tumorigenic nature of ACE/Ang-II/AT_1R axis while anti-tumorigenic role of ACE-2/Ang-II/MASR axis respectively. Besides having such amount of studies that advocates the use of ARBs and ACEIs in adjuvant therapy in cancer management, appropriate human trials are still missing. Thus actual use of ARBs and ACEIs in clinical practice in cancer management need more rigorous investigations and trials.

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Chapter 19
The New Dimension of the Renin-Angiotensin System in the Hallmarks of Cancer

Kena Daza-Galicia, J. Augusto Landetta-Platonoff, and Talia Wegman-Ostrosky

Abstract The renin angiotensin-system, its well-known systemic regulation of the systemic pressure and its role in hypertension has been widely studies, but this system has also a paracrine function. The RAS was been found to be express in almost every tissue, and there is enough evidence to link it to carcinogenesis and cancer progression. With the purpose of facilitating advances in the study of this disease, Weinberg and Hanahan described a series of cellular characteristics that tumor cells can share, which are known as the Hallmarks of cancer. These are defined as "the distinctive and complementary capacities that cells must acquire during the carcinogenesis process that allow them to survive, proliferate and disseminate". Originally there were 6, but currently up to 10 are "officially" accepted and 4 new ones were proposed by Hanahan in early 2022. In this chapter we describe how RAS is related to the 10 classical hallmarks of cancer, defined as a series of cellular characteristics that tumor cells can share, but also to the 4 new hallmarks described recently.

Keywords Renin-angiotensin · Cancer · Hallmarks · Angiotensinogen · Angiotensin · ACE2 · ACE · Epigenetics · Genomics

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Introduction

The hallmarks of cancer are defined as the distinctive and complementary capacities that cells must acquire during the carcinogenesis process that allow them to survive, proliferate and disseminate. They are a tool to understand the complexity of cancer phenotypes and genotypes [1] In two classic papers, Hanahan D, Weinberg RA establish 10 hallmark: sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, and activating invasion and metastasis, reprogramming of energy metabolism, evading immune destruction, genome instability and inflammation, which promote multiple hallmarks [1, 2]. Recently 4 more emerging hallmarks and enabling characteristics were added: unlocking phenotypic plasticity, non-mutational epigenetic reprogramming, polymorphic microbiomes, and senescent cell [3]. In recent years, biochemical pathways involved in diverse mechanisms, such as the Renin-Angiotensin System (RAS) in blood-pressure control, are now being considered for playing a significant role in carcinogenesis. The RAS, besides its well-known systemic regulation of the circulatory homeostasis, has a local or paracrine function. Local expression of the RAS has been described in multiple tissues, such as liver, kidneys, or pancreas; and also in cancer tissues, such as breast cancer, colorectal cancer, and renal cell carcinoma [4]. Recently, efforts have been made to study carcinogenesis from a different point of view, evaluating biochemical pathways of different already known mechanisms, such as the Renin-Angiotensin System (RAS) in blood pressure control. The system is known for regulating the homeostasis of the circulatory system, but lately it has become an object of study due to its local and paracrine properties of action in multiple tissues. Some examples of this are the liver, kidneys or pancreas, but also cancerous tissues, such as breast cancer, colorectal cancer, and renal cell carcinoma [5].

RAS Pathways

The Renin-Angiotensin system is typically known as a serum hormonal system that produces a series of reactions to regulate blood pressure. The axis begins with the hepatic production of a macromolecule known as Angiotensinogen (AGT), which will be released into the bloodstream in response to a decrease in systemic blood pressure or serum sodium level. This hormone will be cleaved at its N-terminus in the juxtaglomerular cells of the kidney, by the aspartyl protease renin, to produce a decapeptide called Angiotensin I (AngI), which is practically inactive. The remaining 98% of this molecule is going to be known as des(angiotensin I) Angiotensinogen (des(AngI)AGT) [6].

AngI is hydrolyzed, usually in the lungs, by Angiotensin-converting enzyme (ACE) to produce Angiotensin II (AngII), a metabolically active octapeptide. This hormone produces some actions, such as:

- Increasing production of aldosterone in the adrenal glands
- Increasing production of antidiuretic hormone or vasopressin
- Promoting vasoconstriction
- Retaining water and sodium
- Increasing thirst and salt appetite.

Together, these actions increase blood volume, blood pressure, and maintain fluid and electrolyte balance. But this is not all yet.

Currently it has been given a new, much more interesting approach. This new perspective focuses on two recently discovered features:

1. That there are multiple pathways for the generation of angiotensin peptides
2. That bioactive angiotensin peptides can be generated not only in the systemic circulation, but also as local hormones in various tissues and organs [7].

Additionally, if one takes into account that AngII is not the only active metabolite in the system, the probabilities become numerous. Angiotensin III heptapeptide (2–8) (AngIII), angiotensin IV hexapeptide (3–8) (AngIV), and heptapeptide (1–7) are some of the AngII-derived products that have similar or different actions to those of its predecessor. All of these hormones produce their intracellular effects through the following membrane receptors:

- Angiotensin II receptors type 1 and 2 (AT1R and AT2R)
- The MAS receptor (MR)
- The insulin-regulated aminopeptidase receptor (IRAR)
- Angiotensin II receptor type 4 and ACE

Ang-(1–7)/ MAS axis and AngII/ ATR2 are antagonists of the ACE/ Ang II/ ATR1 receptor axis, especially under pathological conditions [8].

Ang-(1–7) can be generated by Ang I or Ang II, the first one requires endopeptidases, specifically neutra endopeptidase (NEP) and prolyl oligopeptidase (PEP). Ang II requires mainly ACE2.The formation of ANG-(1–7) from Ang I by ACE2 involves the production of the intermediate Ang-(1–9) and its subsequent cleavage by ACE or NEP [9, 10].

Another important receptor by the RAS regulation is Pro-renin receptor (PRR). This receptor is responsible for AngII formation. There is evidence that PRR is upregulated in leukemia, prostate cancer and pancreas cancer, for this reason it is thought that AT1R and PRR are suggested to have meaningful roles in carcinogenesis [11]. In Fig. 19.1 RAS pathways and their receptors are explained.

The Hallmarks of Cancer and RAS

Cancer is an extremely complex and diverse disease. For this to develop, there must be a series of conditions or alterations that modify the regulatory mechanisms of cell

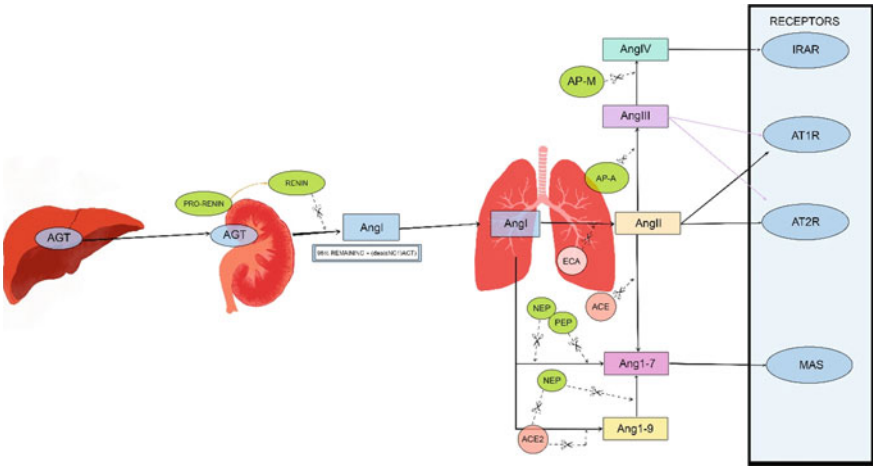

Fig. 19.1 *AGT*, located in chromosome 1, codifies for angiotensinogen, a part of the Renin-Angiotensin System (RAS). Angiotensin II is not the only active substance derived from this chemical cascade, but that there are other biomarkers in this system, such as angiotensin III (2–8) heptapeptide, angiotensin IV hexapeptide (3–8) or the heptapeptide Ang (1–7)

proliferation and homeostasis. The complexity of the disease has led a long way in the search to understand it and find better treatments.

It has been shown that in several of the human tissues, RAS is involved to a greater or lesser degree in the hallmarks of cancer. RAS has activity in different types of tumors like kidney, prostate, bladder, stomach, cervix, brain, pancreas, colon, lung, liver, skin, and hematopoietic cancers [7].

Overexpression of AT1R is typically associated with more aggressive tumor features (larger tumors, higher grade, and higher vascular density) and worse outcomes [12].

The hallmarks of cancer and how the RAS system may be related to each of these characteristics will be described below.

Proliferative Signaling

An essential characteristic of cancer cells is to maintain continuous proliferation. Any type of cell requires growth signaling to enter a proliferative state. These signals come in most cases from the extracellular environment, and will be transmitted through different types of transmembrane receptors and signaling molecules, in a strictly regulated mechanism for cell and body homeostasis. The enabling signals are conveyed by growth factors that bind cell-surface receptors, typically containing intracellular tyrosine kinase domains. The latter proceed to emit signals via branched

intracellular signaling pathways that regulate progression through the cell cycle as well as cell growth.

Therefore, the ability of neoplastic cells to have found a way to imitate these same mechanisms, to make them less dependent on exogenous stimulation and thus produce unregulated cell proliferation is one of the most common.

In summary, tumor cells are capable of producing their own growth signals, which they achieve through 3 mechanisms:

- alteration of extracellular growth signals
- alteration of the transcellular transducers of these signals
- alteration of the intracellular circuits that translate these signals into action.

RAS increases cell proliferation of malignant tumors, due to it directly affects tumor and stromal cells, on the other hand indirectly modulates vascular cell growth through angiogenesis. The intracellular effects of angiotensin II are primarily mediated by two receptors: The Angiotensin type II receptor 1 (AT1R) and the Angiotensin type II receptor 2 (AT2R).

AngII signaling through AT1R: Facilitates cell proliferation and angiogenesis.

AngII signaling through AT2R: Induces antiproliferative signals.

Most of tissues, the proportion of AT1R and AT2R changes dramatically during the postnatal period, with an AT2R being the main one of the adult stages. This observation, together with an AT2R induced stimulation of phosphotyrosine phosphatase activity, suggests that this receptor subtype is involved in the control of cell proliferation and differentiation [13]. Ang II is also involved in the control of proliferation of breast, anterior pituitary, adrenal cortex and endometrium. In fact, it promotes growth in many of our cells by activating phosphatidylinositol via AT1R, which utilizes two main second messengers, triphosphoinositol (IP3) and diacylglycerol, and causes a rise in cytosolic Ca^{2+}, a process linked to mitogenesis in several cell types.

The other intracellular mechanism to control cell growth is through protein tyrosine kinases (PTK) where once again AngII through the AT1R has an important role to modulate tyrosine kinase activity in various tissues either normal or neoplastic; for example, muscle cells, normal and tumor pituitary gland, hormone-independent prostate cancer. Where PTK bind to receptors by various growth factors, thus participating in signal transduction. Breast cancer cell lines and primary breast cancer cells exhibit increased proliferation in response to AngII stimulation that is partly mediated through MEK and PI3K signaling.

Ang (1–7) is thought to be able to inhibit cell proliferation; however, it has the antagonistic effects on some cell types, including fibroblasts, epidermal stem cells, keratinocytes, and hematopoietic progenitors in which this peptide stimulates cell proliferation. This must be considered to understand the role of RAS in the carcinogenesis process and the applicability of RAS blockade as a cancer treatment [6].

Evading Growth Suppressors

Neoplastic cells have the ability to become insensitive to growth inhibition signals. Normally, cell growth and proliferation are controlled by regulatory mechanisms in the cell membrane or exogenous growth inhibitors. For this reason, the ability to ignore or inhibit these signals is vital for cancerous tissues to grow and spread [1]. As described above, we know that AngII is the most bioactive substance in the entire RAS and that it acts in different receptors, among which AT1R and AT2R stand out. Angiotensin type 1 receptors are expressed in all types of tissues, while type 2 is highly expressed on fetal tissues and those who are undergoing a pathological process. Recent research in brain tumors, such as high-grade astrocytomas, has shown the presence of these same two receptors on single tumor cells, which has made us wonder about their involvement in neoplastic processes, as the expression of AT1R and AT2R is highly associated with the degree of malignancy of tumors [13].

So far we know two things: that the selective blockade of AT1R inhibits cell proliferation and neovascularization of different neoplasms, such as gliomas, melanomas, lung carcinomas, prostate adenocarcinomas, kidney and pancreatic cancers and that the activation of AT2R inhibits selectively AT1R related pathways, through direct binding to this receptor, which increases the production of vasodilator substances, inhibition of vascular smooth muscle (VSM) cell growth, and induction of apoptosis [14].

But the presence of both types of receptors in the same cell could also suggest that the pathways of the receptor that have not been inhibited are potentiated, in this case, the AT2R. Therefore, alterations in the ratio of AT1R and AT2R or alterations in their signaling could influence whether cancer cells undergo apoptosis or survive in response to RAS activation [15]. Other substances derived from AngII, such as Ang1-5 and Ang1-7, could mediate proliferative processes in tumor cells. The effects of Ang 1–7 are opposite to those of AngII, which would be vasodilation, diuresis and natriuresis. Although it is known that this hormone acts specifically on *MAS* receptors, the hypothesis that Ang1-7 could participate indirectly with AT1 and AT2 receptors through an interaction with the *MAS* receptor has recently been proposed and supported. In this way, the AT1R signal is reduced and therefore the power and effects of Ang II are too [16]. In addition, Ang1-7 has been shown to inhibit proliferation and induce apoptosis of human lung cancer cells through its MAS receptor (MR) [17].

Resisting Cell Death

The two main mechanisms of cell death are necrosis and apoptosis, which is the cellular mechanism that allows programmed cell death, in order to control the proliferation and cell growth of tissues, whose homeostasis has been lost or threatens to harm other cells. For this reason, the ability to avoid apoptosis is a feature most likely

shared by all types of cancers. Although it is well known that there are two regulatory mechanisms of apoptosis, one intrinsic/ mitochondrial and one extrinsic/mediated by a death receptor, it is also known that there are two classes of components that regulate these processes, sensors and effectors. Sensors regulate and activate effectors when extracellular or intracellular homeostatic conditions are altered and indicate that a cell should be eliminated [1]. For this reason, the mechanisms to avoid cell death through apoptosis are many. An element of the RAS that has been observed to help tumor cells in this regard are the AT1R activated by AngII, which have shown two pathways to prevent apoptosis.

1. The first was observed in colon cancer cells, by suppressing cisplatin-induced apoptosis through the activation of endogenous AT1R and the consequent activation of nF-κb and production of the antiapoptotic molecules BCL-XL and survivin.
2. The second one was found in AngII treatment of MCF-7 breast cancer cells. This therapy suppressed adriamycin-induced apoptosis through the PI3K-Akt pathway and subsequent caspase 9 suppression, but the exact mechanism is not yet fully elucidated [16].

It has also been observed in studies of in vivo models such as xenografts, that the ACE inhibitor captopril induces apoptosis and blocks tumor growth in LNM35 human lung cells. Although this may not be related to the inhibitory effects on ACE, since some studies with lung adenocarcinoma cells show that AngII also induces apoptosis through the activation of AT1R. But although this may seem discouraging, other studies have shown the ability of lisinopril and salarsin to inhibit TNF-α-induced apoptosis [18].

It is also known that the AT2R is involved in the induction of apoptosis, as mentioned above. For example, in prostate cancer cells, an increase in the expression of this receptor induced apoptosis independently of AngII, which is dependent on p38 MAPK, caspase 8 and caspase 3, and its regulatory mechanism depends on the activation of p53. A reduction in procaspase 3 levels was also associated with overexpression of AT2R in a human lung adenocarcinoma cell line, which reduced cell viability and increased apoptosis [19].

Enabling Replicative Immortality

Today it is known that cancer cells need to be able to replicate unlimitedly in order to generate macroscopic tumors. This ability is completely the opposite of the programming of any cell in a homeostatic state, since they can only grow, divide and replicate a certain number of times, determined by senescence and apoptosis programming. But on some occasions, certain cells show unlimited replicative potential, what we call immortalization, a trait that evades senescence and apoptosis [2].

Evidence indicates that telomeres, found at the ends of chromosomes, are centrally involved in the capacity for unlimited proliferation. These telomeres are composed

of multiple tandem hexanucleotide repeats, and will shorten in any physiological cell until eventually the ability to protect the ends of chromosomal DNA from end-to-end fusions is lost. This lack of protection will result in the production of unstable dicentric chromosomes, which can cause an alteration of the karyotype, threatening cell viability. For this reason, the length of telomeric DNA determines how many divisions and replications a cell can go through before the telomeres are largely eroded and lose their protective functions, causing apoptosis.

In order to fulfill this function, it requires the telomerase enzyme complex, the latter consists of the TERT protein, its RNA component (TERC) and other key proteins like DKC1, NOP10, NHP2, and GAR1; generating the extension to TTTAGG(n) nucleotide repeats at chromosome ends. However, this is not enough to prevent telomeres with each incomplete replication of 3' ends of DNA from continuing to be shortened and when they are very short, they are associated with aging and diseases, including cancer. Cancer cells exhibit telomerase activation, enabling replicative immortality [20].

Antihypertensive drugs may affect cellular senescence and telomere length. According to data from the Framingham Heart Study there is a higher frequency of shortening in the length of leukocyte telomeres in hypertensive patients with a higher plasma renin-angiotensin ratio. Furthermore, it was found that treatment with an angiotensin II receptor antagonist could prevent telomere length shortening in spontaneously hypertensive rats by attenuating the aging process, indicating a close link between telomere length and growth and the RAS [21].

To understand this, we need to know two things:

1. Leukocyte telomere length (LTL) records the cumulative burden of oxidative stress and inflammation throughout life.
2. The activation of the RAS is associated with increased oxidative stress and inflammation.

This study found statistically significant relationships of LTL with renin (inverse; $p = 0.005$), aldosterone (positive; $p = 0.002$), and the renin-aldosterone ratio (inverse; $p < 0.001$) in participants with hypertension. But the relationships of LTL with the RAAS biomarkers were not statistically significant in non-hypertensive individuals. These findings raise the possibility that a preponderance of angiotensin II activity relative to aldosterone for blood pressure modulation could promote a greater burden of inflammation and oxidative stress, resulting in a shortened leukocyte telomere length [21]. Besides, Angiotensin II is a potent activator of the p53/p21 pathways, which play a key role in cellular senescence.

Evading Immune Destruction

The immune system is in charge of permanently monitoring cells and tissues in order to recognize and eliminate defective, infected cells or cells with signs of malignancy. For this reason, physiologically and homeostatically, all tumors should be recognized

by this system, in order to be attacked and deprived of further proliferation and complications. In this way it becomes evident that since it is possible for cancer cells to proliferate and grow to produce many types of cancer, there must be mechanisms that make it possible to avoid the recognition and functions of the immune system. At the time this is the only hallmark where RAS has not been involved [1].

Genome Instability

For a cell or tissue to acquire the characteristics that we have been talking about, it is necessary that it suffers genetic alterations that will cause its appearance. These mutations in turn will confer advantages to the mutant phenotypes, allowing uncontrolled growth and eventually tissue invasion and metastasis. But acquiring these characteristics is not easy, since our cells are programmed to detect and repair genetic alterations that allow these characteristics, reducing mutation rates. To become a cancer cell, a way must be found to increase these mutation rates, through genomic instability [1].

Studies have found that the DD genotype of the in/del polymorphic marker of the ACE gene promotes this genomic instability in its carriers. On the one hand, it has been reported that carriers of the D allele have a tendency to increase the frequency of cells with micronuclei. On the other hand, carriers of the homozygous I/I variant tend to have a significantly higher frequency of appearance of cells with karyopyknosis than carriers of the D allele [22].

The in/del polymorphic marker of the ACE gene is associated with apoptotic changes in the cells of children carrying this genotype that have been studied, so it can be used as a prognostic marker of genome destabilization processes in the early stages of development of the human body [17].

Inducing Angiogenesis

Tissues need oxygen and nutrients in order to grow, proliferate and carry out their functions. In fact no cell can be found more than 100 μm from a capillary blood vessel for this very reason. And while the metabolism of cancer cells may vary from that of regular cells, there's no reason to think they don't need it as well [1]. Therefore, it becomes essential to ensure the growth and proliferation of new vessels that nourish tumor tissues, through sustained and unregulated angiogenesis.

Ang II promotes angiogenesis and proinflammatory signaling through AT1R. This signaling results in the activation of transcription factors, such as nuclear factor-κb (nF-κb), molecules of the signal transducer and activation of transcription (STAT) family, and hypoxia-inducible factor 1α (HIF1α). These factors regulate the genes that code for growth factors and cytokines involved in malignancy, so an increase in their expression could trigger a cascade of events that lead to inflammation and

angiogenesis [14]. Another mechanism of activation of angiogenesis through proinflammatory molecules is due to the fact that some inflammatory cells express components of the RAS in the tumor microenvironment, which will increase the secretion of IL-1α, IL-6, IL-8, MCP1, and macrophage colony-stimulating factor (M-CSF) when stimulated by Ang II.AGT belongs to the group of serine protease inhibitors, better known as Serpins, and although it is not an inhibitor as such, it has been seen that it may be involved in the inhibitory processes of angiogenesis. In artificial angiogenic models, AGT and des (AngI)AGT have been shown to inhibit the proliferation of endothelial cells, reducing their ability to migrate and form capillary-like structures. Another RAS metabolite that shares this property is AdAGT, which has shown in in vitro and in vivo studies in mouse cells the ability to suppress intratumoral vascularization and necrosis [6]. Another mechanism involved in Ang II-induced tumor angiogenesis is regulated through growth factors, specifically VEGF. First, the mediation of Ang II in this process has been documented, since it upregulates growth factors such as transforming growth factor-B, insulin-like growth factor-1, basic fibroblast growth, VEGF and angiopoietin 2, all of which are involved in the formation and growth of new blood vessels.

Signaling through vascular endothelial growth factor a (VEGFa) induced by AT1R is a control point in healthy and tumor tissues for neoplastic growth and cancer-associated angiogenesis. This is due to the fact that through the stimulation of AT1R, the expression of VEGF is increased and its blockade inhibits it, for example in gliomas and other tissues. It has also been shown in artificial models that Ang(1–7) can reduce tissue proliferation and angiogenesis by decreasing placental growth factor (PlGF) and VEGF; although it has also been observed in rodent models that this same molecule can promote angiogenesis by stimulating the expression of cardiac VEGF-D and matrix metalloproteinase-9 (MMP-9), which facilitates cardiac repair and ventricular function [6].

For this reason, it would be appropriate to mention that any of the metabolites or substances involved in the RAS system can have actions that favor or harm neoplastic processes, depending on the tissue. Therefore, they should not be described from a single universal point of view as inhibitors or promoters of these processes and hence the complexity of this topic.

Cell Migration, Invasion and Metastasis

Around 90% of cancer deaths in humans are due to the spread of cancer cells to a location other than their origin, that is metastasis. The ability to invade and spread occurs mainly through the lymphatic or blood stream, but also by proximity, which gives tumors the ability to escape the primary focus and occupy new tissues, with their respective nutrients and resources. Seen in this way, it may appear to be simple, but in reality, it is a feature that we still cannot fully understand, due to the complexity and number of processes that are involved in it [16].

Recent studies in humans have shown that genotypic changes or increases in the expression of components of the RAS axis may be involved in the ability of tumor cells to migrate, invade and produce metastasis, but the complex part is that there are some components of this system that favor it and others that inhibit it. The evidence in favor is that a component of the axis that has shown several examples of increasing this capacity in tumor cells is the angiotensin receptor Type 1 (AT1R), since the expression of AT1R correlates with the degree of invasiveness of some tumors. An example of this would be that the increase in the expression of this receptor together with an I/D polymorphism of the ACE (rs1799752), will be related to an increased nodular spread in gastric cancers [16]. Increased expression of AT1R has also been documented to increase the ability to invade new tissues of ovarian adenocarcinomas and malignant borderline tumors, and decreased amounts of expression are associated with benign cystadenomas. Another way of favoring the invasion and tumor migration of the AT1R is through the propagation of a greater expression of VEGF, since in carcinomas with greater invasive capacity a greater expression of this receptor has been documented than in those that do not express it as much.

Ang II stimulation of the AT1R receptor increased the potential for ovarian cancer invasion and has been shown to also induce cell migration in choriocarcinomas through the activation of a PI3K signaling mechanism [6]. On the other hand, it has been observed that the stimulation of breast cancer cells such as T47D and MCF7 with Ang II reduces the invasive and adhesion properties through a process mediated by protein C kinase and the downregulation of α3 and β1 integrins. Traditionally, different types of drugs have been studied and used to inhibit the hypertensive effects of the RAS system, but recent studies on the use of these same drugs in the treatment of neoplastic processes have shown the capabilities of the axis to promote tumor invasion and metastasis. Angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARB) have shown the ability to reduce metastasis. Here are some examples:

- In independent rodent models of non-small cell lung cancer xenograft pulmonary metastasis, use of candesartan (ARB) reduced metastatic burden, while captopril (ACE inhibitor) reduced tumor volume and lymph node metastasis.
- Administration of candesartan in athymic mice transplanted with SKOV-3 also demonstrated reduced peritoneal metastasis [18].

Reprogramming of Energy Metabolism

In tumors, the rate of glucose uptake is dramatically increased and lactate is produced, even in the presence of oxygen and fully functioning mitochondria. This process is known as “The Warburg effect” [23]. It is believed that RAS could be directly or indirectly related to the Warburg effect, since high glucose levels in various tissues can increase the expression of the (pro)renin receptor, AGT,ACE, and AT1R and stimulate renin.

RAS activation by AngII can generate reactive oxygen species (ROS) and subsequent proinflammatory and proangiogenic signaling, which depends on cell-type-specific NADPH oxidases that activate downstream signaling cascades, including the activation of the MAPK and PI3K pathways and other redox-sensitive transcriptional factors such as hypoxia-inducible factor 1α (HIF1α) [6].

Inflammation

Inflammation has a strong relationship with the development of cancer, clear evidence has been obtained that chronic inflammation plays a fundamental role in tumorigenesis, including cell transformation, promotion, survival, proliferation, invasion, angiogenesis and metastasis [1].

Approximately 90% of cancers are associated with somatic genetic variants and environmental factors. Many environmental factors of cancer and risk factors are related to a greater or lesser extent to some form of chronic inflammation, for example; chronic infections, smoking, inhaled pollutants, and dietary factors [20].

RAS is a very important mediator of inflammation. AT1R that controls the transcription of proinflammatory mediators in the tissues and in infiltrating cells, like macrophages.

The inflammatory proteins which are induced by the AT1R are: interleukin-1 beta (IL-1b), tumor necrosis factor-alpha (TNF-α), plasminogen activator inhibitor type 1 (PAI-1), adrenomedullin transforming growth factor-beta (TGF)-β, and signal transducer and activation of transcription (STAT) family members and HIF1α. AT1R expression could be a consequence of stress and cell damage in the stromal cells of the neoplastic cells.

AngII promotes vascular inflammation by exerting pleiotropic actions, such as vasoconstriction, migration, proliferation and hypertrophy, increased extracellular matrix formation, and activation of NADPH oxidases. Which generates endothelial dysfunction as well as structural remodeling. Increased expression of two factors essential for vascular inflammation by reducing LOX-1 expression [5].

Many components of RAS can be found in different cells of the tumor microenvironment, such as endothelial cells, fibroblasts, monocytes, macrophages, neutrophils, dendritic cells, and T cells [24].

Senescent Cells

Cellular senescence refers to cell cycle arrest as a consequence of limited proliferative capacity of normal human somatic cells. Senescent cells are arrested in either the G1 or G2/M phase of the cell cycle. With age the accumulation of senescent cells increases in several tissues. During cell senescence there is a shortening of telomeres that occurs after extensive cell division. It is possible to generate a similar phenotype

know as Stress-induced premature senescent (SIPS) which can be induced by intrinsic as well as extrinsic stress. These deleterious insults include DNA damage, mitochondrial dysfunction, activation of certain oncogenes and inflammatory responses, all of which are classically associated with the pathological aging process and cancer [25].

There are two main signaling mechanisms by which AngII promotes vascular senescence; in the first one, by the AT1R has been demonstrated to induce two types of senescence in human vascular smooth muscle cells (VSMC), namely replicative senescence after a period of 30 days Ang II stimulation compared to a dose-dependent increase in premature VSMC senescence after 24 h stimulation interestingly, AT1R blockers (ARB) inhibit Ang II induced vascular cell senescence, therefore showing an anti-aging effect [19]. A relevant point, although the participation of the AT2R continues to be an enigma, in recent years it has been shown to generate the opposite signaling output as previously described for AT1R, thus, functionally antagonizing AT1R-mediated vascular senescence. In this study, Min et al. showed that deleting the AT2R enhances vascular senescence through methyl methanesulfonate 2 (MM2) inhibition [6].

Unlocking Phenotypic Plasticity

Cancer cells are plastic—they can assume a wide range of distinct phenotypes. Plasticity is integral to carcinogenesis and progression, as well as to the emergence and maintenance of intratumoral heterogeneity [3]. RAS proteins and peptides are expressed in many cell types and also in cells of the tumor microenvironment, such as endothelial cells, fibroblasts, monocytes, macrophages, neutrophils, dendritic cells, and T cells [10]. In a model of focal segmental glomerulosclerosis, monitoring origin lineage cells with reported mice showed that administration of the ACE inhibitor enalapril or the AT1R inhibitor losartan stimulated proliferation of renin lineage cells in the juxtaglomerular region. Another study, prolonged treatment with RAS inhibitors was shown to activate renin-producing cells and lead to arterial hypertrophy, a concentric thickening of intrarenal arteries and arterioles in both mice and humans. In conclusion, it has been considered that cells of the renin lineage play a role in the regeneration of different types of glomerular cells after injury [26].

Microbioma

Approximately 100 trillions of microbes including bacteria, virus, phage, yeasts and fungi inhabit ecological niches in humans like lung, gut, skin, genital tract and mouth, this creates a symbiotic. The human microbiota represents the microbiome taxa and this is changing all the time and the catalog of this microbes as well as their genes is known as the human microbiome is. If an imbalance occurs between

human-microbiome, known as dysbiosis, there is a high probability of developing cancer [3]. Also RAS has an important relationship with gut microbiota alterations in RAS shift microbiota composition and metabolic activity, while gut microbiota derived metabolites wich modulates the gut RAS. Through regulation of intestinal amino acid transport, prior research reported that ACE2 plays a key non-catalytic role in gut biology and modulation of the gut microbiota [3, 27].

Epigenetics

As we have already seen, the instability and mutation of the genome (DNA) is a fundamental component in the formation and pathogenesis of cancer. But recently it has been observed that cancer progression is possible without the presence of a genetic mutation.

This problem has led us to wonder what could be the mechanism behind this evolution. For this reason, purely epigenetic programming of characteristic cancer phenotypes was proposed, to which increasing evidence has supported and accepted that there may be epigenetic alterations that contribute to the acquisition of distinctive capacities during tumor development and malignant progression.

But first things first. Epigenetics is defined as "the study of heritable changes in gene expression that cannot be explained by alterations in DNA sequence". This genetic expression through the transcription of genes and organization of the nucleus will be regulated by different factors, among which the following stand out:

- DNA methylation
- Histone post-translational modifications (PTMs)
- Histone positioning.

The modulators of these processes are going to be proteins that place or remove chemical modifications from DNA nucleotides and within the amino-terminal regions of histones [28].

Recently, ncRNAs have become an active area of study since it has been discovered that they can be scaffolding elements for transporting proteins with epigenetic functions, which could make them epigenetic modifiers [7].

MicroRNAs (miRNAs) are short ncRNAs, with 19–23 nucleotides that function in post-transcriptional gene regulation and RNA silencing by base-pairing with a complementary sequence [28].

As such, miRNAs are not considered epigenetic components, but some of them are modulated by epigenetic mechanisms and are interesting for this chapter, because there is increasing evidence that miRNAs are involved in RAS regulation and that their expression could be altered in some types of tumors, since they can target the same genes and have oncogenic or carcinostatic functions [29].

MicroRNAs That Promote Cancer

miR-155

Various studies have shown that miR-155 disregulates the expression of the Angiotensin 1 receptor (AT1R) gene mRNA, which is strongly associated with the malignant transformation of B cells into tumors.

miRNA21

One of the important new players found in lung cancer is miRNA21. A positive correlation between the EGF receptor (EGFR) and this type of MicroRNA has been demonstrated. Within these findings, it was also detected that EGFR-tyrosine kinase inhibitors (EGFR-TKI) can suppress miRNA-21, suggesting that miRNA-21 expression is positively regulated by EGFR. In turn, increased expression of miRNA-21 was found to be associated with acquired resistance to EGFR-TKIs in non-small cell lung cancer (NSCLC). Therefore, the hypothesis has been suggested that miRNA21 is the culprit of an NSCLC.

miRNA-221 and *miRNA-222.*

miRNA-221 and miRNA-222 are involved in cell proliferation by inhibiting the cell cycle regulator, p27kip.

miR-141 and *miR-200a.*

Enhanced expression of miR-141 and miR-200a has been shown to mimic p38α deficiency and increase tumor growth in mouse models, but also improves chemotherapeutic response. Higher levels of miR-200a were found in high-grade human ovarian adenocarcinomas along with low concentrations of p38α and increased oxidative stress.

MicroRNAs Against Cancer

miRNA-155.

Angiotensin II enhanced vascular smooth muscle cell (VSMC) viability in a dose-dependent manner in cultured cells from the aorta, which were incubated with AngII. Besides, miRNA-155 prevented this effect of AngII on VSMCs, and further decreased ATR1 gene and protein expression. The antiproliferative effects of this MicroRNA have also been demonstrated in human extravillous trophoblast-derived HTR-8/SVneo cells by downregulation of the cyclin D1 pathway and induction of apoptosis. This effects were also explored in mice.

miRNA 205.

miRNA 205 is a tumor suppressor miRNA, so experimental studies found that Olmesartan (an angiotensin II receptor antagonist) caused overexpression of miRNA-205 and decreased VEGF-A levels, which contributed to the antitumor effect in cervical cancer cells.

Further studies are needed to reveal the role of specific miRNAs in many types of tumors and malignancies and their corresponding interaction with RAS, considering that RAS blockade may be a therapeutic option in certain circumstances [29].

Antihypertensive in Cancer

Drug repurposing consists of indication of existing drugs with a new purpose. The antihypertensives in cancer have great advantages comparted to the development of new drugs since lower the economic standards, shortening the time to include a new treatment in patients; another of their advantages is the oral administration, this drugs are well tolerated and cheaper due to the lacks of patent [18].

Antihypertensives involved in RAS and cancer treatment can be classified according to the hallmarks of cancer which interact, they are described below.

1. Resisting cell death.

   The categories of the antihypertensives in this hallmark are: Aldosterone antagonist and β -blocker.

   Aldosterone antagonist like spironolactone act by reducing survivin mRNA expression and increases protein degradation by proteasomes.

   The main β-blocker that has been used is propanolol wich act in the downregulation of Bcl-2 and Upregulation of Bax and other pro-apoptotic molecules.
2. Deregulating cellular energetic.

   The only group of antihypertensive who attacks this hallmark is β -blocker: The Atenolol by inhibition of respiratory chain breast cancer cell lines, thus reducing oxygen consumption, this drug make synergism with Metformin. Intead the Propanolol by the inhibition of hexokinase 2 and GLUT1 transporter and make synergism with Vemurafenib.
3. Sustaining cellular energetic.

   This hallmark is affected by four groups of antihypertensives: Angiotensin converting enzyme inhibitor (ACEI), Angiotensin receptor blocker (ARB), Aldosterone antagonist and β -blocker.

   The main drug used in ACEI is Captopril through hyper segmentation and induction of cytotoxic activity of tumor associated neutrophils, mediated by mTOR. And increases anti-tumor T cells and reduces immunosuppressive cells.

In case of ARB the drugs are Valsartan, Candesartan participating in the upregulation of antitumoral T cells (CD3 + and CD8 +) and reduction of immunosuppressive cells activity, also make synergism with Anti-PD-L1 and anti-CTLA4 antibodies.

In the Aldosterone antagonist exist evidence that spironolactone increased surface expression of NKG2DL, recognized by NK cells. This is mediated by RXRγ rather than the MR.

β -blocker, in this case propanolol through Inhibition of adrenergic signaling upregulates tumor-infiltrating $CD8^+$ T cells.

4. Evading growth suppressors.

   ACEI like Perindopril and fosinopril: Downregulation of cyclin D1, arresting cell cycle at G1.

   ARB like losartan: Inhibits production of cyclin D1, preventing progression across the G1 phase of the cell cycle.

   β-blocker in this case propranolol increases the fraction of cells in G0/G1.

   ACEI for example captopril: -Hypersegmentation and induction of cytotoxic activity of tumor-associated neutrophils, mediated by mTOR. And the other hand Increases antitumor T cells and reduces immunosuppressive cells.

   ARB for example Valsartan, Candesartan: Upregulation of antitumoral T cells ($CD3^+$ and $CD8^+$) and reduction of immunosuppressive cells activity.

   Aldosterone antagonist like spironolactone: Increased surface expression of NKG2DL, recognized by NK cells. This is mediated by RXRγ rather than the MR.

   β-blocker in this case propranolol through inhibition of adrenergic signaling upregulates tumor-infiltrating $CD8^+$ T cells.

5. Activating invasion and metastasis.

   ACEI like Captopril, perindopril and fosinopril: Direct inhibition of matrix metalloproteinase activity and Fosinopril decrease TFG-β activity.

   ARB in this case losartan: Downregulation of TFG-β and has synergism with FOLFIRINOX.

   Aldosterone antagonist like spironolactone: Activation of RXRγ, which promotes the expression of antimetastatic gens *TIMP2* and *TIMP3.*

   β-blocker like Propranolol: Inhibition of stress-induced metastasis, mediated by M2 macrophages and Downregulation of MMP-2 and MMP9.

6. Inducing angiogenesis.

   ACEI like Perindopril, benazepril, captopril: Downregulation of vascular endothelial growth factor transcription. This drugs have synergism with Gemcitabine and Sorafenib.

β-blocker like Propranolol: Inhibition of tubulogenesis of endothelial cells and MMP-9 secretion. reduces the mRNA expression of vascular endothelial growth factor (18).

Conclusions

RAS is a complex system that has many interactions in the process of carcinogenesis. Generally, the AngII/AT1R axis is considered to promote tumor growth, whereas AngII/AT2R and Ang(1–7)/MAS signaling have opposing effects. In this chapter we find a connection between most of the Hallmarks of cancer and RAS. This connection could help to develop new cancer treatment opportunities, but also strategies for cancer prevention.

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Chapter 20
Renin-Angiotensin System in Hematological Malignancies

Nidhi Gupta, Shraddha Kapoor, Aparna Sharma, and Alpana Sharma

Abstract The regular functioning of the human body is the result of interactions between numerous organs and biological systems, with the Renin-Angiotensin System (RAS) playing a crucial role in maintaining homeostasis for human existence. Though RAS is mainly known for regulation of blood pressure, however, its role in cancer development and progression has been emerging nowadays wherein this system was found to promote angiogenesis and inflammation in tumor niche. RAS comprises of numerous elements, of which angiotensin converting enzyme (ACE) is of utmost significance which converts angiotensin I (ATI) to angiotensin II (ATII), which then undergoes downstream signaling via binding to AT receptors. This signaling aids in hematopoiesis and the associated malignancies like leukemia, myeloma and lymphoma. ACE and the downstream signaling cascades upregulation could be seen in cancer models which are linked to the activation of multiple signaling events involving NF-κB, PI3K, MAPK, etc. The importance of RAS in hematological malignancies led to the exploration of RAS inhibitors for the cancer treatment. There are certain categories of RAS inhibitors which include ACE inhibitors, Angiotensin receptor blockers or renin inhibitors which have been tested in vitro either alone or in combination for therapeutics of various cancers including hematological malignancies. The local RAS and its association with cancer might opens up new avenues for investigation and development of novel therapies for hematological malignancies.

Keywords Renin-angiotensin system · Cancer · Therapeutics · Hematological malignancies · Signaling · ACE · ACE inhibitors

Nidhi Gupta and Shraddha Kapoor, equal contribution

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Introduction

The normal functioning of human body is interplay of the interactions among various organs and biological systems, out of which the role of Renin-Angiotensin system (RAS) is inevitably significant for maintaining homeostasis in human being's survival [1, 2]. Since, from numerous years it has been well studied in context with heart-kidney axial system and also, as a key player for regulating blood pressure in the body [3]. In addition to systemic blood pressure regulation, it maintains tonicity of vascular tissues and extracelluar volumes, ion concentration gradient and vasomotor functions [1, 4]. Tissue specific expression of local RAS is responsible for diversity in functionality and processes [3]. Augmented levels of RAS activation have been evident in many organ specific tumors like kidney, pancreas, brain, cervix, etc. and also in hematopoietic cancers [5].

Apart from the role documented for "classical" RAS, the data has emerged discussing the revolutionary involvement of RAS in plethora of physiological as well as pathological conditions. One of the relevant areas of study is the implication of RAS in the biology of cancer. Cancer in itself is a broad area in focus for association of RAS, however, the scope of this chapter highlights the role of RAS in relation to Hematological Malignancies, mainly leukemia (Acute Myeloid Leukemia, Chronic Myeloid Leukemia, and Chronic Lymphoid Leukemia etc.), myeloma and lymphoma, and scope of treatment by targeting RAS in context to hematological malignancies which is less known till date.

Componential Organization of RAS

RAS system involves complex network of its multiple peptides, receptors and enzyme components; the key step of significance in circulating RAS is production of Angiotensin II (ATII) via two-step process involving Renin and Angiotensin-Converting Enzyme (ACE) [6] as shown in Fig. 20.1.

Renin is produced by the kidneys in response to decreased arterial pressure, reduced sodium in the distal tubule or sympathetic nervous system activity via the β-adrenergic receptors. Renin is secreted into the bloodstream by juxtaglomerular cells, where it encounters angiotensinogen (AGN), which is ordinarily produced by the liver. Renin catalyses the conversion of angiotensinogen (AGN) to angiotensin I (ATI), which is rapidly cleaved by angiotensin converting enzyme (ACE) to create ATII. ATII causes the adrenal glands to release aldosterone, which accelerates sodium and water reabsorption, increasing blood volume and blood pressure. ATII also causes vasoconstriction of the arterioles in several organs of the body by acting on smooth muscle. Furthermore, ATII stimulates the posterior pituitary gland to release antidiuretic hormone, which causes water retention and activates the thirst reflex [7, 8].

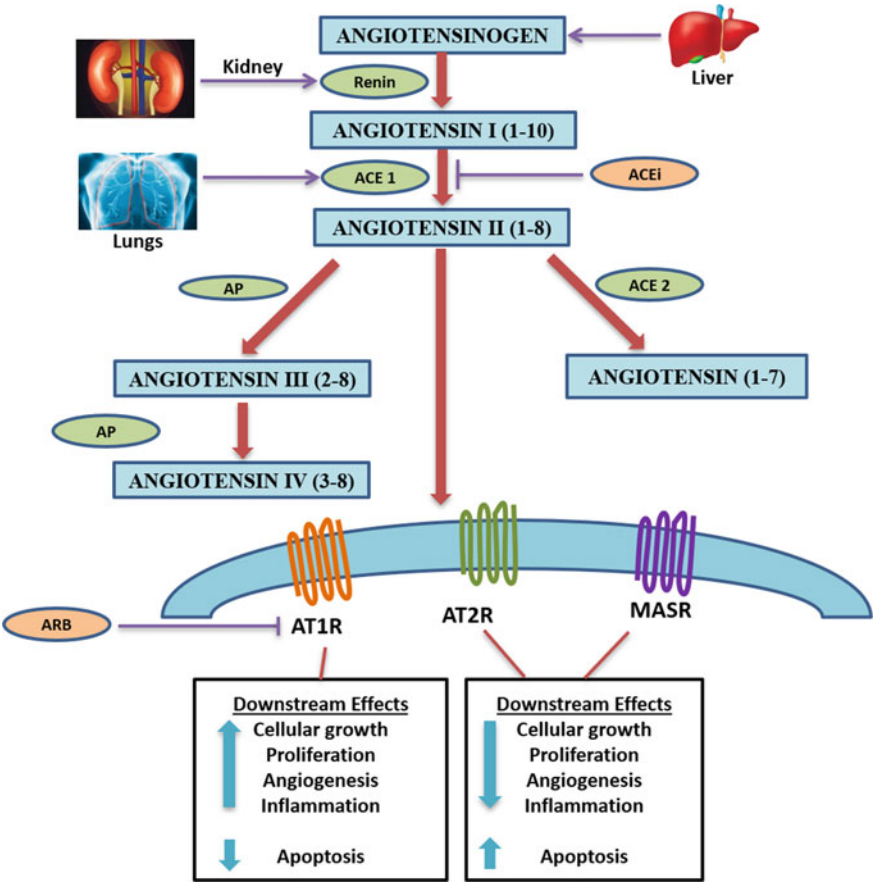

Fig. 20.1 Componential organization of RAS involving numerous active peptide and receptor. Renin is secreted into the bloodstream by the juxtaglomerular cells of kidneys, where it interacts with angiotensinogen (AGN), which is produced by the liver. Renin catalyzes the conversion of angiotensinogen (AGN) to angiotensin I (ATI), which is then cleaved into ATII by angiotensin converting enzyme (ACE). The active biopeptide ATII elucidates its effect via AT1R and AT2R receptors. ACE2 also fragments ATII, resulting in AT (1–7) that bind with the MAS receptor (MASR). ATIII and ATIV are also created in the sequential processing of ATII. [RAS: Renin-Angiotensin system; ACEi: ACE inhibitor; ARB: Angiotensin Receptor Blockers; AP: Aminopeptidase]

The active biopeptide ATII elucidate its effect via AT1R and AT2R receptor through downstream signaling. Also, ATII is fragmented by ACE2 to AT (1–7) which further interact through MAS receptor (MASR). Further ATIII and ATIV are also produced in stepwise processing of ATII [9].

RAS and Cancer: What's Known Till Date?

There has been a recent upthrust of RAS in relation to cancer and malignancy implicated by various studies wherein the involvement of RAS in induction of cell proliferation, maintaining growth stimulatory signals, promoting angiogenesis and inflammation, combating apoptosis etc. were shown as the attributes of RAS functionality indicating its characteristics synchronous to cancer progression [10]. It has been found that impaired regulation of RAS in case of tumorigenesis might be crucial for studying its significance in cancer.

Carcinogenesis being a multi-factorial disease involves various hallmarks such as angiogenesis, inflammation, telomerase activity, etc. In addition, tumor microenvironment is a pre-requisite for the continuous proliferation of cancerous cells. Our group had reported the involvement of these factors in different cancers ranging from solid tumors to hematological malignancies [11–15]. In relation to RAS, the signaling promoted by peptide Angiotensin II is observed by studies to enhance angiogenesis mediated by vascular endothelial growth factor (VEGF) via growth of cellular vasculature [16]. Besides, other ways of stimulation of pathogenesis mechanism for evasion and growth of tumor mainly comprises of aggravation of ATII expression and comparative expression of Angiotensin converting enzymes-1 and 2 [17].

RAS Signaling: Role in Oncogenesis

Numerous findings have highlighted that the downstream regulation of cellular RAS signaling pathways may be associated with pace of tumor development or regression. Some evident molecule with anti-carcinogenic effect is AT2R receptor-interacting proteins (ATIP) that work in conjunction with AT2R receptors whose downstream function is generated by action of peptide ATII. These ATIP mainly ATIP-1 and ATIP-3 collectively promote tumor inhibitory actions by blocking receptor tyrosine kinase activation, thus, halting cellular proliferation [5, 18].

While interaction of ATII with AT1R has been contradictory to action with AT2R; tumor cells were found to have exacerbated expression levels of these AT1R receptors. ATII bio peptide also has cellular proliferation promoting effects in turn by ultimate increased levels of transcription factor like Nuclear Factor κB (NF-κB). This may be attributed by enhancement of apoptosis via AT2R receptor downstream signaling events supporting its anti-apoptotic activity while ATII binding to AT1R receptor in cancerous cells inhibits cell death and promotes cell growth and survival [19, 20]. Moreover, the MAPK or PI3K mitotic cascade events by AT1R activation via binding to ATII leading to NF-κB stimulation resulted in promotion of Bcl-xL and Bcl-2 anti-apoptotic molecules [6]. The amelioration of these mitotic effects by inhibiting molecules like ATII and NF-κB have been principally observed

and utilized for RAS blockage in order to enhance apoptosis and decrease cell proliferation [5, 21].

Though multiple signaling pathways including PI-3 K, MAPK and JAK-STAT are deregulated in carcinogenesis and shown to be regulated by RAS to favour tumor progression. Another pathway such as adenosine monophosphate (AMP)-activated protein kinase (AMPK) signaling system is also reported to be relevant in oncogenesis. With the stability of p53 and the cyclin-dependent kinase inhibitors p21WAF1 and p27CIP1, AMPK pathway possess tumour suppressor role. This pathway also limits the production of mTOR-1 and hypoxia-inducible factor-1 (HIF-1), as well as fatty acids, triglycerides, cholesterol, glycogen, and proteins, resulting in cell growth inhibition. The association between RAS and AMPK signaling pathway was observed by in vivo studies in uninephrectomized (UNX) rat experimental model. AMPK expression reduced in carcinogenesis with increase in mutant p53 and Ki-67, however, antagonization of RAS by inhibitors resulted in elevated AMPK, hence, proves to be protective against malignant transformations.

Inhibition in levels of molecules like hypoxia-inducible factor-1α (HIF-1α) and mTOR-1 are also responsible for regulation of AMPK signaling pathway [5, 22].

Hematopoietic RAS

According to previous molecular biological investigations, the existence of local intrinsic bone marrow RAS was proved which was found to be regulatory in scenarios involving physiological and pathological hematopoiesis; one of earliest experimental evidence relating hematopoiesis production and differeniation in bone marrow to the components of RAS system [23]. The major effector peptide of RAS, angiotensin II (ATII), stimulates hematopoietic progenitor cell proliferation via binding to angiotensin type 1a (AT1a) receptors present on $CD34^{+}$ hematopoietic stem cells. The existence of ACE in human primitive lympho-hematopoietic cells, embryonic, foetal and adult hematopoietic tissues has also been described [24, 25]. The elements of RAS system have been observed to be modulating in proliferative processes mainly; myelopoiesis, erythropoiesis, thrombopoiesis and even process involving formation of other immune cell linages [19].

The presence of a regulatory tissue RAS system has eased the investigations such as tracing the level of specific levels of glycoproteinaceous enzyme ACE would help in studying of pathophysiological aspect of many diseases [26].

RAS in Hematological Malignancies

Since RAS elements function in hematopoietic processes throughout the development, hence, there has been data suggesting the importance of this system in hematological malignancies which mainly comprises of leukemia, myeloma and lymphoma.

The present chapter discussed the involvement of RAS in these types of hematological malignancies.

RAS and Leukemia

There are many studies elucidating association between presence of local bone marrow RAS with hematopoietic functioning and production of the neoplastic blood cells [23]. In the report by Aksu et al. the group evaluated the level of surface antigen marker ACE (CD143) isolated from bone marrow of Acute Myeloid Leukemia (AML) patients and found to be upregulated in leukemic cells of myeloid blast origin [27]. The levels of renin and ACE were investigated in both bone marrow aspirate and peripheral blood samples of leukemic and control patients. Unlike Renin, levels of ACE have shown to be increased significantly in bone marrow samples as compared to peripheral blood [28]. Regulatory role of an ACE-Acetyl-N-Ser-Asp-Lys-Pro (AcSDKP) system is also under scrutiny check in vicinity of bone marrow microenvironment for its role in involvement of hematopoiesis. The degradation of AcSDKP or goralatide is facilitated by ACE, hence, enhanced degradation could be observed due to increased production of ACE in leukemia [29–32]. The mechanism of AcSDKP to act as negative regulator of hematopoiesis is by keeping the S-phase population of hematopoietic and progenitor cells in non-dividing quiescent phase [19, 33] (Fig. 20.2). Role of analogs of AcSDKP are under study which remain unaltered in terms of degradation by ACE thus, playing important function in preventing hematopoiesis [34] (Fig. 20.3).

The evidence of disease specific expression and activation of local RAS such as a differentially altered activity in leukemia and lymphoma was indicated by study of Uz et al. The comparative expression monitoring in case of groups of myeloid or lymphoid blood malignancies for the important components of RAS mainly ACE1, ACE2, Renin and Angiotensin have been carried out [35]. The expression levels of Renin mRNA was found to be significantly elevated in lymphoid compared to myeloid classified disease, whereas for ACE1 and ACE2 expression elevation were found vice versa. The study also mentioned mRNA expression of RAS elements with active and without active disease status in patients [35].

The major RAS components' (Renin, ACE and angiotensinogen) specific gene expression levels using real time quantitative PCR analysis method was studied in K562 experimental cell line to evaluate role of RAS in leukemia and further paving way for it to act as in vitro model system for finding effect of RAS system effecting drugs and association between respective leukemic cell proliferation pattern [36, 37].

While specific with quantitative expression of ACE, renin and angiotensinogen in case of AML, the patient had relevantly higher mRNA expression in the bone marrow samples [38]. A contemporary study to the work of Beyazit et al., traced the occurrence of AT receptors and other RAS components in cultured line of marrow stromal cells as well as in lineage of bone marrow cells, concluding the presence of local RAS supported autocrine and paracrine mechanism of hematopoiesis due to auto regulatory synthesis of marrow stromal cells mediated synthesis of Angiotensin II [39].

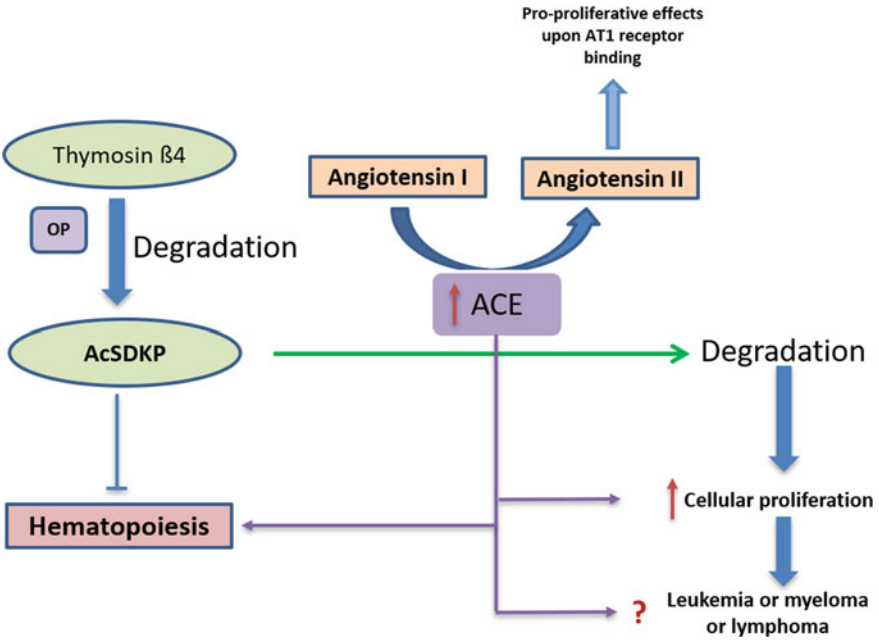

Fig. 20.2 Scrutiny check by ACE-AcSDKP system on hematopoiesis and the associated malignancy. Thymosin β4 peptide is produced by various cell types whose degradation by oligopeptidase (OP) produce a smaller peptide AcSDKP (Acetyl-N-Ser-Asp-Lys-Pro) which inhibits hematopoiesis in bone marrow microenvironment. The degradation of AcSDKP by ACE promotes cellular proliferation and enhances the process of hematopoiesis and might also play a crucial role in hematological malignancy

RAS and Lymphoma

Angiotensin Converting Enzyme (ACE) being an important enzyme for octapeptide ATII peptide production and RAS functioning, also associated with certain neoplastic conditions and while regulated by AcSDKP peptide negative regulator vis-à-vis affecting cellular proliferation in bone marrow environment [27, 36, 37, 40]. Thus, due to numerous significant role of this enzyme, ACE expression levels were mapped in macrophages of lymphoma linked proliferative disorder Hodgkin's disease in lymph nodes to decipher its relationship in RAS functionality and thus, mechanism of tumorigenesis. This study demonstrated the ACE expression in intratumoral residual macrophages in lymph nodes of patients suffering from Hodgkin's lymphoma (HL) versus negligible ACE expression obtained in case of controls and lymphoid hyperplasia. The study has prominent drawback of not demarcating in quantification of level of ACE expression in relation to the strength of staining and unable to differentiate between nodular sclerosing and mixed cellular sub categorized cases of Hodgkin's lymphoma. ACE expression was reported to be directly proportional to macrophages infiltration is lymph node in HL which is linked to

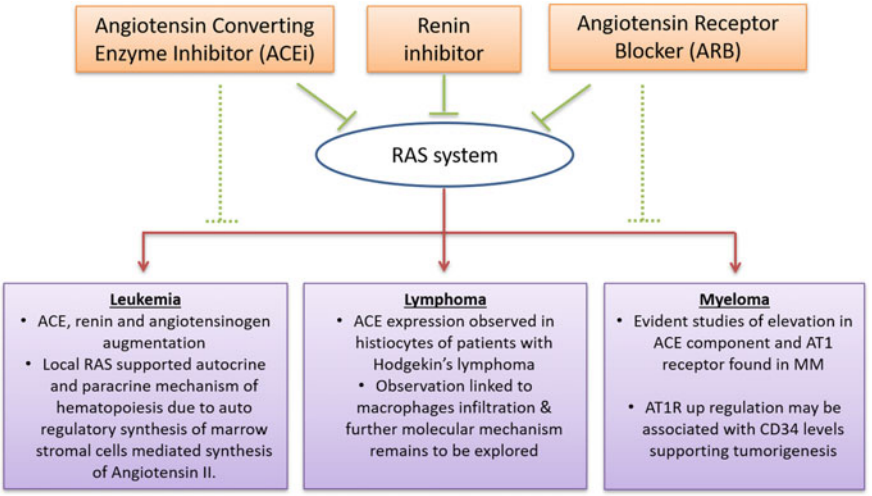

Fig. 20.3 Disease specific expression observed in hematological malignancies. The association between local bone marrow RAS, hematopoiesis and neoplastic conditions such as leukemia, lymphoma and myeloma have been elucidated as per the literature. Various inhibitors of RAS contribute have been tested in vitro for cancer therapeutics and hence, possess the potential of combating and managing the hematological malignancy

interaction between RAS system and thereby, enhancing evidence of lymphoma generation [41]. The other molecular and cellular evidences available with relevance to lymphoma (both Hodgkin's and non-Hodgkin's) generation in turn with tissue RAS system is least explored and further require experimental and clinical elucidation. The pronounced disease prevalence of Hodgkin's Lymphoma in pediatrics population as compared to adult counterpart and treatable nature of these lymphoma provides an urge to be explored with greater scientific upthrust in the field to ensure disease free life to these individuals (Fig. 20.3) [42].

RAS and Multiple Myeloma

The levels of circulatory ACE enzyme important for investigation of systemic and portal disease was found to be augmented significantly in clinical cases of Multiple myeloma in bone marrow compared to controls. However, intra group staging of these MM patients according to International Staging System (ISS) criteria reported non-significant variation in ACE levels [24, 26]. Similarly other studies provide evidence of elevated RAS component expression levels directly relating to activation of local bone marrow RAS functioning in MM disease state (Fig. 20.3). Further reports indicate enhanced AT1R type receptor in MM and correlated with CD34 activation in bone marrow supporting the idea of AT1R receptor promotion of tumorigenic

activity and angiogenesis (as mentioned before in the chapter under section RAS signaling) [43].

RAS Inhibitors as Cancer Therapy: Single or Combinatorial

The role of RAS in tumor progression and pathogenesis has been documented in numerous cancers. ATII signaling in RAS system drives neovascularization and boosts cell proliferation. An increasing amount of evidence demonstrates that ATII signalling increases VEGF-mediated angiogenesis in malignancy by directly impacting tumour and stromal cells as well as indirectly altering vascular cell development during angiogenesis. Hence, blocking of this system might be beneficial to combat the malignancy. Thus, the class of drugs that modulates the RAS functionally has been observed to be efficacious in in vitro and in vivo cancer models [6]. Suppressing the action of RAS by administration of Angiotensin Receptor Blockers **(ARB)**, which acts through inhibiting the activity of AT1R; or ACE inhibitor **(ACEi)** which inhibits enzyme ACE, thus preventing synthesis of ATII; or directly renin inhibitor; all function as RAS antagonists, block the RAS influence on angiogenesis, reducing the growth of cancer and possibly lowering cancer risk over time and has proved to be preventive and helpful in treatment of certain types of cancers [6, 44]. However, these medications can impact additional potential pathways mediated by ACE internalisation and endonuclear localization in distinct cell types, which may be related to certain aspects of carcinogenesis. ACEi apparently inhibited tumour growth in both cancer patients and animal models, as well as the carcinogenesis process via inhibiting angiogenesis (Table 20.1) [16, 45, 46].

RAS inhibitors have been tested in hematological malignancies to evaluate their efficacy for treatment. ACEi captopril and trandolapril and ARB losartan decreased cell proliferation, increased apoptosis, and decreased c-myc expression in leukemic cell line in vitro [47, 48]. Captopril also reduced tumor burden in mice with colorectal liver metastasis following partial hepatectomy and hence, reported as an adjunct therapy [49]. Another ACEi Enalapril induced apoptosis in HL60 acute promyelocytic leukaemia cells via downregualtion of STAT5A [50]. Enalapril also significantly slowed the progression of pancreatic cancer precursor lesions by inhibiting NF-kB in tumour cells [51, 52]. Other in vivo studies have shown that perindopril, another ACEi has a potential inhibitory effect on tumor growth due to suppression of VEGF-induced angiogenesis in head and neck squamous cell carcinoma & renal cell carcinoma [53]. In addition, Perindopril or fosinopril or ARBs losartan inhibited the progression of diethylnitrosamine-induced hepatocellular carcinoma in mice via inactivation of NF-κB [21]. ARBs such as Telmisartan appears to downregulate Bcl-2, an anti-apoptotic protein, and activate caspase-3, causing cell death of renal cell carcinoma [54]. Moreover, the basic mechanism of action of ARB like Irbesartan

may act by inhibiting the proliferating activity of bioactive angiotensin element which may in turn inhibits proliferating-cell nuclear antigen (PCNA) and cyclin D1 expression and modulate p53 expression via AT1R receptor in experimental MCF-7 breast cancer cell line [19, 55]. Furthermore, Aliskiren, a renin inhibitor, reduced cell proliferation via decreasing Notch1 and KRT6 expression, and regulating apoptosis in renal carcinoma cells [56].

Besides, the single treatment of RAS inhibitors, their combination with standard therapy has also been found effective in cancer treatment. ACEi Enalapril was found to prevent the cardiotoxic side effects associated with doxorubicin treatment in lymphoma patients [57]. Further, the combination treatment of ARB losartan and standard drug, doxorubicin resulted in an increase in sensitivity of drug treatment in AML cell lines [58].

Conclusion and Future Prospects

The multifactorial interplay of local tissue RAS concerning many peptides, enzymes, receptors and feedback regulation as well as its involvement in growth of tumor tissue, metastasizing tendency, autocrine and paracrine regulation of mechanism relevant to cellular proliferation, cell signaling, immune responses, extracellular matrix formation, apoptosis, angiogenesis are characteristics which favors the study of local RAS as a therapeutic target for its significant role in carcinogenesis. Most of the clinical reports correlated the levels of different local bone marrow RAS components in case of normal or altered hematopoiesis processes and neoplastic disorders to investigate its disease specific altered state.

The mechanism involving hematopoiesis regulation or dysregulation via local bone marrow RAS modulation in normal or pathological state may be characterized by changing functionality of transcriptional factors dependent signals involved in turn in regulation of gene expression; and BM microenvironment and stromal cells mediated growth factors signals. Moreover, targeting the components involved in hematopoietic regulation of local RAS might be of therapeutic significance in treatment modalities related to cancers. The local RAS and its relationship to pathogical conditions involving malignancy paves a new way for exploration and delineating into its molecular mechanism which could be investigated further to gain an in-depth insight into this area for utilization for novel therapeutics of hematological malignancies.

Table 20.1 Different types of Renin-Angiotensin system inhibitors tested in cancer along with the involved mechanism

Drug	Category	Disease(s) tested	Mechanism reported	Reference
Captopril	ACEi	Myeloid leukemia, breast cancer, melanoma, cervix adenocarcinoma, colorectal cancer	Decreased c-myc expression, Angiotensin-II induced Smad activation	[39–41]
Trandolapril				
Losartan	ARB			
		Acute myeloid leukemia	Increases sensitivity of doxorubicin	[51]
Enalapril	ACEi	Acute promyelocytic leukaemia	Downregulation of STAT5A	[42–44]
		Pancreatic Cancer	Inhibition of NF-kB	
		Lymphoma	Prevents cardiotoxic effects of doxorubicin	[49]
Perindopril	ACEi	Head and neck squamous cell carcinoma and renal cell carcinoma	Suppression of VEGF-induced angiogenesis	[45]
		Hepatocellular carcinoma	Inactivation of NF-κB	[46]
Fosinopril	ACEi	Hepatocellular carcinoma	Inactivation of NF-κB	[46]
Telmisartan	ARB	Renal cell carcinoma	Downregulate Bcl-2 and activate caspase-3	[47]
Irbesartan	ARB	Breast Cancer	Inhibits PCNA and cyclin D1 expression and modulate p53 expression	[48]
Aliskiren	Renin Inhibitor	Renal carcinoma cells	Decreases Notch1 and KRT6 expression and regulates apoptosis	[49]

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Chapter 21
Renin Angiotensin System (RAS): The Common Thread Between Cancer and Heart Failure

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Abstract Cancer is a chronic illness that directly or indirectly affects many individuals worldwide. As the second most commonly diagnosed cancer worldwide, breast cancer is a major public health concern affecting 1 in 8 women in their lifetime. Breast cancer, along with many other cancers, is typically treated using a combination of surgery, chemotherapy, radiation, and/or targeted therapies. Despite the beneficial effects of anti-cancer therapy in reducing overall patient morbidity and mortality, these treatments are associated with cardiotoxic side effects. Although the renin-angiotensin system (RAS) plays an important role in cardiovascular and renal homeostasis, RAS inhibitors (RASi) may be used in the treatment of cancer and chemotherapy mediated cardiotoxicity. The following chapter explores the role of various RASi as adjunctive treatment options for women with breast cancer and as future anti-cancer agents with a potential use in both the prevention and treatment of chemotherapy mediated cardiotoxicity.

Keyword Renin-angiotensin-system · Cancer · Breast cancer · Chemotherapy · Anthracycline · Angiotensin converting enzyme inhibitor · Cardiotoxicity · Cardio-Oncology

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Cancer: A Brief Overview

Cancer is the leading cause of death, affecting more than 10 million individuals worldwide. In 2021 alone, cancer was responsible for the death of over 84,600 Canadians [1]. Cancer is a multistep disease process characterized by uncontrolled division of cells, owing to the breakdown of cellular replication machinery, leading to formation of tumors that can eventually invade and destroy other healthy tissues within the body [2]. This spontaneous cell transformation from normal to a cancerous phenotype is due to the combination of intrinsic genetic factors and extrinsic environmental exposure to physical, chemical, and biological carcinogens [3, 4].

The progression of cancer as a chronic disease state is broadly divided into four stages. Stages-I and II are indicative of more benign forms of tumors up to 5 cm in size while stages-III and IV are indicative of metastatic tumors >5 cm in size [4]. Correctly diagnosing the stage and type of cancer is of paramount importance in deciding on the appropriate treatment plan directed towards meeting the specific needs of the individual afflicted with cancer.

In women, breast cancer is the second most diagnosed cancer in the world affecting the lives of 230,000 women in 2021 [5]. In North America, breast cancer is a major public health concern affecting 1 in 8 women during their lifetime. Approximately 15 Canadian women die of breast cancer every day [5]. Unfortunately, demographic trends predict that this epidemic will continue to worsen over the next 15 years. Population growth, rising obesity rates, and the increasing number of the aging population greatly contribute to the rise in the incidence of breast cancer.

In both sexes, there are many socioeconomic and biological factors that correlate with a higher risk of developing breast cancer including advancing age, genetic mutations, lack of physical activity, hormone replacement therapy, frequent alcohol use, as well as family history of breast cancer [6]. While individuals of all ages may develop cancer, breast cancer is more prevalent in women with a mean age of 42 years and above. Heritable gene mutations are the most reliable predictor of breast cancer development. Mutations in tumor suppressor genes including *BRCA1* (Breast Cancer gene 1) and *BRCA2* (Breast Cancer gene 2) that are present on chromosomes 17 and 13, respectively, increases an individual's susceptibility of developing breast and ovarian cancer by greater than 80% during their lifetime [7, 8]. Approximately 0.1–0.5% of women carry a mutation in either the *BRCA1* or *BRCA2* gene, and development of breast cancer is certain in individuals with simultaneous mutations in both alleles [9]. Breast cancers with a *BRCA1* variant tend to be "triple negative cancers" with no surface hormone receptor targets for estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth receptor (HER-2) [10]. Triple negative cancers present a challenge for both the patient, as well as the oncologist, in terms of poorer prognosis for the patient and difficulty in determining the most appropriate treatment plan.

Certain lifestyle choices including frequent alcohol consumption, smoking, and a sedentary lifestyle not only increase the risk of developing breast cancer but also exacerbate the side effects of chemotherapy. Active tobacco smoking, as well as

passive second-hand smoke, correlates with a significant increase in breast cancer risk. Alcohol use displays a linear dose dependant relationship with increased breast cancer risk even with moderate consumption of 3–6 drinks per week [11]. Interestingly, it is the cumulative amount of alcohol intake, not the frequency of alcohol consumption, that increases the risk of breast cancer, therefore making binge drinking a risky behavior [12]. Accumulation of excess adipose tissue, owing to a predominantly sedentary lifestyle, not only pre-disposes individuals to conditions like diabetes, but also increases their chances of developing breast cancer [13]. Moreover, overweight (BMI 25–29.9 kg/m^2) and obese (BMI $\geq$ 30 kg/m^2) individuals have increased morbidity and mortality due to cancer [14]. This is primarily due to the unique genetic composition of ever-dividing cancer stem cells that are derived from adipose tissue, which lead to a chronic state of inflammation primarily observed in obese individuals. Molecular markers involved in endocrine and paracrine dysregulation of adipose tissue form the common thread between obesity and cancer. These markers include the insulin-IGF-1 axis, adipokines, and sex hormones including estrogen and progesterone [15]. Overall, inhibition of these markers via pharmaceuticals may play a crucial therapeutic role in slowing down breast cancer progression in obese individuals.

The recommended screening mammography guidelines in Canada include optional screening for women between 40 and 49 years of age with identified risk factors, and biennial screening mammography for women between the ages of 50 and 74 [5]. While mammography is a useful tool for non-invasive screening, the analysis of biopsy tissue is mandatory in providing insight into tumor grade, staging, and aggressiveness of the neoplasm. The combination of surgery, radiation, and chemotherapy comprise the central pillars of breast cancer treatment.

Approximately 80% of breast cancers are diagnosed when the cancer is still in early stages, which often allows for early life saving treatment. Surgical interventions include mastectomy and breast conserving lumpectomy. Among these, lumpectomies are the preferred surgical method for most patients and oncologists [16]. However, as surgery by itself fails to guarantee absence of breast cancer reoccurrence, it is often performed in combination with radiation and chemotherapy.

Radiation therapy is often used in the treatment of breast cancer before or after chemotherapy and/or surgical intervention. In the neoadjuvant setting, radiation therapy reduces overall tumor size thus increasing the success rate of surgical tumor resection. In the adjuvant setting, radiation therapy has been shown to reduce both the recurrence rate following breast conserving therapy by 50%, and the breast cancer death rate by approximately 17% [17].

Chemotherapy is the most common anti-cancer regimen used for the treatment of early-stage breast cancer (EBC). Amongst the variety of chemotherapy drugs and regimens that are prescribed, dependent upon the stage of breast cancer, third generation regimens are the most frequently prescribed to women with EBC [18]. These regimens include a combination of anthracyclines and taxane classes of anti-cancer drugs. Doxorubicin (DOX) and Trastuzumab (TRZ) are combined with Paclitaxel (PCL) or Docetaxel (DCL) [18, 19]. While they are very effective in the breast cancer setting, their use is associated with increased risk of cardiotoxicity. [20, 21]

In recent years, renin angiotensin system (RAS) antagonists have gained popularity as anti-cancer agents in the clinical setting and are under investigation to solidify their role in inhibition of cancer metastasis, growth, and angiogenesis [22]. RAS acts on both systemic and local levels to maintain homeostasis. On a systemic level, RAS is the regulator of blood pressure and electrolyte balances, while on local levels, it participates in various pathological processes including breast cancer [23]. Overall, RAS-inhibitors (RASi), including ACE inhibitors (ACEi) and AT1 receptor blockers (ARB), could potentially have a beneficial role in the treatment of breast cancer.

RAS Pathway: Function and Suppression

The RAS pathway is one of the body's primary regulators of sodium and fluid balance. It plays a vital role in many body systems including the renal and circulatory systems [24–26]. The pathway starts with angiotensinogen, the precursor to all angiotensins, which is released from the liver. In the rate-limiting step of the pathway, renin, an enzyme released from the juxtaglomerular cells in the kidneys, converts angiotensinogen to angiotensin I [24–29]. Angiotensin I (Ang-I) is then converted by angiotensin converting enzyme (ACE) to the biologically active form of angiotensin II (Ang-II) [24–26, 29]. Once Ang-II has been produced, it elicits vasoconstriction, sodium and water retention, growth and remodelling, pro-inflammatory effects, and adrenal synthesis of aldosterone [24, 26]. In addition to the actions of Ang-II, aldosterone acts on mineralocorticoid receptors to promote sodium resorption in the kidneys, and tissue remodelling in the heart, blood vessels, and eyes [24].

This cascade also triggers the release of aldosterone which has numerous effects throughout the body. In the epithelial tissues of the kidneys, colon, salivary glands, and sweat glands, aldosterone modulates the expression of ion transporters through interaction with mineralocorticoid receptors (MR) to favour the retention of water and sodium, and the excretion of potassium [26, 28]. In non-epithelial tissues including the brain, vascular smooth muscle, myocardium, retina, fibroblasts, macrophages, and adipocytes, aldosterone plays a key role in energy metabolism and mediation of inflammation. [28, 30–32]

To maintain blood pressure and fluid balance, the liver continuously releases angiotensinogen into the bloodstream, allowing renin levels to be the rate-limiting factor in the RAS pathway [28]. When systemic blood pressure or sodium levels drop, renin synthesis and release is triggered to convert angiotensinogen to Ang-I thus starting the biochemical cascade towards Ang-II to increase blood pressure via retention of sodium and water, and stimulation of vasoconstriction [26, 28]. This action of Ang-II primarily occurs in the kidneys and vasculature, but has impacts in other organs including the heart.

In the kidneys, the RAS mediates sodium retention, vasoconstriction, inflammation, and fibrosis [24, 26]. Primarily, it causes vasoconstriction of the interlobular artery, afferent arterioles, and efferent arterioles, thus increasing the filtration fraction. The pathway also causes increased sodium resorption through the renal tubules

with Ang-II acting mostly in the proximal tubule and loop of Henle while aldosterone is more active in the distal convoluted tubule and collecting duct [28]. The increased sodium resorption pulls water back into the circulatory system thus increasing blood volume to maintain blood pressure [26]. Activation of the RAS occurs in a circadian periodicity causing a variation in urine flow rate, urinary electrolyte excretion, and blood pressure throughout a 24-h period. [28, 33]

Over-expression of the RAS terminal proteins can lead to severe issues in numerous organ systems. Consequently, RAS suppression is used to treat many diseases such as hypertension, heart failure, renal diseases, ophthalmological diseases, and diabetes among others [24, 26, 28]. While RAS suppression using ACEi, ARB, and mineralocorticoid receptor antagonists (MRA) is an effective treatment for many diseases, it is important to perform a comprehensive assessment of RAS function during these treatments. It is especially important with ACEi because while ACE levels may suggest effective RAS suppression, Ang-II and aldosterone can break through treatment in some cases [28, 34].

When functioning properly, the RAS induces vasoconstriction to maintain blood pressure. However, with excess activation of the RAS, the vasoconstrictive effects of Ang-II cause vascular endothelial dysfunction through nitric oxide synthase inhibition, increased endothelin (ET) expression, cyclooxygenase-2 (COX-2) activation, and generation of reactive oxygen species [28]. In response to vascular damage, there is a cascade of inflammation and fibrosis that is amplified by RAS activation [24]. This vascular damage, associated with the increased blood pressure caused by RAS over-activation, increases the risk of developing atherosclerosis. The atherosclerotic process and hypertension, either alone or in conjunction, can lead to arrhythmias, myocardial infarction, heart failure, stroke, and/or sudden death. [35]

Due to the RAS control over vascular tone, RAS suppression via ACEi, ARB, and MRA, alone or in conjunction, is used in the treatment of hypertension. Since each class of RAS antagonism has a different target within the system, patients with resistant hypertension already receiving ACEi or ARB can be treated with adjuvant MRA for improved blood pressure control. [28, 36–38]

In the heart, the RAS plays a local role in cardiomyocyte contractility, but can also be associated with pathological inflammation, fibrosis, and hypertrophy [24]. RAS suppression has become a mainstay treatment for heart failure with reduced ejection fraction (HFrEF) [34, 39]. Although ACEi alone is beneficial in treatment of systolic heart failure, combination treatment can be more effective. Addition of a MRA to treatment with an ACEi or ARB shows significant benefit in the treatment of HFrEF, due to broader suppression of the RAS, such that treatment guidelines recommend that MRA use should be standard of care [28]. ß-blockers are another class of medications often used to treat heart failure in combination with ACEi. While ß-blockers are not typically considered RAS antagonists, they inhibit renin secretion, further decreasing the activity of the RAS [35]. Even in cases of severe heart failure, the negative impact of ACEi on kidney function is minimal [28]. RAS suppression is also used in the field of Cardio-Oncology, as a treatment for chemotherapy induced heart failure [40], as will be discussed in more detail later in this chapter.

With cardiovascular disease being one of the leading causes of death worldwide, it is critical to develop effective therapies for treating all stages of the disease [41]. Due to the intricate involvement of the RAS in cardiovascular function, modulation of the RAS is an effective treatment for hypertension, vascular endothelial damage and atherosclerosis, post-MI cardiac dysfunction, and heart failure [35]. RAS suppression can be used in both the prevention and treatment of heart failure. As hypertension and coronary artery disease (CAD) typically precede heart failure, treatment of these conditions using RAS antagonists can prevent the development of heart failure [42]. If heart failure cannot be prevented, RAS antagonists continue to be used to reduce symptoms and improve patient outcomes [42]. Despite the effectiveness of RAS suppression in the treatment of cardiovascular disease, this is still an important area for further research to reduce the extremely high morbidity and mortality associated with cardiovascular disease.

RAS: Development, Prevention, and Treatment of Breast Cancer

Despite the increased advances in breast cancer treatment to date, a significant proportion of women either do not respond to the available treatment options or develop resistance. This reinforces the need for alternate and adjunct treatment strategies with improved patient outcomes and minimizing side effects to improve quality of life. This opens the door for the adjunct use of RAS inhibitors (RASi) as anti-neoplastic agents in conjunction with regular chemotherapy regimens in the prevention and treatment of breast cancer.

There are many biological and physiological factors such as the level of sex hormones, steroid receptors, and obesity that impose effects on the regulation of the RAS resulting in the production of angiotensin peptides. These peptides not only regulate vascular tone but are also involved in tumor growth and permeability. In particular, Ang-II stimulates angiogenesis in response to increased estrogen production or decreased plasma renin. This results in inhibition of renin secretion from the juxtaglomerular apparatus of the kidneys ultimately resulting in suppression of RAS. Ang-II is a multifunctional octapeptide of RAS that is responsible for stimulating tumor cell differentiation in breast cancers via the interaction with its G-protein coupled receptor AT1-R [23]. AT1-R is present in the cytoplasm of both ductal carcinoma in situ (DCIS) and breast hyperplasia. In microvascular endothelial cells, AT1-R overexpression results in upregulation of vascular endothelial growth factor (VEGF), stimulating the release of angiopoietin-2 promoting angiogenesis [43]. Additionally, AT1-R transactivates epidermal growth factor receptor (EGFR) in breast cancer cells leading to activation of extracellular regulated kinase (ERK), phosphorylation of signal transducers, and activation of protein kinase-C (PKC) which

initiates cell proliferation in invasive breast cancer cells. Therefore, blockade of AT1-R could be an effective anti-cancer strategy in preventing breast cancer tumorigenesis and metastasis.

Many experimental and clinical studies highlight the role of sex hormones in the development of breast cancer [44]. Sex hormones, including estrogen and progesterone, affect RAS such that plasma renin activity is higher in post-menopausal women than in pre-menopausal women with breast cancer. The lower estrogen levels in post-menopausal women with breast cancer results in increased ACE activity contributing to higher Ang-II and upregulation of AT1-R [46]. However, further investigation into the benefits of the adjunct use of RASi in decreasing tumorigenicity holds tremendous potential to benefit the breast cancer population.

Steroid receptors comprise of a family of nuclear receptor polypeptides including estrogen (ER), progesterone (PR), androgen (AR), glucocorticoid (GR), and mineralocorticoid (MR) receptors, which are all expressed normally by healthy human mammary gland cells [45]. However, development of breast cancer is marked by overexpression of these nuclear receptors, which can serve as target sites for endocrine therapy agents. Tamoxifen, an ER antagonist, is one of the most clinically used selective estrogen receptor modulators (SERM). It is administered as a first line defence against ER positive breast cancer as a competitive inhibitor of estrogen that prevents ligand binding to its receptor, resulting in apoptosis of ER+ breast cancer cells [46]. The use of tamoxifen reduces the risk of recurrence by over 40% and decreases the likelihood of death by 34% when compared to non-tamoxifen compounds [22, 45, 47]. Unfortunately, long term use of tamoxifen leads to a concentration dependent development of self-resistance due to the upregulation of various RAS components via induction of the endothelin pathway in ER+ breast cancer cells. [48] One of the possible mechanisms of resistance development involves tamoxifen induced upregulation of endothelins (ET-1, ET-2 and ET-3) and its receptors which in turn activates Ang-II hypertrophic signaling leading to metastasis of the malignancy [48, 49]. Therefore, a combinatorial therapy including ACEi and ARB with tamoxifen may be beneficial in preventing the development of acquired tamoxifen resistance.

Obesity is a key risk factor that contributes to the development of breast cancer. Obesity related metabolic alterations, including inflammation and overactivation of adipose RAS, are linked with progression of breast cancer [50]. In obese individuals with triple negative breast cancer, the concentration of adipocytes is increased, resulting in higher amounts of Ang-II production and secretion. This increase in Ang-II level causes metabolic dysregulation by altering insulin signaling. Ultimately, the secretion of proinflammatory, prothrombic, and angiogenic biomarkers is upregulated resulting in obesity-associated tumor growth, proliferation, and metastasis to adjacent breast tissues. [22] Moreover, activation of Ang-II/AT1-R signaling in the tumor microenvironment triggers infiltration of tumor-associated macrophages (TAM) resulting in reoccurrence of breast cancer [22, 47, 50]. RAS inhibitors including ACEi, ARB, and MRA can down-regulate Ang-II production, restraining tumor growth and the TAM response.

Various bioactive RAS components including ACE, Ang-II, and AT1-R are associated with breast tumour development, progression, and metastasis. As a result,

RAS inhibition becomes an additional strategy in treating breast cancer. RAS inhibiting agents including captopril (an ACEi), and losartan (an ARB) are commonly prescribed to downregulate the bioactive components of the RAS. Downregulation of cancer associated fibroblasts (CAFs) and profibrogenic pathways, via RAS inhibition, helps normalize the desmoplastic environment, which positively affects the immune response. [51] Oral administration of captopril generates hypoxia and acidity in the tumor microenvironment, which leads to decreased tumor perfusion, making cancer cells more susceptible to attack by the immune response [52, 53]. Similarly, losartan improves distribution of chemotherapy drugs by inhibiting collagen-I synthesis in CAFs, which decreases the proinflammatory signaling response of TGF-β. [51] This attenuates tumor progression, and enhances T-cell infiltration, providing antitumor immunity while improving the delivery of anticancer drugs [51, 54]. Therefore, ARB's have a dual effect on tumor microenvironment and efficacy of chemotherapy drugs. Overall, combinatorial use of ACEi and ARB appears to be an effective strategy, especially for highly desmoplastic breast cancers.

Aldosterone binds to the MR in response to activation of Ang-II. During puberty, aldosterone is known to play a role in mammary gland development and differentiation. The presence of MR is correlated positively with triple negative breast cancer and axillary nodal absence [55]. Prolonged use of MRAs, including spironolactone, amrinone, and eplerenone, results in downregulation of proinflammatory and angiogenic biomarkers, such as VEGF, nuclear factor kappa beta (NF-κβ), and cAMP response element binding protein (CREB) [45]. Hence, MRAs lead to improved overall outcomes, including survival and quality of life, for triple negative breast cancers that overexpress MR.

Neoadjuvant combinatorial chemotherapies composed of anthracyclines (DOX), receptor targeted therapy (TRZ), and taxanes (PCL) are effective agents used to eliminate breast cancer cells. However, these treatments have many off-target effects including alteration of the expression of various RAS components in breast cancer patients. In comparison to women not receiving neoadjuvant chemotherapy, the alterations include higher levels of Ang-II catabolism, resulting in higher concentrations of aminopeptidase A and N, which converts Ang-II to Ang-III and Ang-IV [22, 56]. Interestingly, Ang-III and Ang-IV result in production of a lower affinity ligand for AT1-R, thus relatively decreasing downstream pro-cancer effects of Ang-II [57]. Additionally, these effects on RAS are demonstrated to be sustained for a duration of up to 2 years. [54] Therefore, use of RASi, concomitant with chemotherapy, could enhance the efficacy of cancer treatments (Fig. 21.1).

Cardio-Oncology: Heart Protection via RAS Inhibition

Cardio-Oncology focuses on the prevention, management, and treatment of chemotherapy induced cardiotoxicity. This novel field of research emerged as a result of the increasing prevalence of heart failure in patients following chemotherapy treatment. Anthracycline-based chemotherapies, such as Doxorubicin (DOX), cause

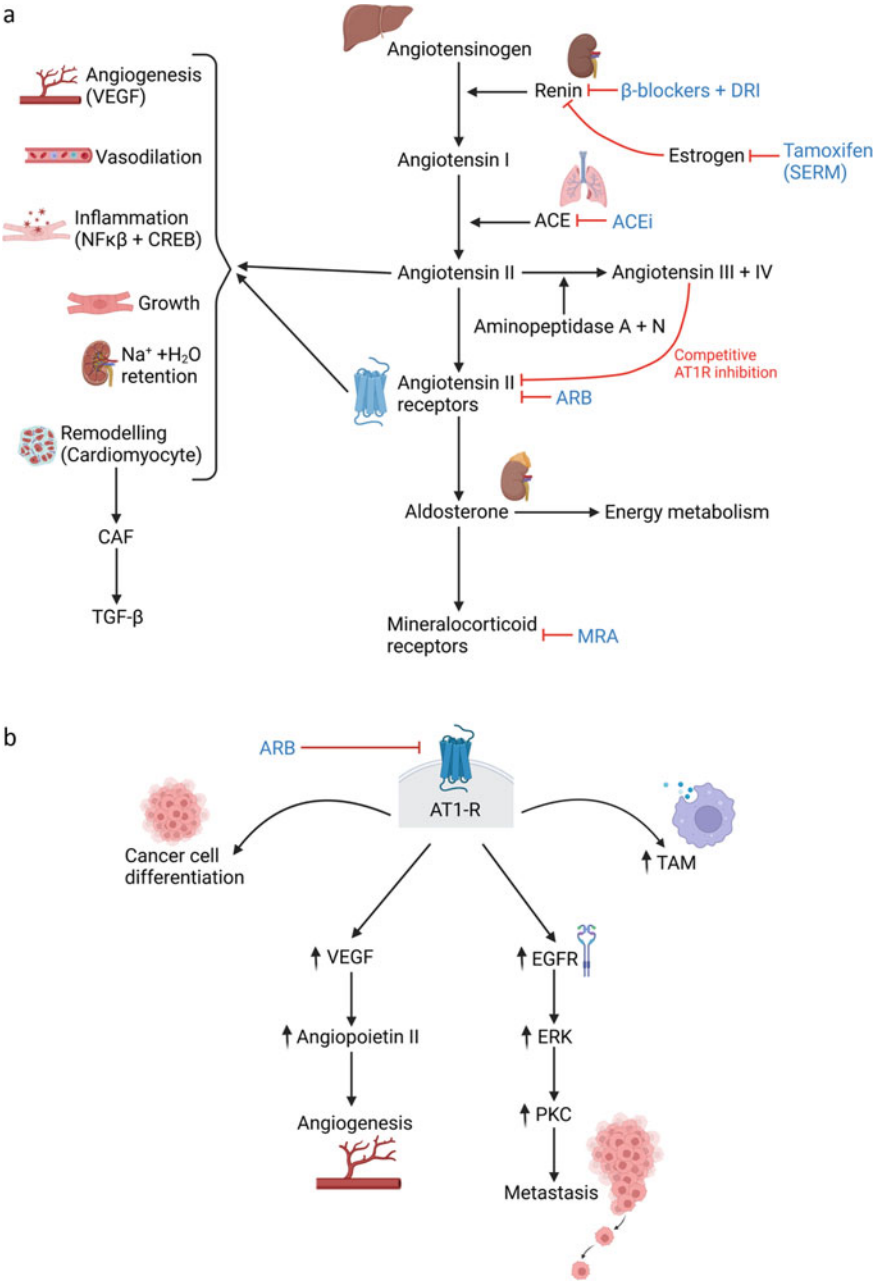

Fig. 21.1 A mechanistic pathway representing the RAS pathway and its downstream effects in cancer cell proliferation, differentiation, and metastasis mediated via Ang-II (Panel A) and the AT1-R (Panel B). The concomitant use of RASi and SERM lead to downregulation of the RAS pathway

Table 21.1 Comparison between the features of type 1 and type 2 cardiotoxicity

	Type 1 cardiotoxicity	Type 2 cardiotoxicity
Prototypical Agent	Anthracycline based chemotherapy	Trastuzumab
Dose dependency	Yes	No
Mechanism	Oxidative stress, topoisomerase inhibition	Variable
Reversibility	Irreversible	Functional recovery after discontinuation is common
Ultrastructural change	Vacuolar swelling, myofibrillar disarray, and cell death	May not lead to apoptosis in isolation
Rechallenge after cardiotoxicity	Not safe	May not result in further cardiotoxicity

dose-dependent type-I cardiotoxicity. This type of toxicity causes permanent damage through necrosis and apoptosis of cardiac myocytes followed by myocardial fibrosis [40, 58]. Trastuzumab (TRZ), a monoclonal antibody targeting the overexpressed HER2 receptor in HER2+ breast cancers, is another anti-cancer therapy with potential cardiotoxic side effects. Alone, TRZ causes reversible type-II cardiotoxicity observed as a reduction in left ventricular ejection fraction (LVEF) that often leads to temporary discontinuation of the targeted biological therapy. [59, 60] When used in conjunction with anthracycline-based chemotherapy, however, TRZ potentiates the type-I cardiotoxicity further limiting the maximum dose of chemotherapy that can be safely administered in the breast cancer setting [40, 61]. (Table 21.1)

Chemotherapy mediated cardiotoxicity is major contributor to long-term morbidity and mortality among cancer survivors [62]. It often manifests as congestive heart failure; however, it can also present as ischemia, hypertension, pericarditis, and/or arrhythmias [40, 63]. Chemotherapy mediated cardiac dysfunction is defined as an absolute LVEF of <53% or a 10% drop in LVEF when compared to the patient's baseline value [62].

Although serial echocardiograms, including radionuclide angiography (MUGA) and cardiac magnetic resonance imaging (CMR), can be used to measure changes in LVEF as a non-invasive measure of cardiac dysfunction, these changes only present later in the process of myocardial damage. In recent years, increased emphasis has been placed on detecting early chemotherapy mediated cardiotoxicity using novel echocardiographic techniques in conjunction with cardiac biomarkers of injury and cardiac inflammation. Specifically, the echocardiographic measurement of myocardial strain analysis, global longitudinal strain (GLS), is more effective for the early detection of chemotherapy mediated cardiotoxicity as it is predictive of future reductions in LVEF [59, 60, 62, 64, 65]. Additionally, cardiac biomarkers including troponin, N-terminal pro-B type natriuretic peptide (NT-pro-BNP), and interleukin-6 (IL-6) have been demonstrated to predict early development of chemotherapy mediated cardiotoxicity [62, 66].

In women with breast cancer receiving DOX + TRZ as their anti-cancer therapy, up to 1 in 4 are at risk of developing chemotherapy mediated cardiotoxicity [65, 67]. DOX induced RAS dysregulation is implicated in the development of cardiotoxicity via effects in the vasculature, myocardium, and hypothalamus. This indicates that anthracycline-induced cardiotoxicity both directly damages the cardiovascular system and affects the central regulation of cardiovascular function leading to sympathoexcitation [40, 68, 69]. The RAS mechanisms associated with anthracycline-induced cardiotoxicity are initiated by an increase in renin activity. This increase converts more angiotensinogen to Ang-I, increased ACE activity converting Ang-I to Ang-II, ultimately resulting in upregulated Ang-II levels [40, 68].

In addition to its physiological functions, Ang-II can induce inflammation and reactive oxygen species production leading to cardiac hypertrophy and fibrosis [40]. While many of the mechanisms remain elusive, overexpression of Ang-II is linked to two established mechanisms of DOX induced myocardial damage. First, it can cause inflammation that leads to cardiac remodelling and fibrosis which causes hemodynamic dysfunction resulting in heart failure. Second, it can induce oxidative stress in cardiac cells which promotes necrosis and apoptosis [40]. This effect can be improved through RAS inhibitors with strong anti-oxidant properties, which affect both the development of chemotherapy mediated cardiotoxicity and the subsequent remodelling process. [40]

In the emerging field of Cardio-Oncology, various pharmacological therapies may prevent and/or treat chemotherapy mediated cardiotoxicity including anti-oxidants, beta blockers, statins, and RAS antagonists [60, 62, 70–81]. RAS antagonists are currently the most effective option for the prevention of cardiotoxicity and the reduction of cardiovascular risk following chemotherapy. These pharmacological agents, often administered in conjunction with ß-blockers, help preserve LVEF especially when used in the pre-treatment setting [40].

There are a number of potential mechanisms for the cardioprotective effects of ACEi in the setting of chemotherapy mediated cardiotoxicity. Through their primary action of blocking ACE activity and thereby reducing levels of Ang-II, ACEi reduce total peripheral resistance and blood pressure thereby decreasing afterload which increases stroke volume. This is one possible mechanism through which ACEi are effective at preventing anthracycline-based cardiotoxicity [40]. A second mechanism is the preservation of myocardial contractility through reducing bradykinin degradation and enhancing NO synthesis to maintain Ca^{2+} ion concentrations in the sarcoplasmic reticulum. A third mechanism is the diminution of reactive oxygen species via reduced production and increased free-radical scavenging [79]. Through these mechanisms, treatment with ACEi prior to or during chemotherapy has been shown to improve hemodynamic function and decrease ventricular hypertrophy, biomarkers of myocardial damage, heart failure, and mortality. [40, 82]

In pre-clinical studies, ACEi were found to attenuate many of the cardiotoxic changes typically associated with chemotherapy [40, 83]. Prophylactic administration of the ACEi perindopril and/or ARB valsartan decreased mortality and attenuated adverse cardiac remodeling in a chronic in vivo murine model of both DOX + TRZ mediated mediated cardiotoxicity. [80, 81] ARB have also been shown to reduce

mortality in a murine model following chemotherapy through the cardioprotective effects of decreasing blood pressure, increasing myocyte fractional shortening, reducing oxidative stress, decreasing the percentage of apoptotic cells, preserving LVEF, and preventing adverse cardiovascular remodeling [40, 84]. Cardioprotective effects are more pronounced with earlier initiation of treatment with ARB suggesting that these treatments should be used before or during anthracycline chemotherapy rather than waiting until symptoms of cardiotoxicity become apparent [40, 85].

Translating these results into the clinical setting, the effects of RAS inhibition on cardiotoxicity have been examined through retrospective studies and randomized controlled trials [59, 60, 86–88]. Studies in various cancer settings have shown that patients receiving ACEi starting at least 24 h before DOX treatment and continuing for 6 months had lower incidence of reduced LVEF, heart failure, and death [87, 88]. Patients who receive DOX and/or TRZ show reduced LVEF and global longitudinal strain (GLS) parameters, with increased biomarkers of cardiac injury, inflammation, and remodelling [59, 60]. In a clinical trial, prophylactic treatment with the ACEi perindopril prevented both systolic and diastolic dysfunction induced by DOX + TRZ mediated cardiotoxicity [59, 60]. Since DOX + TRZ are often used in conjunction, ACEi are an effective treatment for chemotherapy mediated cardiotoxicity. The MANTICORE trial examined the efficacy of ACEi (perindopril) and ß-blockers (bisoprolol) in the prevention of trastuzumab mediated type II cardiotoxicity, and found that they were effective for preventing declines in LVEF [60]. Additionally, ARB during chemotherapy have been shown to prevent the early echocardiographic signs of developing heart failure. This protective effect has been demonstrated by reduced serum levels of reactive oxygen species indicating antioxidant properties, and reduced interleukin-6, interleukin-1ß, tumor necrosis factor alpha (TNF-α), and monocyte chemoattractant protein 1 (MCP-1) indicating anti-inflammatory properties [40, 79, 89, 90]. The PRADA trial examined the efficacy of ARB (candesartan) and ß-blockers (metoprolol) in the prevention of type I and type II cardiotoxicity, and found that ARB were effective for protecting against early declines in LV function [91]. Finally, MRA have been shown to have antioxidant effects against anthracycline induced oxidative stress and decreased markers of myocardial injury as well as protection of systolic function, diastolic function, and LVEF in the clinical setting. [40, 92]

Although cardioprotective strategies are not recommended for all cancer patients receiving anthracyclines, due to the still limited supporting evidence, it is recognized that RAS antagonists have high cardioprotective potential [40, 93–95]. One recommendation to assess the need for cardioprotective agents is to perform cardiovascular risk stratification prior to chemotherapy to protect high-risk patients, monitor patients to ensure early identification of cardiotoxicity, and immediately start therapy if cardiotoxicity is diagnosed [40, 95]. Specifically considering the cancer patient population, RAS inhibition does not interfere with the anti-tumor efficacy of chemotherapy, does not affect cancer cell proliferation or metastasis, and may even be beneficial in cancer treatment and patient survival [40, 96, 97]. Although further research is required to validate the benefits of RAS inhibition in the clinical prevention and treatment of cardiotoxicity, the existing data provides support for

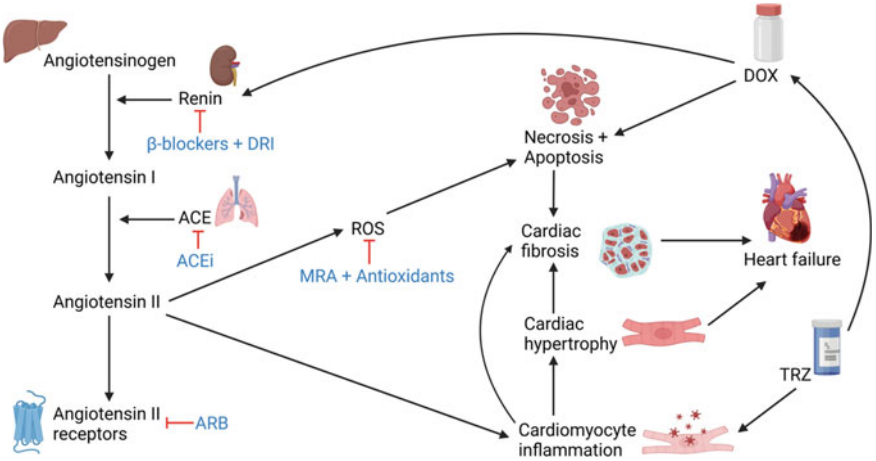

Fig. 21.2 The mechanistic pathway representing use of various pharmacological agents including β–blockers, direct renin inhibitors (DRI), angiotensin converting enzyme inhibitors (ACEi), angiotensin receptor blockers (ARB), and mineralocorticoid receptor antagonists (MRA) in preventing DOX + TRZ mediated cardiotoxicity

the early introduction of ACEi, ARB, and MRA in patients at risk of chemotherapy mediated cardiotoxicity [40].

Treatment of chemotherapy induced cardiomyopathy typically relies on the same approaches used with non-chemotherapy induced heart failure, including diuretics, ß-blockers, and RAS inhibitors. [40, 62] ACEi and ARB are recommended as the first line of treatment for anthracycline-induced cardiotoxicity [40, 62]. In the early stages of chemotherapy induced heart failure, treatment with RAS antagonists is effective in improving LV remodeling, afterload, fractional shortening, and blood pressure. For these reasons, the Canadian Cardiovascular Society guidelines strongly recommend the use of RAS antagonists as early as possible in the setting of chemotherapy mediated cardiotoxicity [62]. However, over time, these treatments become less effective and LV function parameters decline, so treatment of chemotherapy induced cardiomyopathies continues to be an active area of research [40, 98]. (Fig. 21.2)

Conclusion: Patient Experiences with RAS Inhibition

As the leading cause of death worldwide, many individuals will develop cancer within their lifetime. The risk of developing cancer is influenced by biological factors, socioeconomic factors, and environmental exposures. Following a diagnosis of breast cancer, patients undergo combination treatment of surgery, radiation, chemotherapy, and targeted biological therapy. While effective as anti-cancer therapies, these treatments increase the risk of developing cardiotoxicity. A newer approach to cancer

treatment considers the systemic and local roles of the RAS in order to use RAS antagonists in anti-cancer therapy. The RAS primarily functions to regulate sodium and fluid balance in the kidneys, thus maintaining blood pressure. While the RAS is essential for physiological functioning, over-expression of the terminal proteins can be detrimental, and RAS inhibition is therefore used to treat many diseases. RAS antagonists have been shown to enhance the efficacy of cancer treatments by improving the delivery of chemotherapy, by preventing the development of resistance to SERM, and by regulating the tumor microenvironment. Since upregulation of RAS induces pathological angiogenesis, inflammation, differentiation, proliferation, and metastasis, and increases the likelihood of cancer recurrence, RAS antagonists demonstrate the potential to become the next generation of anti-cancer agents. Following cancer treatment, there is a risk of developing chemotherapy mediated cardiotoxicity. Part of the mechanism of anthracycline mediated cardiotoxicity is RAS upregulation, which starts a cascade of inflammation induced fibrosis and oxidative stress induced necrosis and apoptosis ending with heart failure. RAS antagonists can therefore be used in both the prevention and treatment of cardiotoxicity through their anti-inflammatory and antioxidant mechanisms.

While cancer treatment has increased overall survival in the last decade, additional research is required to develop more effective treatments with less adverse side effects, likely through the integration of RAS antagonists during and after cancer treatment.

Appendix

See Table 21.2.

Table 21.2 A comprehensive list of all the abbreviations used in the chapter

Abbreviation	Description
ACEi	Angiotensin converting enzyme inhibitors
Ang-I	Angiotensin I
Ang-II	Angiotensin II
Ang-III	Angiotensin III
Ang-IV	Angiotensin IV
AR	Androgen receptor
ARB	AT1 receptor blockers
BMI	Body mass index
BRCA1	Breast cancer gene 1
BRCA2	Breast cancer gene 2
CAD	Coronary artery disease

(continued)

Table 21.2 (continued)

Abbreviation	Description
CAFs	Cancer associated fibroblasts
CMR	Cardiac magnetic resonance imaging
COX-2	Cyclooxygenase-II
CREB	Camp response element binding protein
DCIS	Ductal Carcinoma in Situ
DCL	Docetaxel
DOX	Doxorubicin
DRI	Direct renin inhibitors
EBC	Early breast cancer
EKR	Extracellular regulated kinase
ER	Estrogen receptor
ET	Endothelins
GLS	Global longitudinal strain
GR	Glucocorticoid receptor
HER-2	Human epidermal growth receptor
HFrEF	Heart failure with reduced ejection fraction
IL-6	Interleukin-6
LVEF	Left ventricular ejection fraction
MCP-1	Monocyte chemoattractant protein 1
MR	Mineralocorticoid receptor
MRA	Mineralocorticoid receptor blockers
MUGA	Multigated acquisition scan
NF-κβ	Nuclear factor kappa beta
NT-pro-BNP	N-terminal pro-B type natriuretic peptide
PCL	Paclitaxel
PKC	Protein kinase C
PR	Progesterone receptor
RAS	Renin angiotensin system
RASi	Renin angiotensin system inhibitors
SERM	Selective estrogen receptor modulators
TAM	Tumor-associated macrophages
TNF-α	Tumor necrosis factor alpha
TRZ	Trastuzumab
VEGF	Vascular endothelial growth factor

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Chapter 22
Renin-Angiotensin System: A Potential Therapeutic Target for Colorectal Cancer

Lokesh Kumar Bhatt, Niraj Parihar, and Kedar S. Prabhavalkar

Abstract The Renin-Angiotensin System (RAS) is associated with regulation of blood pressure, electrolyte balance, and hemostasis. It is implicated in cancer hallmarks because of its local expression in almost all of the body's tissues. RAS has recently been implicated in the progression of colorectal cancer (CRC) and subsequent liver metastasis. This review summarizes various RAS-associated cellular signaling pathways in colon cancer.

Keywords Renin-angiotensin system · Colorectal cancer · Liver metastasis · Angiotensin · Angiotensin-converting enzyme inhibitors · Angiotensin receptor blockers

Introduction

Colorectal cancer is the world's third most prevalent malignancy and the fourth leading cause of cancer-related deaths, with approximately 9,40,000 new cases and 5,00,000 deaths reported each year [1]. CRC develops from the epithelial cells that line the colon and rectum. Colon cells replicate at a very high rate, with 10^{10} epithelial cells changed every day. This rapid replication rate increases susceptibility of colon epithelium to mutation and, as a result, carcinogenesis [2]. CRC pathogenesis is a complex interaction of genetic predisposition and lifestyle factors. CRC subtypes are hereditary and non-hereditary, with the majority being sporadic and caused by a somatic mutation in response to environmental factors [3]. It can develop from a serrated hyperplastic polyp or an adenomatous polyp (AP) through the adenoma-carcinoma sequence, as well as from spontaneous mutations and inherited conditions. Although a single BRAF (B-Raf proto-oncogene serine/threonine kinase) mutation can cause CRC in serrated-type hyperplastic polyps, the bulk of colon cancers today are caused by an AP through the adenoma to carcinoma sequence [4]. Colonoscopy is

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used routinely by physicians to detect the presence of APs, and lesions can be studied by histological evaluation. Further, histological results are used to determine the cells' malignant potential [5]. Surgical resection offers the best chance of recovery. But, only about 20–25% of patients are candidates for surgery, and recurrence rates range from 40 to 70%. For the vast majority of these patients, palliative systemic chemotherapy is the preferred treatment option [6]. The primary cause of death from CRC is metastasis to the liver, which accounts for more than 70% of all deaths. In up to 30–40% of individuals with advanced CRC, the liver is the primary site of metastasis. 20–25% of CRC patients have detectable synchronous liver metastasis while primary diagnosis and another 40–50% will develop metachronous liver metastasis within three years of primary tumor resection [7]. Recent studies revealed variations in the compositions of the intestinal microbiota between CRC patients and healthy individuals [8].

Components of Renin-Angiotensin System (RAS), are renin, angiotensinogen (AGT), angiotensin I (Ang I), angiotensin-converting enzyme (ACE), angiotensin II (Ang II), angiotensin II type 1 receptor (AT1R), and angiotensin II type 2 receptor (AT2R). Recently, counter-regulatory axis of RAS is identified which comprises of angiotensin 1–7 (Ang 1–7), MAS Receptor (MASR), and angiotensin-converting enzyme 2 (ACE-2) [9]. Overactivation of RAS, which causes hypertension, raises the risk of cancer, cancer progression, and mortality in cancer patients [10]. Chronic inflammation and high levels of Vascular Endothelial Growth Factor (VEGF) during hypertension lead to endothelial dysfunction and angiogenesis, which may then act as auxiliary factors during cancer development [11]. Several studies have been conducted to investigate the role of RAS in the pathophysiology of CRC [12, 13]. Many renin-angiotensin system inhibitors have been shown to reduce the risk of cancer [14]. ACE inhibitors (ACEIs)/angiotensin II receptor blockers (ARBs) are RAS inhibitors that are used to treat hypertension. However, as more studies on RAS inhibitors have been conducted, it has been discovered that RAS inhibitors play a role not only in the treatment of hypertension but also in the treatment of colorectal cancer [15, 16].

Renin-Angiotensin System-Associated Cellular Signaling Pathways in Colorectal Cancer

In terms of cancer etiology, the mechanism of cancer development is a complex multi-stage process that involves sequential mutational events that occur concurrently with cancer progression [17]. Mutations in signaling pathways and genes that interact with RAS are involved in several tasks, including cell growth and survival. As a result, in a cancerous cell, these events will be interrupted [18]. The EGFR/MAPK, PI3K, and Wnt/β-catenin signaling pathways affect a variety of biological activities, including cell proliferation, differentiation, angiogenesis, apoptosis, and survival. These are some of the primary signaling pathways through which RAS protein functions.

RAS and EGFR/MAPK in Colorectal Cancer

When a ligand binds to receptor tyrosine kinase, the pathway is activated for the first time. For example, epidermal growth factor (EGF), whose receptor is EGFR. EGF releases from cell surface before it can bind with EGFR. This is accomplished through the use of the TACE/ADAM-17 enzyme, which cleaves transforming growth factor-α and amphiregulin, two EGF family ligands [19]. Following ligand binding, the receptor dimerizes and becomes phosphorylated. Following that, inside a cell, a protein complex is formed, with growth factor receptor-bound protein 2 (GRB2) attaching to the receptor while being bound by son of sevenless (SOS) [20]. SOS exhibits guanine nucleotide exchange factor activity after being attached to RAS. The RAF proteins (A-RAF, B-RAF, and C-RAF) can be recruited to the cell surface by active GTP-RAS [20]. It has been proposed that the Ras–Raf–ERK signaling pathway regulates cell growth, differentiation, and survival [20]. Malignant transformation and tumor progression results, if this pathway is faulty. A mutation in the RAS protein is one of the most common ways that the MAPK is activated; mutations in the KRAS protein are found in approximately 40% of all colorectal cancer (CRC) cases, whereas NRAS mutations are less common, accounting for 5% of all CRC cases. The most common mutations in KRAS and NRAS are found at codons 12, 13, and 61. These mutations can be found in early adenomas as well as cells with little potential for malignancy. Silencing these mutant codons results in a diminution of the tumorigenic features of diseased cells, according to both in vitro and animal studies [17]. BRAF mutates in the MAPK pathway, which appears to occur in about 5% to 10% of all colon cancer patients. The V600E mutation is the most common BRAF mutation in all cancers, including colorectal cancer [21]. As previously stated, the EGFR/MAPK signaling pathway has been linked to oncogenic processes and thus plays an important role in tumor growth and CRC progression [22]. This pathway's aberrant expression has been identified as a potential target for CRC treatment.

RAS and PI3K in Colorectal Cancer

PI3K/Akt is a critical intracellular signal pathway that regulates a wide range of cellular activities including cell growth, proliferation, differentiation, and migration. PI3K is divided into three classes, I–III, established on structural and functional differences. Type class IA contains two PI3K subunits, one regulatory (p85) and one catalytic (p110). Three genes, PIK3R1, PIK3R2, and PIK3R3, encode different isoforms of p85 as well as different types of p110 such as alpha, beta, gamma, and delta, which produce PIK3CA, PIK3CB, and PIK3CD, respectively [23]. Stimulating Ras activation or extracellular factors through RTK, causes PI3K activation. Cancer has been treated by inhibiting the PI3K/Akt pathway [24]. Akt phosphorylation has been linked to the downregulation of cell proliferation and apoptosis in human CRCs. Akt controls downstream targets like mTOR. Phosphatase and tensin

homologue protein (PTEN), a tumor suppressor molecule, dephosphorylates PIP3 and thus inhibits the PI3K pathway [23]. P110 is encoded by the gene PIK3CA. RAS mutations frequently coexist with PIK3CA exon 9 and 20 mutations. 15–25% of CRCs are thought to have PIK3CA mutations, which might lead to higher PI3K activity [25]. Overall, the PI3K signaling pathway has been shown to play a carcinogenic role in the initiation and progression of CRC. Several studies have found that inhibiting this pathway specifically causes a decrease in CRC cell growth and an increase in apoptosis [24].

RAS and Wnt/β-Catenin in Colorectal Cancer

The Wnt/β-catenin signaling pathway has shown its role in a variety of biological processes, including embryonic development, tissue homeostasis, and carcinogenesis [26]. Excess Wnt/β-catenin signaling causes abnormal cell proliferation and inhibits apoptosis, which cause progression of colorectal cancer [27]. β-Catenin degradation is usually mediated by the destruction complex, which is made up of the adenomatous polyposis coli protein (APC) and numerous other proteins in the absence of Wnts. Further, Wnt phosphorylation of LRP6 disrupts this destruction complex. The active β-catenin then translocates into the nucleus, where it binds to the TCF/LEF to trigger the production of target oncogenes namely cyclin D1 and c-Myc [28, 29]. Over 80% of CRCs have APC loss-of-function mutations, and roughly 5% have β-catenin activating mutations, which result in constitutive activation of the Wnt/β-catenin pathway and thus contribute to oncogenesis [30]. Recent research has shown that silencing Wnt3 significantly reduced the activation of the Wnt/β-catenin pathway and the proliferation of CRC cells with APC or β-catenin mutations [31]. The 350-amino-acid protein, (Pro)renin receptor ((P)RR) was first identified as a key component of the RAS and is widely expressed in the human body. Additionally, it performs the role of an adapter protein that associates with the Wnt receptor complex and aids in the activation of Wnt/β-catenin signaling independently of the renin-angiotensin system. Aberrant (P)RR expression, through the Wnt/β-catenin signaling pathway, enhances CRC carcinogenesis in the presence of constitutive pathway component mutations [32].

Role of Renin-Angiotensin System in Colorectal Cancer

Novel physiologic functions of the renin-angiotensin system in the renal and cardiovascular systems have been well established. Recent research has looked into and identified RAS receptors and mediators in other tissues and organs of the body [33]. Components of the RAS system were also found in the lamina propria, lending credence to the hypothesis that the RAS also performs paracrine functions. AT2R is primarily stimulated at low Ang II concentrations, which stimulates colonic water

and sodium absorption. When exposed to high levels of Ang II, the AT1R-mediated pathway is primarily activated, inhibiting sodium reabsorption and/or stimulating sodium secretion [34]. The activation of AT1R may also influence colonic motility. Losartan, an AT1R inhibitor, downregulates the effect competitively, requiring a higher concentration to produce the same contractile effect [35]. Ang II stimulates sodium and water absorption and secretion via the AT1R and AT2R, respectively. It was discovered that RAS components are dysregulated in CRC, indicating that RAS plays a role in CRC pathology (Fig. 22.1). The liver is the site of most CRC metastasis, and angiotensinogen production there is normal, raising the possibility of RAS and its role in CRC metastasis. ACE, Ang II, and MASR were found to be upregulated in CRC liver metastasis, while AT1R was found to be inhibited when compared to normal liver tissue. Angiotensinogen generated by the liver is converted by ACE into Ang II, which induces angiogenesis and metastasis via the AT1R by upregulating VEGF and TGF-β, respectively [34]. Ang II inhibits cell growth, invasion, and apoptosis in several CRC-derived cell lines. It has also been shown to increase cell growth and invitro invasion into type IV collagen while decreasing apoptosis in a dose-dependent manner. Although AT1R was found to be decreased in the liver during CRC metastasis, Kupffer cells (KCs) overexpressed AT1R, which promotes CRC liver metastasis [36]. AT1R deletion in CRC animal models resulted in decreased liver metastasis and downregulated TGF-β production in KCs, showing that KCs increases liver metastasis by inducing TGF-β production via AT1R signaling [36]. RAS inhibitors have been linked to lower risk and mortality from CRC. Treatment with ACEIs and ARBs was found to improve recurrence-free survival in early-stage CRC and left-sided CRC cases. Overexpression of the AGTR1 gene, on the other hand, was associated with poor recurrence-free survival in the advanced stages [37]. Furthermore, ARBs were able to improve both overall survival and progression-free survival (PFS) in individuals with metastatic CRC who received first-line chemotherapy [38].

Renin-Angiotensin System in Colorectal Cancer Liver Metastasis

In up to 30–40% of individuals with advanced illness, the liver is typically the primary metastatic location for CRC, it may be the only site of spread (Fig. 22.2) [39]. In response to tissue injury and hypoxia, the local RAS is up-regulated in the liver [40]. Ang II promotes the expression of a variety of growth and pro-angiogenic factors, including VEGF. Other RAS components mediate counter-regulatory effects of Ang II/AT1R signaling, which has proliferative and angiogenic effects. Activation of the AT2R, which is expressed more than the AT1R in primary CRC, inhibits angiogenesis and cellular proliferation [41]. In liver injury, the expression of an ACE homologue, ACE2, is increased [42]. This enzyme directly produces the peptide Ang-(1–7) from Ang II and indirectly from Ang I. Ang-(1–7) antagonizes some Ang

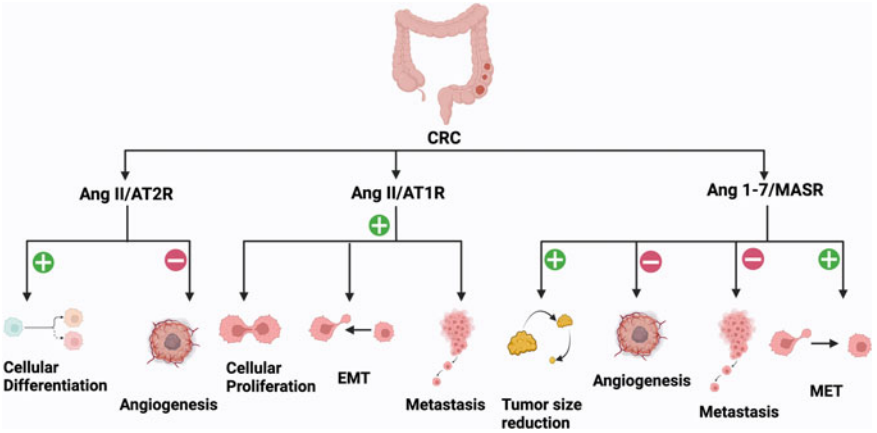

Fig. 22.1 The role of renin-angiotensin system components in colorectal cancer. CRC-colorectal cancer, MET- mesenchymal-epithelial transition, EMT- epithelial-mesenchymal transition, Ang I-angiotensin I, Ang II-angiotensin II, AT1R-angiotensin II type 1 receptor, AT2R-angiotensin II type 2 receptor, Ang 1–7-angiotensin 1–7, MASR-MAS Receptor

II-induced effects, via the MASR [43]. CRC cells with angiotensin-activating ability produce abundant Ang-2 from AGT in the liver and cause liver metastasis [44]. Several RAS components are expressed in primary CRC, and blocking the RAS inhibited tumor growth in a mouse model of CRC liver metastasis [45]. RAS expression changes significantly in the tumor-bearing captopril-treated liver and in CRC metastasis. Captopril treatment reduced ACE expression in CRC liver metastasis. Following captopril treatment, angiotensinogen expression is lower in CRC liver metastasis and lower in the liver surrounding tumors [1]. Treatment with captopril alters the spatial and temporal infiltration of tumor lymphocytes expressing $CD3^+$ and $CD4^+$. It modulates T cell subpopulations that infiltrate into tumor and liver tissues in different ways [46]. In a mouse model of CLM, both captopril and irbesartan significantly inhibited tumor growth [45]. Anti-hypertensive drugs that target the renin-angiotensin system (anti-RAS) in combination with bevacizumab have been shown to significantly improve anti-angiogenic efficacy in CRC liver metastasis. Anti-RAS blocks the contraction of fibroblasts and the deposition of ECM, which prevents liver metastasis from hardening and increasing bevacizumab's anti-angiogenic effect [44]. Diabetes has been linked to angiotensin activation and the progression of CRC liver metastasis. The expression of renin and chymase in CRC cells provides an angiotensin activation mechanism. Cathepsin D produces Ang I from AGT instead of renin in cardiac myocytes, fibroblasts, and vascular smooth muscle cells. Renin expression was found in both HT29 and CT26 cells, and it was dose-dependently related to glucose concentration. Levels of intracellular Ang II are dramatically raised by a high glucose concentration in cardiac fibroblasts by increasing renin levels. Clinical data showed high tumoral renin concentrations, high tumoral Ang II concentrations, and liver metastasis in diabetic cancer patients [47].

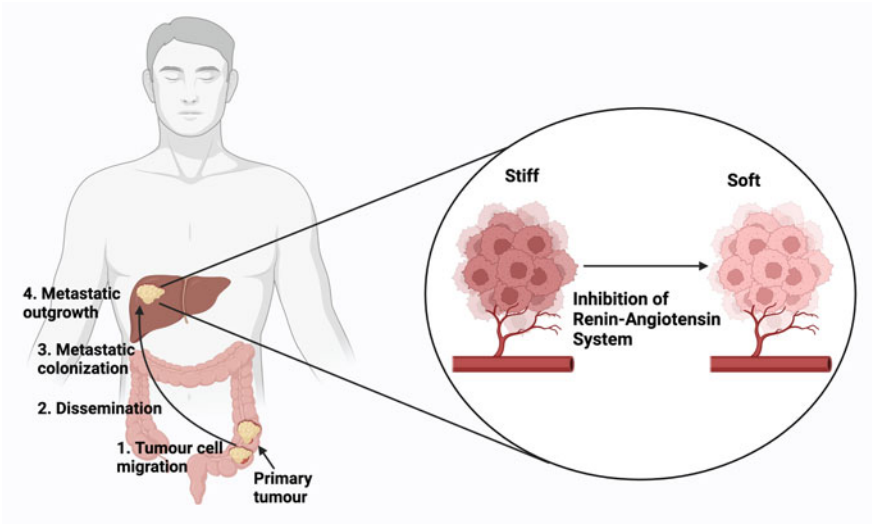

Fig. 22.2 Schematic representation of the role of the renin-angiotensin system in Colorectal cancer liver metastasis. Colon cancer metastasis to the liver occurs when tumor cells migrate and colonize from the colon to the liver, resulting in metastatic spread. The renin-angiotensin system is involved in liver metastasis, and inhibiting it reduces metastatic stiffness, hence boosting the anti-angiogenic effect

Renin-Angiotensin System Targeting Therapy for Colorectal Cancer

According to recent research, drugs that target paracrine hormone systems that promote tumor formation may give an alternate or supplementary therapeutic option in CRC patients. Long-term inhibition of the renin-angiotensin system in hypertensive patients is related to a lower incidence of numerous human malignancies [16]. The angiotensin I converting enzyme is a critical enzyme in the RAS, converting the physiologically inactive angiotensin (Ang) I precursor to Ang II, the RAS's primary effector peptide. Renin-angiotensin system inhibitors' potential protective impact is gaining attention due to their potential involvement as chemopreventive medications against colorectal cancer. A variety of renin-angiotensin system inhibitors have been shown to reduce the risk of colorectal cancer (Table 22.1) [48, 49]. Recent research shows that Ang II/AT1R signaling has proliferative and angiogenic effects, while additional RAS components have counter-regulatory effects. Activation of the AT2R, which is expressed more than the AT1R in primary CRC, for example, suppresses angiogenesis and cellular proliferation [41].

The renin-angiotensin system has been widely studied in the context of liver and renal fibrosis. Ang II, the major effector of the RAS, has been demonstrated to stimulate TGF-β1 and NF-κB signaling pathways in inflammatory diseases such as hepatic and renal fibrosis [50]. Studies on renal fibrosis showed that multiple

Table 22.1 List of renin-angiotensin system inhibitors tested for colorectal cancer

Targets	Drugs	Study outcome	Disease model	Reference
ACE	Captopril	Despite increasing Wnt signaling, captopril lowered the expression of downstream Wnt target genes, including c-myc and cyclin-D1 in a mouse model of colorectal cancer liver metastasis	MoCR-treated CBA mice	[52]
		Through anti-angiogenesis and pro-tumor apoptosis, captopril therapy regresses CRCLM in the regenerating liver without affecting liver recovery following large partial hepatectomy	MoCR-treated CBA mice	[54]
	Enalapril	Enalapril significantly increased the sensitivity of CRC cells to 5-FU at therapeutically acceptable dosages without causing additional damage, and the synergistic effect is due to synergistically decreasing proliferation, angiogenesis, and NF-κB/STAT3-regulated proteins	SW620-treated BALB/c nude mice; HCT116 and SW620 cell lines	[55]
ARB	Candesartan	Candesartan decreased tumor growth by decreasing fibrosis and increasing ROS generation when combined with 5-FU. Modulating Cyclin D1, MMP3/9, and E-cadherin inhibited tumor cell growth and migration	CT26 cells-treated BALB/c mice; CT26 and SW480 cell lines	[56]
	Telmisartan	Telmisartan suppressed cell growth and lowered cell viability in colon cancer cell lines in a dose-dependent manner	HT29, SW480, and SW620 cell lines	[53]
		Telmisartan inhibits cell proliferation by blocking HB-EGF-CTF nuclear translocation and blocks cell growth in colon cancer cells	HT29, HCT116, CaCo2, and SW480 cell lines	[57]

(continued)

Table 22.1 (continued)

Targets	Drugs	Study outcome	Disease model	Reference
	Losartan	Losartan inhibited tumor development while increasing tumor cell necrosis. Losartan-treated animals had less metastasis and angiogenesis, as evidenced by decreased tumor vasculature and suppressed matrix metalloproteinase-2 and -9 activity	CT26 cells-treated BALB/c mice; CT26 cell line	[58]
	Irbesartan	Irbesartan reduces the development of colitis-associated CRC by inhibiting MCP-1 production and the concentration of $CCR2^+$ inflammatory monocytes and fibrocytes in the inflamed colon	AOM/DSS-treated mice	[59]

ACE-angiotensin-converting enzyme, ARB-angiotensin receptor blockers, CRC-colorectal cancer, CRCLM-colorectal cancer liver metastasis, NF-κB-nuclear factor kappa-light-chain-enhancer of activated B cells, STAT3-Signal transducer and activator of transcription 3, ROS-reactive oxygen species, 5-FU-5-fluorouracil, MMP3-matrix metalloproteinase-3, HB-EGF-CTF-Heparin-binding epidermal growth factor-like growth factor carboxy-terminal fragment, MCP-1-Monocyte chemoattractant protein-1, DSS-dextran sulfate sodium

RAS genes in renal tissue are regulated by the Wnt/β-catenin pathway. Furthermore, ICG-001, a small molecule inhibitor of Wnt signaling, was able to reduce kidney fibrosis [51]. In a mice model of colorectal cancer liver metastasis, RAS inhibition by captopril could modify the Wnt/β-catenin pathway and EMT/MET in the context of liver regeneration following partial hepatectomy [52]. AT1R blocker, Telmisartan had antiproliferative and apoptotic effects in human colon cancer cells at therapeutic blood concentrations, and telmisartan had potency at least similar to pioglitazone, a complete PPARγ agonist. PPARγ blockade with GW9662 did not completely reverse pioglitazone's antiproliferative and apoptotic effects in human colon cancer cells. PPARγ inhibition boosted antiproliferative and apoptotic effects in the telmisartan-treated cells [53].

Conclusion and Future Perspectives

In most malignancies, the ACE/Ang II/AT1R axis plays a tumorigenic role, whereas the ACE-2/Ang 1–7/MASR axis plays an antitumorigenic role. Furthermore, ACEIs and ARBs have been shown to enhance colorectal cancer outcomes. RAS activation

is a critical component of colorectal cancer for disease development and liver metastasis. In addition, Renin-angiotensin system blockade is a promising therapeutic for colorectal cancer liver metastases. Because of their potential role as chemopreventive drugs against CRC, RAS inhibitors' potential protective impact is gaining attention, however, there are still few studies that examine this association. More research is needed to confirm the significance of the renin-angiotensin system in colorectal cancer and its potential as a therapeutic target.

Acknowledgements Figures 22.1 and 22.2 were created with BioRender.com.

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Chapter 23
Anticancer Role of Natural Phenolic Acids by Targeting Angiotensin-Converting Enzyme (ACE)

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Abstract Recently, it has become obvious that renin-angiotensin system (RAS) plays an important role in cancer progression through angiotensin converting enzyme

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(ACE), involving activation and upregulation of multiple oncogenic molecules. Accordingly, suppression of ACE and its subsequent downstream cascades has received considerable attention as a possible way to combat oncological diseases. In this book chapter, the role of a several plant-derived phenolic acids (Ellagic acid, Gallic acid, Caffeic acid) on inhibition of ACE is demonstrated, and involvement of this action in chemopreventive and chemotherapeutic properties of these natural compounds is discussed, with a special focus on antiangiogenic and antiinflammatory activities.

Keywords Angiotensin-converting enzyme · Cancer prevention · Phenolic acids · Ellagic acid · Gallic acid · Caffeic acid

Introduction

Due to a rapid increase in cancer incidence in the last few decades, researchers all over the world have focused more and more on developing new efficient strategies for combating this dreadful disease. In fact, while there were an estimated 12.7 million new cancer cases and 7.6 million cancer-related deaths in 2008 [1–8], these numbers were increased more than 1.5- and 1.3-fold, respectively, by the year 2020 and are expected to grow further over the coming decades [9–12].

Natural products have fascinated researchers already for more than half a century, leading to identification of several clinically used chemotherapeutic drugs, such as paclitaxel, vinblastine and vincristine, as part of the US National Cancer Institute screening program initiated in 1960 [4, 10, 11, 13–19]. Driven by this success, later on, a wide variety of plant-derived phenolics have been investigated and demonstrated to exert some anticancer efficacy in diverse preclinical models of different human cancer types, displaying antioxidant, antiinflammatory, immunomodulatory, antiproliferative, apoptotic, antiangiogenic and antimetastatic activities [20]. Moreover, molecular mechanisms underlying these effects have also become increasingly clear, revealing the ability of plant phenolics to affect multiple molecular targets and diverse cellular signaling pathways [21]. Among plant-derived phenolic agents, phenolic acids constitute an important class, with Ellagic acid, Gallic acid, Caffeic acid and Ferulic acid being the major representatives of these phytochemicals [22].

Increasing evidence has recently shown that angiotensin-converting enzyme (ACE) can be a crucial target for anticancer intervention. Both ACE inhibitors as well as angiotensin receptor blockers have actually led to reduced proliferation and invasion of cancer cells in vitro, besides impeding also tumor growth and metastasis in vivo [23–25]. Therefore, compounds with ACE-inhibiting activity can be attractive as potential novel anticancer agents. In this chapter, the role of ACE as a molecular target for cancer prevention and therapy is thoroughly described at molecular level, whereupon the ability of plant-derived phenolic acids to intervene in the ACE-driven cellular pathways is analyzed.

ACE as a Molecular Target in Cancer Prevention and Therapy: Regulated Cellular Mechanisms

In the recent times mortality rates due to cancer have seen a significant decline attributing to improvement in treatment options, availability of newer and advanced treatment options as well as early diagnosis [26]. However, despite tremendous success in the cancer research and targeted therapies it still remains a leading cause of death worldwide after other major non-communicable diseases. Numerous factors play a crucial role in development and progression of cancers major being environmental and lifestyle modifications and somatic mutations, further success with the available cancer therapies till today remains limited and challenging resulting in diverting the attention of researchers towards developing novel targeted therapeutics with minimum toxicity profiles and identification and repurposing of natural compounds offering a rich source of anti-tumor properties [26, 27].

In the recent past the involvement of renin-angiotensin system (RAS) which plays a pivotal role in the maintenance of cardiovascular homeostasis and fluid/electrolyte balance in cancer has come into light. It is observed that RAS is expressed in many tissues where it mediates cell proliferation, growth, and metabolism, numerous clinical and experimental studies have demonstrated the role of RAS through angiotensin converting enzyme (ACE) in cancer progression via involvement, activation and upregulation of multiple oncogenic molecules [28, 29]. Studies have demonstrated the role of ACE in conversion of angiotensin I to angiotensin II (AngII) the main effector molecule which exerts many regulatory and counter regulatory effects via its receptors. Inhibition of ACE and its subsequent downstream molecules has recently received considerable attention in the field of oncology (Fig. 23.1). Studies have demonstrated that ACE/AngII signaling system promotes VEGF-mediated angiogenesis in solid tumors [30–33]. It has been demonstrated that in cancer, ACE mediated angiotensin II activation up-regulates angiotensin receptor 1 (AGTR1), which in turn activates the extracellular signal-related kinase/protein kinase B pathways, resulting in increased VEGF production. Treatment with ACE inhibitors not only resulted in reduced VEGF expression and decreased neovascularization in-vitro and in-vivo. A number of studies have also shown that ACE mediated signaling can increase the production and release of several pro-inflammatory cytokines in tumor cells resulting in cascade of events in tumor progression [33–37].

Chemistry of Phenolic Acids

Phenolic acids are ingredients of the complex phenolics complexes (Fig. 23.2), taking place in food plants as esters or glycosides coupled with other usual complexes such as flavonoids, alcohols, hydroxyfatty acids, sterols, and glucosides. Mostly they have been related with varied functions, such as nutrient uptake, protein synthesis, enzyme activity, photosynthesis, structural components, and allelopathy. Structurally

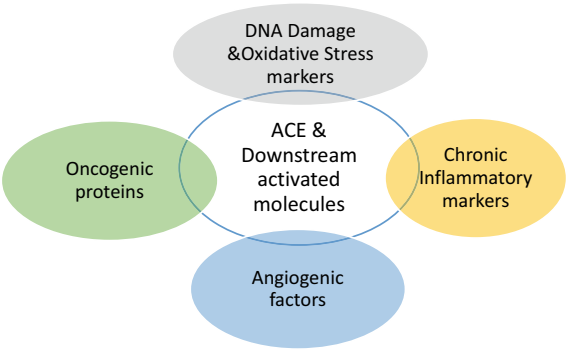

Fig. 23.1 ACE and its activated pathways/molecules potential targets of Caffeic acid

phenolic acids or phenolcarboxylic acids are phenols having phenolic ring and at least one organic carboxylic acid function. Provisional on the carbon units of the lateral chain attached to the phenolic ring, the phenolic acids can be separated into C6-C3, C6-C2, and C6-C1 complexes (Fig. 23.3), the utmost vital C6-C3 (derived from the hydroxycinnamic acid) and C6-C1 (compounds with a hydroxybenzoic structure). The basic skeleton relics the same but phenolic acids are found to vary in the number and location of the hydroxyl groups on the aromatic ring. The common of phenolic acids are linked through ester, ether, or acetal bonds. Many phenolic acids like cinnamic and benzoic acid derivatives exist in all plant and plant-derived foods (e.g., fruits, vegetables, and grains). However, only a minor fraction exists in the free acid form [38–41].

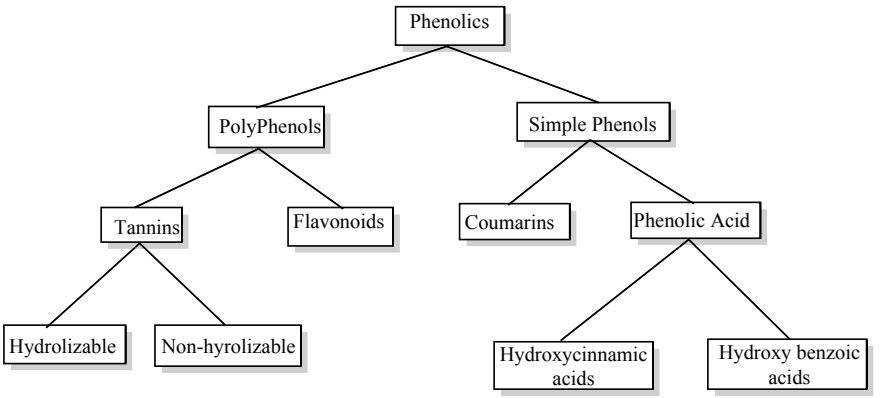

Fig. 23.2 Schematic representations of classification of phenolics compounds

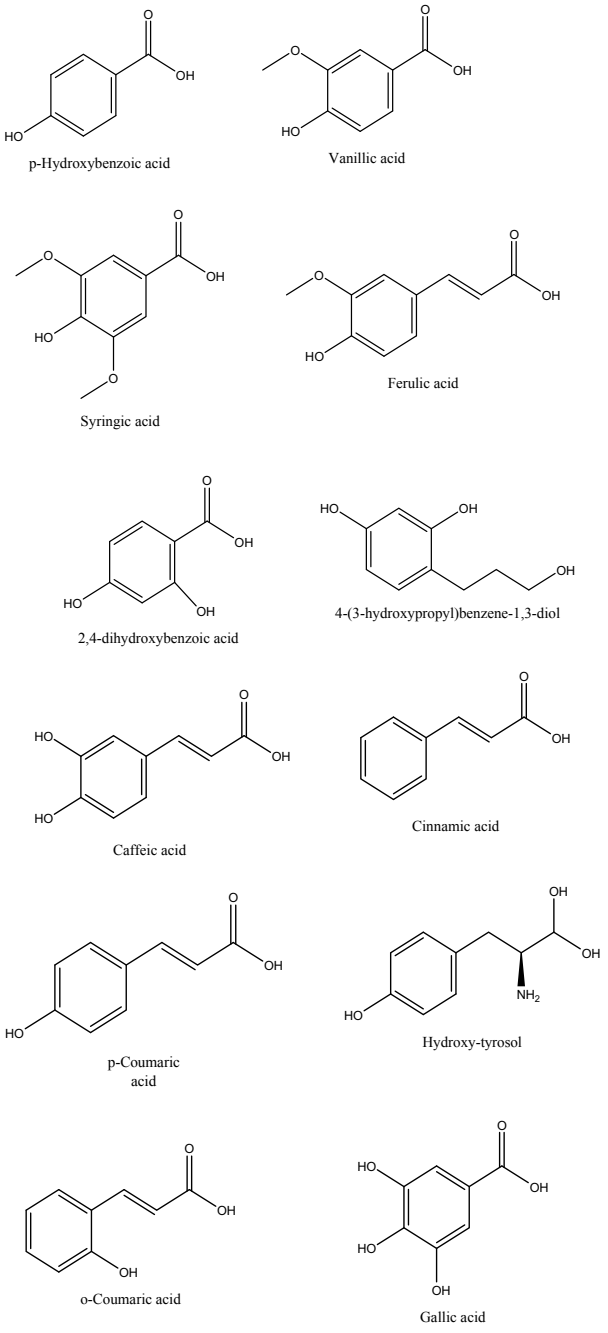

Fig. 23.3 Chemical structure of different groups of the phenolic compounds in plants

Involvement of Phenolic Acids in Chemoprevention and Therapy Through Inhibition of ACE

Ellagic Acid

A retrospective study was performed on 5207 patients attending the Glasgow Blood Pressure Clinic between 1 January 1980 and 31 December 1995 and a lower-than-expected incidence of breast and lung cancer in hypertensive patients undergoing long-term treatment with ACEIs was reported [42–45]. After the findings of these reports in vitro [46] and in vivo studies [47] were performed to clarify their action of mechanism in cancer progress, identifying the role of Ang II in cell proliferation and migration based on the angiogenesis progress in neoplasm [48], as well as in several experimental models of angiogenesis, suggests that this peptide might be involved in certain important steps of carcinogenesis [49, 50].

The two meta-analysis revealed the association of ACE inhibition and cancer risk [51, 52]. After these meta-analysis a cohort clinical trial has increased the concern of this relationship [53] showing an increased risk of lung cancer associated with ACEI (ACE Inhibitor)s compared with angiotensin receptor blockers (ARBs). Furthermore, in a clinical trial on 9652 lung cancer cases matched to 190 055 controls with a follow up of 15 years, especially use of high cumulative ACE Inhibitor doses was associated with modestly (33%) increased odds of lung cancer although use of lower doses showed neutral associations [54]. However, the biological evidence on ACE inhibition was conflicting with these finding revealing that the ACEIs and ARBs reduce the tumor growth and metastasis in several cancer types [44, 55, 56] and the action of mechanism was mainly related to the effect of AgII on vascular endothelial growth factor (VEGF) in most of the studies [57, 58].

Taking into account that the role of AgII and VEGF in cancer progression in biological studies in addition to the investigations of these ACEIs and ARBs in the market [25], the scientists also investigated the possible effects of polyphenolic compounds which were associated with these systems. Ellagic acid which is found in high levels in Rosacea family plants such as raspberry, strawberry and other berries [59] and pomegranate (*Punica granatum*), [60] was one of these compounds that has been investigated for its possible action of mechanism in cancer progress over this pathway. Ellagitannins as the complex polymers of ellagic acid and the metabolites of ellagic acid such as urolithins have shown to be protective in endothelial dysfunction in ischemic conditions [61]. Furthermore, several studies have also reported the antihypertensive mechanisms of ellagic acid such as vasodilation, amelioration of endothelial dysfunction, and suppression of NLRP3 inflammasome activation in in vitro and in vivo experimental models [62–64]. Looi et al. [65] investigated the inhibitory effect of ellagic acid and other ellagitannin metabolites on ACE inhibition via in vitro and in silico evidences and revealed that ellagic acid has a similar ACE inhibitory effect with Captopril.

Ellagic Acid has shown to have anticancer effects via different action of mechanisms in different cancer cell types were well-reviewed by Ceci et al. [66]. The

most related mechanisms those might be summarized as its effects via angiogenesis includes; decreased levels of pro-matrix metalloproteinase-2 (pro-MMP-2), pro-MMP-9 and vascular endothelial growth factor-165 (VEGF-165) in breast cancer [67]; inhibited tumor growth and VEGF receptor 2 (VEGFR-2) phosphorylation in MDA-MB-231 xenografts [68]; inhibiting angiogenesis tested with a microarray panel [69]; reducing the angiogenic factors in prostate cancer [70]; inhibiting the metastasis and angiogenesis in vivo followed by a bioluminescence assay [71]; reducing tumor growth and spreading as well as tumor-associated angiogenesis in nude mice bearing human bladder cancer xenografts [72]; decreasing serum levels of hepatocarcinogenesis markers including the angiogenic and metastatic factors [73]; inhibiting HIF-1α-induced VEGF/VEGFR-2 signaling by abrogating PI3K/Akt and MAPK activity via downregulation of PI3K, PDK-1, p-Akt (ser473), mTOR, p-ERK, and p-JNK [74]. In conclusion, there are evidences that ellagic acid acts as an ACEI which might be a potential action of mechanism in tumor environment antiangiogenic factors of this molecule, which deserves to be investigated by further preclinical and clinical studies.

Gallic Acid

Medicinal plants or herbs are great reservoir of bioactive compounds. It will not be an exception to say that the pharmacological properties of several herbs greatly depend on the presence of flavonoids, anthocyanins, and polyphenolic compounds [75–77]. Among polyphenols, gallic acid is a low molecular weight natural phenolic small molecule with its widespread occurrence in plant kingdom [78]. Structurally, gallic acid is a planar molecule comprising one aromatic ring, three phenolic hydroxyl groups, and a carboxylic acid group [78] while physically, gallic acid is yellowish-white compound with a molecular mass of 170.12 g/mol. The melting point of this small molecule is 250 °C and its water solubility is 1.1% at 20 °C [79]. The biosynthesis of gallic acid takes place by two different biosynthetic pathways. One of which uses the amino acid phenylalanine and the second one uses an early shikimate intermediate, which is 3-dehydroshikimate. The latter one involves the enzyme known as shikimate dehydrogenase [78].

Being a part of renin–angiotensin–aldosterone (RAAS) system, which efficiently controls the cardiovascular homeostasis, ACE is extensively involved in regulating the blood pressure [80–82]. However, the existence and involvement of ACE in cancer cannot be disregarded [83]. Scientifically, gallic acid is extensively studied for its chemotherapeutic and chemopreventive effects [15] via targeting multiple cellular targets. However, studying the anti-cancer effects of any pharmacophore or small molecule including gallic acid in the context of ACE inhibition are of extreme interest. The molecular mechanism underlying the inhibition of ACE by phenolic compounds including gallic acid depends upon the generation of chelating complexes between free hydroxyl group of their phenolic moiety and the zinc ion located in the active site of ACE. Therefore, the formation of such chelating complexes renders

the aforesaid enzyme inactive [84]. Previously, Hidalgo et al., (2012) demonstrated gallic acid mediated inhibition of ACE activity. Gallic acid inhibited ACE activity marginally with an IC_{50} value of 332.4 μM [85]. Similarly, Hassani et al. [86] studied the time dependent ACE inhibitory activity of gallic acid nanoparticles followed by antioxidative potential. Gallic acid nano particle mediated inhibition of ACE was 69% at 90 min. While gallic acid alone could inhibit ACE by 54%, demonstrating a significance of sustained release of bioactive gallic acid. It is not worthy that oxidants are potential causative agents of carcinogenesis and preventing their synthesis and progression is an impending strategy to curb carcinogenesis [87]. Furthermore, the polyphenols of jaboticaba seeds incorporated in a yogurt model exerted significant antioxidant activity and modulated gut microbiota of 1,2-dimethylhydrazine-induced colon cancer in rats as a consequence of inhibition of ACE. Of note, gallic acid is one of the major phenolic compounds prevalent in jaboticaba seeds [88].

It is clear that ACE inhibition by gallic acid offers ample avenues ~~to open~~ to combat the initiation as well as progression of very complex human impairments including but not limited to cancer. However, one of the major hurdles in using phytochemicals as therapeutic agents is their lesser bioavailability [89]. To some extent, this is true for gallic acid as well. However, such issues may be countered by using advanced drug delivering strategies such as nanoencapsulation and other targeted drug delivery approaches [75, 90].

Caffeic Acid

In the recent times search for plant derived-natural compounds with higher therapeutic potential and newer and more potent properties has seen a tremendous rise. Since ages phytochemicals have been extensively used for treating different diseases, many studies and reports have demonstrated that approximately 60% of global population till today relies on phytochemicals to meet their healthcare needs. Numerous clinical studies using plant derived therapeutic compounds have demonstrated beneficial and positive results in treating multiple diseases [91, 92]. The potential of phytochemicals in treating numerous chronic diseases such as cardiovascular diseases, diabetes, infections, cancer etc. along with their low or negligible toxicity upon systemic administration have gained significant attention over synthetic drugs which have high toxicity profiles and affect health organs of body such as liver, brain and kidney etc. [93, 94]. It is well known that the current chemotherapeutic drugs available for cancer treatment suffer from high toxicity potential due to the burden of catering to the aggressive behavior of cancer cells and also due to the drug resistance offered by them resulting in the usage of either higher dose of chemotherapeutic drugs of using excessively potent drugs with side effects. However, studies have shown that phytochemicals such as phenolic acids are capable of targeting different oncogenic molecular pathways such pro-inflammatory, angiogenesis etc., have low toxicity profiles to organs and are also able to tackle the problem of drug resistance to cancer therapy. Different separate studies have also shown positive and beneficial

results of co-administration of different form of phenolic acids with chemotherapeutic agents such as cisplatin, docetaxel, paclitaxel and doxorubicin in different cancer therapies via targeting multiple oncogenic pathways [94–97].

Many recent studies have thrown light on the involvement and therapeutic potential of caffeic acid (CA), in chemoprevention and therapy; CA is a widespread phenolic acid occurring naturally in many plants and agricultural products such as different fruits, vegetables, wine, olive oil, honeybee propolis etc. [27]. Numerous experimental evidences have demonstrated the therapeutic potential of caffeic acid and its derivates such antioxidant, antiviral, antibacterial, anti-inflammatory, antitumor, antihypertensive, anti-atherosclerotic, cardioprotective, antiproliferative, immunomodulatory activity etc. Different non clinical and experimental studies have thrown light inhibitory and antagonistic activities of CA such suppressing lipid peroxidation, blocking production of reactive oxygen species (ROS) and the xanthine/xanthine oxidase system, inhibiting NF-κB binding activity, COX-2 expression [98–102]. Further, studies on several cancer cell lines and animal models have thrown light on apoptosis inducing activity of CA thereby inhibiting tumor growth. Besides being an antioxidant, different biological studies have thrown light on the numerous activities of CA and its derivatives against angiogenesis, tumor invasion, metastasis, proliferation, apoptosis and macrophage polarization in different cancers such as human pancreatic, colon, oral cancer etc. via targeting multiple pathways [103–105]. Different isolated nonclinical and in vitro studies have thrown light on the efficacy of CA in ACE inhibition [106–108]. Ganiyu et al. [108] in their study on wistar rats demonstrated the inhibitory potential on CA on ACE activity and its downstream activated molecules, further Henryk et al. in their review have effortlessly summarized the inhibitory potential of caffeic acid on ACE along with other phenolic acids. In an in-silico study Bare et al. had elaborately described the mode of action of CA in ACE inhibition, the study demonstrated that the inhibitory potential of CA on angiotensin converting enzyme (ACE) activity occurs as a result of interactions between its phenolic hydroxyl groups and enzymes active site amino acids via hydrogen bonds, they also showed that three hydrogen bonds are formed in CA and ACE interaction resulting in ACE inhibition. It is seen that 4 amino acid residues bind with CA, this interaction prevents ACE from facilitating the synthesis from angiotensin I to angiotensin II [109]. In different experimental studies CA has demonstrated its inhibitory potential on cancer cells via inhibiting and suppressing the activity and expression of ACE activated molecules such VEGF, proinflammatory cytokines, pro-oxidants, NF-KB, oncogenic proteins etc. [110, 111]. The therapeutic potential of caffeic acid in chemoprevention and therapy through inhibition of ACE needs further investigation and attention to be used alone or as an adjunct therapeutic option in cancer treatment.

Safety Profile of Phenolic Acids in Humans

As natural products, phytochemicals are generally considered safe. To prove this, several studies have been carried out, showing no mortality, treatment-related clinical signs or histopathological changes in F344 rats supplemented with ellagic acid at doses of 9.4–39.1 g/kg bw for male and 10.1–42.3 g/kg bw for female animals during 90-day experimental period [112]. Also, acute oral administration of gallic acid at a dose as high as 5 g/kg bw did not cause any signs of toxicity or mortality in Swiss albino mice, whereas gallic acid at a dose of 1 g/kg bw for a period of 28 days produced no significant alterations in hematological and biochemical parameters [113]. Similarly, in a more recent study, oral administration of gallic acid to albino mice at doses of 100–900 mg/kg bw for 28-days period revealed no alterations in hematological, morphological or behavioral parameters, or in tissue histology [114]. Moreover, oral exposure of female ICR mice to Caffeic acid at doses of 0.15–150 mg/kg/day from premating to implantation day caused no maternal toxicity, fetal teratogenesis or post-natal effects on pup development, only implantation and fetal body weight gain were somewhat affected at high doses [115]. Therefore, the use of plant-derived phenolic acids is probably safe, even in relatively large doses, encouraging further studies of these phytochemicals to realize their high anticancer potential.

Current Bottlenecks and Future Perspectives

Cancer is the second leading cause of mortality worldwide. Phenolic acids (such as carnosic, caffeic, ferulic, p-coumaric, Gallic, vanillic, rosmarinic) are a class of phytochemical compounds that are isolated from a variety of plants and are recognized for their various pharmacological activities. Clinical research has concentrated on a variety of in vitro (in human cell lines) and in vivo (clinical animal models) studies over the last decade to investigate the health-protective benefits of phenolic acids against various cancers. The consumption of phenolic-rich cereals, vegetables, and fruits reduces cancer risk and improves human health. Plant phenolic acids have acquired importance as cytotoxic anti-cancer drugs, inducing apoptosis, lowering proliferation, and targeting many features of cancer, in the ongoing search for safer and more effective treatments than chemotherapy or radiotherapy. The use of medications to treat human cancers includes inhibitors of the angiotensin-converting enzyme (ACE), which are one of the most important classes.

Concerning future prospects in this domain, the authors would like to point out that although plant-derived phenolic compounds have been widely researched for their ability to inhibit tumor cell proliferation in vitro and in vivo, many gaps remain, necessitating further research. For example, cinnamic acid derivatives, caffeic acid, and ferulic acid have been shown to inhibit survival and proliferation signaling pathways such as the PI3K-Akt and MAPK pathways. However, the exact mechanism

by which these cinnamic acid derivatives slow down various signaling pathways is unknown. As a result, more research is needed to address these concerns. In addition, experimental data is needed to examine differences in treatment responsiveness among people who eat a phenolic acid-rich diet. To improve the therapeutic efficacy and tumor cell selectivity of phenolic compounds, more research is needed. Since benzoic and cinnamic acid derivatives are only effective at larger dosages (about 10–20 mg/kg body weight), strategies to lower dose and toxicity are desperately needed. Thus, future research should concentrate on the development of inhibitors targeting angiotensin-converting enzyme containing anti-tumor phenolic compounds. Finally, the authors would like to highlight that although the non-toxic profile and pharmacokinetics of phenolic acids make them safer for clinical studies, in vivo human trials should be performed to further explore their potential for extensive pharmaceutical applications.

Conclusions

Renin–Angiotensin–Aldosterone system is normally associated with the cardiovascular diseases especially the hypertension. The RAAS dysfunction is related to vascular functions especially based on the Angiotensin II and its receptors in cardiovascular system. The underlying mechanisms related to angiogenesis in cancer progression arouse the question whether Angiotensin converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) has any effect on cancer progress. After the findings of in vitro and in vivo studies revealing the effects Captopril on cancer progression, revealing the possibility that ACE inhibition may have protective effect against cancer. Further studies are needed to clarify their action of mechanism in cancer progress, identifying the role of Ang II in cell proliferation and migration based on the angiogenesis progress in neoplasm. However, several experimental models of angiogenesis, suggests that this peptide might be involved in certain important steps of carcinogenesis. Couple of meta-analysis revealed the association of ACE inhibition and cancer risk. However, the biological evidence on ACE inhibition was conflicting with these finding revealing that the ACEIs and ARBs reduce the tumor growth and metastasis in several cancer types and the action of mechanism was mainly related to the effect of AgII on vascular endothelial growth factor (VEGF) in most of the studies.

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Chapter 24
Angiotensin-(1–7): A Prospective Cancer Therapeutic

Ana Clara Melo, E. Ann Tallant, and Patricia E. Gallagher

Abstract Angiotensin-(1–7) is a heptapeptide hormone of the renin-angiotensin system with anti-proliferative, anti-inflammatory, anti-oxidant, anti-angiogenic, and anti-fibrotic properties. This chapter summarizes published preclinical and clinical research assessing the use of angiotensin-(1–7) as a chemotherapy for cancer and cancer-related pathologies. Animal studies demonstrate that the heptapeptide hormone activates a unique angiotensin receptor mas1 to attenuate lung, breast, prostate, nasopharyngeal, and liver tumor growth by regulating multiple signaling pathways critical for carcinogenesis initiation and progression as well as reducing tumor-associated angiogenesis, inflammatory response, and fibrosis. Clinical benefit with limited side effects was observed in cancer patient trials assessing Ang-(1–7) alone or in combination with standard of care therapies. The published results thus far indicate that angiotensin-(1–7) may serve as a first-in-class, targeted monotherapy for the treatment of cancer or as an effective adjuvant to enhance reductions in cancer progression and reduce toxicity of current cancer therapeutic regimens.

Keywords Angiotensin-(1–7) · Cancer · Proliferation · Angiogenesis · Fibrosis · Metastasis · Clinical trials · Analogs

Introduction

Angiotensin-(1–7) [Ang-(1–7)] is a seven amino acid peptide hormone of the renin-angiotensin system (RAS), a primary regulator of blood pressure, electrolyte balance, cardiovascular and renal function, and cell growth [1–4]. In the RAS cascade, the non-functional precursor protein angiotensinogen is cleaved by the aspartyl protease renin to form the decapeptide angiotensin I (Ang I) (Fig. 24.1). Subsequently, one of three enzymes, neprilysin, thimet oligopeptidase, or prolylendopeptidase, convert Ang I to Ang-(1–7) depending on the physiological compartment. In a two-step process,

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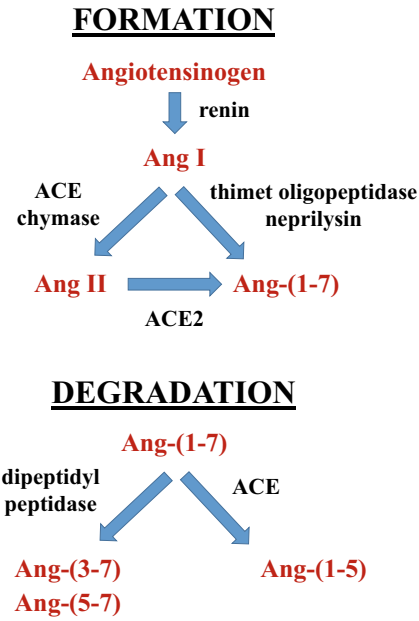

Fig. 24.1 Enzymatic synthesis and catabolism of Ang-(1–7)

Ang-(1–7) is produced by the cleavage of Ang I to the octapeptide Ang II by the dipeptidyl-carboxypeptidase angiotensin converting enzyme (ACE) or the endopeptidase chymase. The monocarboxypeptidase ACE2 then cleaves Ang II to Ang-(1–7). The heptapeptide hormone is ultimately degraded by two potential enzymes: ACE catabolizes Ang-(1–7) to the inactive degradation product Ang-(1–5) [5], while dipeptidyl peptidase 3 degrades the heptapeptide hormone to Ang-(3–7) or Ang-(5–7) (Fig. 24.1) [6, 7]. It is important to note that the RAS cascade is highly regulated with the proteolytic enzymes playing a critical role in maintaining the balance of tissue and circulating Ang II and Ang-(1–7) concentrations for normal physiological function.

The biological actions of peptide hormones are mediated by binding and activating cell-surface receptors, to initiate cascades of downstream reactions that modulate physiological processes. Ang II is the endogenous ligand for two G-protein coupled receptors (GPRCs), angiotensin 1 (AT1) and angiotensin 2 (AT2) receptors, while Ang-(1–7) binds and activates a unique 325 amino acid GPCR mas1, encoded by the MAS1 gene [1–4]. Ang-(1–7) interaction with the mas1 receptor promotes downstream signaling through generation of cyclic adenosine monophosphate (cAMP) [8, 9] to reduce cellular proliferation [8, 9]. Incubation with Rp-CAMPS, an inhibitor of the cAMP-dependent protein kinase A (PKA), in VSMCs abrogated the Ang-(1–7)-mediated reduction in cell proliferation, indicating that activation of PKA by cAMP is necessary for the heptapeptide hormone to exert inhibitory effects on cell growth [10]. Subsequent decades of research demonstrated that the heptapeptide

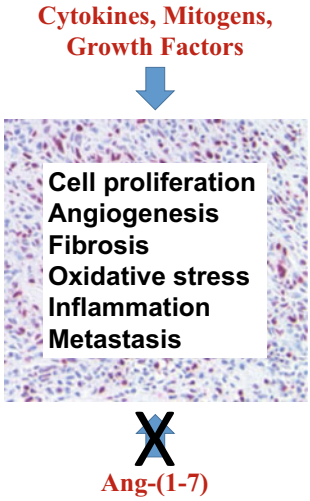

Fig. 24.2 Ang-(1–7) blocks biological processes stimulated by cytokines, mitogens and growth factors. The background shows a breast tumor section stained for the proliferation marker Ki67 (red-brown stain)

hormone through activation of mas1 receptor counter-regulates the actions of mitogens, cytokines, and growth factors to prevent dysregulated vasoconstriction, cell proliferation, oxidative stress, fibrosis, inflammation, angiogenesis, and thrombosis, depending on the cell type and tissue (Fig. 24.2).

Summarized below are published studies assessing the anti-cancer properties of Ang-(1–7). This article will focus on the in vivo preclinical research investigating the effect of the heptapeptide hormone on various types of cancer in animal models, the clinical trials reported using Ang-(1–7) as a chemotherapeutic drug, and the use of Ang-(1–7) in combination with standard of care therapies. The research described below highlight the use of Ang-(1–7) or viral vectors producing Ang-(1–7). In vivo studies that overexpress ACE2 to enhance production of Ang-(1–7) were not included as this enzyme has multiple physiological substrates complicating the ability to assess the precise molecular mechanism producing the observed effect in the animal or patient.

Ang-(1–7) and Cancer in Preclinical Animal Models

Lung Cancer

The first evidence that Ang-(1–7) inhibited cancer cell proliferation was reported in lung cancer cells by Gallagher and Tallant [11]. Sub-nanomolar concentrations of the heptapeptide hormone significantly reduced DNA synthesis and proliferation of human adenocarcinoma SK-LU-1 and A549 cells as well as non-small lung cancer SK-MES-1 cells as determined by attenuated serum-stimulated [^{3}H]-thymidine

incorporation, decreased cell number and ERK1/ERK2 dephosphorylation. Treatment with the heptapeptide hormone also decreased MAP kinase signaling and Akt and PI3K activation in platinum-resistant human lung cancer cells [12]. Delivery of Ang-(1–7) by lentiviral transduction resulted in reduced lung cell proliferation with an associated decrease in DNA synthesis and the replicative protein Cdc6 [13, 14], suggesting that the heptapeptide hormone regulates multiple proliferative signaling pathways. No reduction in lung cancer cell proliferation was observed following incubation with Ang I, Ang II, Ang-(2–8), Ang-(3–7) or Ang-(3–8) [11], demonstrating peptide specificity. Inhibition of proliferation by Ang-(1–7) was blocked by the mas1 receptor antagonist [D-Alanine^{7}]-Ang-(1–7) (D-ala). Incubation with the Ang II type 1 receptor antagonist losartan or the Ang II type 2 receptor antagonist PD123319 did not attenuate the reduction in lung cancer cell proliferation by Ang-(1–7), supporting a selective receptor-mediated process.

Administration of the heptapeptide hormone by osmotic mini-pump [15], subcutaneous injection [16] or adenoviral vector delivery [13, 14] caused a significant reduction in lung tumor xenograft volume and wet weight as well as a decrease in the immunostaining of proliferation marker Ki67 in tumors from mice as compared to saline-treated control rodents. A similar attenuation of tumor volume and weight was observed following Ang-(1–7) treatment of mice harboring platinum resistant lung tumors [12]. No adverse reactions in the animals or tissue and organ abnormalities were observed with Ang-(1–7) administration, indicating that the heptapeptide hormone was well-tolerated and may have limited quality-of-life effects when administered to patients. A marked decrease in COX-2 mRNA and protein was observed in tumor tissue from Ang-(1–7) treated mice as compared to controls, suggesting that regulation of prostaglandin synthesis by the heptapeptide hormone may play a role in the attenuation of lung tumor growth. In support of these in vivo findings, Ang-(1–7) or the microRNA miR-513a that is upregulated by Ang-(1–7) inhibited COX-2 production and activity in serum-stimulated human A549 lung cancer cells with an associated decrease in the anti-inflammatory prostaglandin E2 [11, 17, 18].

Ang-(1–7) also inhibited angiogenesis in human A549 tumor xenografts [16] and platinum-resistant lung cancer xenografts [12] with a concomitant decrease in the pro-angiogenic cytokine VEGF. Treatment of the parent A549 lung cancer cells or platinum-resistant human lung cancer cells with the heptapeptide hormone also resulted in a reduction in VEGF protein and mRNA. Additionally, the heptapeptide hormone markedly reduced neovascularization of the chick chorioallantoic membrane and endothelial tubule branching, two models of angiogenesis. Collectively, these studies suggest that Ang-(1–7) could serve as an anti-angiogenic drug to reduce lung cancer growth.

Migration and invasion of human A549 lung cancer cells in vitro were attenuated significantly following treatment with nanomolar concentrations of Ang-(1–7) [19]. A concomitant inactivation of Akt/pKB and MAPK signaling and a decrease in metalloproteinase 2 and 9 activity was observed, suggesting that the heptapeptide hormone blocks migration and invasion by inhibiting the degradation of the extracellular matrix by these two enzymes. Lung cancer cell invasion was attenuated by Ang-(1–7) or miRNA-149-3p, a microRNA upregulated by the heptapeptide hormone, with an

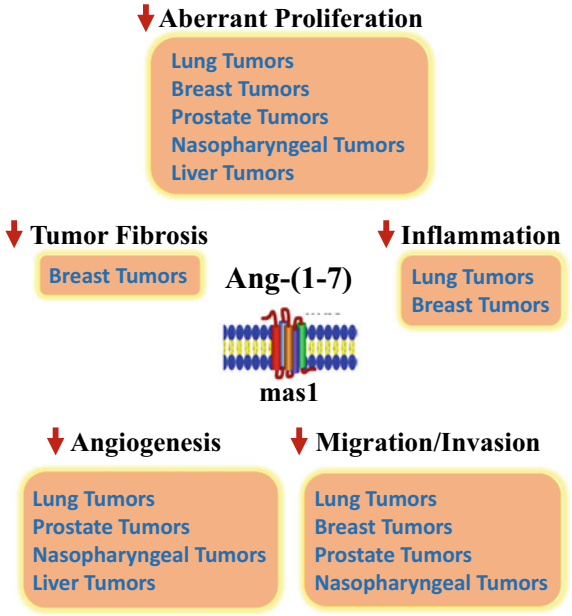

Fig. 24.3 Summary of the characteristics of tumors reduced by Ang-(1–7) activation of the mas1 receptor

associated decrease in integrin subunit beta, a key protein in proliferation and extracellular matrix interaction [17, 18]. Knockdown or antagonist blockade of the mas1 receptor prevented the decrease in migration and invasion by Ang-(1–7), indicating a receptor-mediated process. In addition, epithelial-mesenchymal transition in which epithelial cells gain migratory properties was inhibited in human lung cancer cells following transfection with a lentiviral construct to over-express the heptapeptide hormone [13, 14]. The results of these studies suggest that Ang-(1–7) may inhibit multiple steps in the metastasis cascade (Fig. 24.3); however, animal studies are needed to determine whether the heptapeptide hormone effectively reduces lung cancer metastasis in vivo.

Breast Cancer

Ang-(1–7) inhibited the growth of human estrogen receptor positive tumors or human estrogen receptor positive, HER2-overexpressing tumors in the mammary fat pad of ovariectomized female mice receiving estrogen replacement therapy, as compared to tumors from control animals [20]. A concomitant decrease in interstitial and perivascular fibrosis and collagen I deposition was observed in tumors from mice infused with the heptapeptide hormone, in addition to the reduction in tumor volume and weight. This suggests that one mechanism whereby Ang-(1–7) attenuates tumor growth is by preventing the proliferation of growth-promoting cancer-associated

fibroblasts [21]. In support, the heptapeptide hormone inhibited in vitro proliferation of fibroblasts isolated from orthotopic breast tumors with an associated reduction in the pro-fibrotic proteins fibronectin and transforming growth factor β (TGF-β) [20]. A decrease in MAP kinase phosphorylation and an accompanying increase in the phosphatase DUSP1 was observed in tumor-associated fibroblasts incubated with Ang-(1–7), suggesting an attenuation of MAP kinase signaling to reduce fibroblast proliferation.

Luo et al. [22] showed that treatment with tamoxifen caused an up-regulation of the mas1 receptor in estrogen-receptor positive MCF-7 cells but not triple-negative basal A MDA-MB-468 cells. Cell proliferation and invasion were inhibited but apoptosis was enhanced significantly in estrogen-receptor positive MCF-7 cells, triple-negative basal A MDA-MB-468 cells, and triple-negative basal B 4T1 cells incubated with Ang-(1–7). These effects were prevented in all cell lines by knockdown of mas1 with an siRNA. Further, increased tumor growth was observed following injection of 4T1 cells transfected with the mas1 siRNA as compared to 4T1 cells containing a control siRNA. Taken together, these results demonstrate the receptor-mediated action of Ang-(1–7) in the regulation of proliferation and suggest that mas1 may serve as a negative regulator of tumor growth (Fig. 24.3).

Ang-(1–7) also prevented the migration of human triple negative MDA-MB-231 cells; migration was equivalent to control levels following co-administration of the mas1 receptor antagonist A-779 with the heptapeptide hormone [23]. Incubation of MDA-MB-231 cells with Ang-(1–7) resulted in increased E-cadherin, a cancer metastasis suppressor, and reduced ZEB1, TWIST1 and Snail1, negative transcriptional regulators of E-cadherin, supporting the potential anti-metastatic actions of the heptapeptide hormone. Further, a marked decrease in NF-κB p65, a transcriptional regulator of Snail1, phosphorylation of PAK1, a regulator of Snail1 nuclear translocation, and the activity of the intracellular Ca^{++} regulator SOCE was observed in MDA-MB-231 cells after incubation with Ang-(1–7). Taken together, these results suggest that the heptapeptide hormone modulates the PAK1/NF-κB/Snail1 signaling pathways by decreasing SOCE-mediated Ca^{2+} influx to reduce metastasis.

Prostate Cancer

Krishnan et al. [24] demonstrated a marked reduction in the proliferation of human LNCaP prostate cancer cells with an associated decrease in secreted angiogenic factors VEGF and PlGF following incubation with Ang-(1–7). Further, the transcription factor HIF-1α, a primary regulator of VEGF family signaling, was also reduced, suggesting that the heptapeptide hormone could inhibit prostate tumor growth in part by attenuating angiogenesis. This was supported by in vivo studies in that the volume and weight of human LNCaP prostate xenograft tumors as well as tumor angiogenesis was diminished following infusion of Ang-(1–7) for 54 days. Ki67 and MAP kinase Erk1/2 phosphorylation were decreased in tumors from mice treated with

the heptapeptide hormone, demonstrating the anti-proliferative actions of Ang-(1–7). In addition, tumors from mice administered Ang-(1–7) had reduced VEGF and PlGF protein and mRNA as well as VEGF receptors Flt-1 and Flk1 but enhanced concentrations of sFlt-1. SFlt-1 is a soluble decoy receptor that traps VEGF and PlGF to reduce circulating concentration of the angiogenic factors. Additionally, s-Flt-1binds to the membrane VEGF receptors and disrupts proliferative signaling. Taken together, these results suggest that Ang-(1–7) disrupts VEGF family signaling to reduce prostate tumor angiogenesis, leading to an attenuation of tumor growth (Fig. 24.3).

Ang-(1–7) attenuated the migration of human PC3 cells in vitro, an effect blocked by the mas receptor antagonist D-alanine-Ang-(1–7), suggesting that activation of mas by the heptapeptide hormone may block an early step in metastasis [25]. In support, infusion of Ang-(1–7) into mice one week prior to the injection of human PC3 cells into the aortic arch prevented the formation of tumors at metastatic sites, while 100% of the control mice developed tumors in the submandibular bone, the spinal column or the long bone of the leg. The heptapeptide hormone also prevented the growth of tumors produced by injecting prostate cancer cells directly into the tibia. The inhibition of tumor proliferation was associated with a decrease in osteoclastogenesis, indicating that the heptapeptide hormone inhibited the formation of osteolytic pits required for tumor cell engraft and grow in the bone microenvironment [26]. These studies suggest that Ang-(1–7) may serve as an effective anti-metastatic agent in men with prostate cancer.

Nasopharyngeal Carcinoma

The Ang-(1–7) receptor mas was increased in nasopharyngeal carcinoma cell lines and patient tumors as compared to an immortalized nasopharyngeal epithelial cell line and normal human nasopharyngeal epithelial tissue, suggesting that nasopharyngeal carcinomas may be susceptible to the anti-proliferative effects of the heptapeptide hormone [13]. Transduction of nasopharyngeal carcinoma cells with a lentiviral construct producing Ang-(1–7) significantly reduced cell proliferation and migration as compared to cells harboring the control construct. The effect was blocked by the mas receptor antagonist A-779. Similar to the observations of Krishnan et al. [25] in prostate tumors, the transduced nasopharyngeal carcinoma cells producing Ang-(1–7) had reduced phosphorylation of the MAP kinases ERK1/2 and p38, decreased angiogenic factors VEGF, PlGF, transcription factor Hif-1a, VEGF receptors Flt-1 and Flk-1, and increased the soluble decoy VEGF receptor sFlt-1. These data indicate that the heptapeptide hormone could attenuate nasopharyngeal carcinoma growth by inhibiting angiogenesis. Injection of an adenoviral vector construct that produced Ang-(1–7) significantly decreased the weight of nasopharyngeal xenograft tumors with an associated reduction in the proliferation marker Ki67. A significant reduction in vessel density was observed in the tumors from mice injected with the Ang-(1–7) adenoviral construct as well as a decrease in tumor VEGF and PlGF, HIF-1α, as

well as Flt1 and Flk1 as compared to tumors from control mice. Conversely, the sFlt-1 was increased with the enhanced production of the heptapeptide hormone, suggesting that Ang-(1–7) attenuates nasopharyngeal cancer through inhibition of angiogenesis.

Daily subcutaneous injection of Ang-(1–7) also reduced nasopharyngeal carcinoma xenographs with a concomitant reduction in the proliferation marker Ki67 [27]. In addition, the phosphorylation of p38, Akt, p-GSK3-β, and mTOR was decreased significantly in tumors from mice treated with the heptapeptide hormone, while an increase in the autophagy proteins PI3K, LC3-II, and Becline-1 was observed. Co-administration of the mas1 receptor antagonist A779 prevented the attenuation of tumor growth, the inactivation of the PI3K/Akt/mTor and p38 signaling pathways and the induction of autophagy by the heptapeptide hormone. Ang-(1–7) reduced proliferation, migration, and invasion of nasopharyngeal carcinoma cells in vitro as well as activated autophagy, further supporting the in vivo observations. The results of the two studies published thus far indicate that the heptapeptide hormone may reduce nasopharyngeal carcinomas by multiple mechanisms and warrant further investigation (Fig. 24.3).

Liver Cancer

The growth of mouse hepatic tumor xenografts was inhibited significantly following subcutaneous infusion of Ang-(1–7) as assessed by tumor volume and weight [28]. The effect was blocked by the mas receptor antagonist A779, demonstrating a receptor-mediated process. The decrease in hepatic tumor proliferation was associated with an increase in apoptotic cells and caspase 3 activity. Administration of the heptapeptide hormone to tumor-bearing mice also markedly attenuated angiogenesis in the liver tumors with a concomitant reduction in VEGF mRNA and protein. Reduced proliferation and increased apoptosis following incubation with Ang-(1–7) was observed in hepatic H22 cancer cells. The effect was blocked by co-administration of A779, further supporting the in vivo results.

Mao et al. [29] also demonstrated attenuation of hepatic tumor growth in mice using adenoviral delivery of Ang-(1–7). A significant reduction in tumor size and weight was observed in mice administered the heptapeptide hormone as compared to animals receiving the control vector. Inhibition of cancer cell proliferation was demonstrated by a reduction in immunostaining of Ki67 and Cdc6 (cell division cycle 6 protein) in tissue sections of tumors from Ang-(1–7) treated mice as compared to controls. In addition, a marked decrease in the mRNA of the angiogenic factors VEGF and PlGF as well as the VEGF receptors Flt-1 and Flk-1 was observed in tumor samples from mice receiving the heptapeptide hormone. Taken together, these two studies demonstrate that Ang-(1–7) reduces liver cancer in mice by pleiotropic mechanisms and suggest that the heptapeptide hormone may effectively inhibit liver cancer in patients (Fig. 24.3).

Preclinical animal studies support a cytostatic mechanism of action for Ang-(1–7). While the heptapeptide hormone inhibits tumor growth as compared to the tumors from untreated control animals, the tumor weight or size is generally not decreased to baseline measures prior to initiation of Ang-(1–7) treatment [12, 15, 16, 20, 25, 27, 30, 31]. Thus, it may be possible to reduce doses of cytotoxic chemotherapeutics in combination with Ang-(1–7) and still obtain positive outcomes.

Clinical Trials in Cancer Patients Administered Ang-(1–7)

Subcutaneous injection of Ang-(1–7) in escalating doses (100, 200, 400, and 700 μg/Kg) was administered to cohorts of 3 patients with solid tumors refractory to standard of care therapy for five consecutive days of a 21 day cycle [32]. Serum concentrations of the heptapeptide hormone prior to study initiation were equivalent to healthy adults [33]. The mean half-life was between 25 and 37 min, similar to previous report by Rodgers et al. [34] in breast cancer patients, with a maximum bioavailability of 1 h post injection. Attenuation of tumor growth for at least three months was observed in four of the 15 evaluable patients, while one patient with metastatic sarcoma receiving the highest dose of Ang-(1–7) had a mixed response without disease progression for 10 months. The heptapeptide hormone was well tolerated with mild toxicities for most patients. The maximum tolerated dose (MTD) was defined as 400 μg/kg since serious adverse events, including vascular abnormalities or neuropathy, were observed in two of six patients at the 700 μg/kg dose. Prevention in tumor growth was associated with a decrease in circulating concentrations of the angiogenic factor PlGF [32, 35], suggesting a reduction in angiogenesis by Ang-(1–7).

In a Phase II trial, patients with advanced metastatic sarcoma self-injected 20 mg/day of Ang-(1–7) in a 21-day cycle [36]. This dose was approximately equivalent to the MTD of 400 μg/kg designated in the Phase I trial [32]. Response Evaluation Criteria in Solid Tumors (RECIST 1.1) [37, 38], an assessment of tumor burden, was used to evaluate patient response to the heptapeptide hormone. In 9 of 20 patients, tumor progression was attenuated for more than three months; two of these patients had disease stabilization for 10 and 19 months. No progressive decrease in plasma PlGF or VEGF with Ang-(1–7) administration was observed in any patient. One patient experienced a grade 3 deep vein thrombosis, which was resolved with anticoagulants. The primary endpoint of a 10% response rate (percentage of patient's cancer reduction after treatment) was not reached with this small cohort.

Based on these two studies, further clinical trials with Ang-(1–7) as a monotherapy are warranted, as stabilization of tumor progression was observed in a significant percentage of the small number of patients assessed. Similar to animal studies described above, the clinical trial results described thus far suggest that the heptapeptide hormone has cytostatic actions and may be more efficacious when administered with cytotoxic therapeutics.

Combination Therapy with Ang-(1–7) in Preclinical Models

Summarized below are studies assessing Ang-(1–7) with radiation or standard of care chemotherapeutics. Cancer therapy regimens generally consist of multiple procedures, including surgery, radiation, and cancer drugs. Studies in animal models are vital to determine whether a combination therapy is more efficacious without the addition of side effects and there are no antagonistic actions.

Circulating white blood cells as well as bone marrow myeloid, erythroid and megakaryocyte progenitor cells were increased markedly following Ang-(1–7) administration to mice receiving total body irradiation [39]. Co-administration of A779, the mas1 receptor antagonist, blocked the improvement in hematopoietic recovery induced by the heptapeptide hormone. Surprisingly, treatment with Ang-(1–7) several days after total body irradiation still invoked an improvement in hematopoietic recovery [40]. In fact, a more pronounced enhancement of bone marrow progenitor cells was observed when Ang-(1–7) treatment was delayed until after cessation of the myelotoxic damage induced by irradiation. Treatment with the heptapeptide hormone also reduced pathological fibrosis induced by radiotherapy. Interstitial and perivascular fibrosis was increased significantly in the skeletal muscle of mice exposed to clinically equivalent doses irradiation in the hindlimb as compared to non-irradiated mice [41]. Infusion of Ang-(1–7) prior to radiation effectively prevented the pathological fibrotic effect. The radiation-induced increase in the profibrotic cytokines TGF-β and connective tissue growth factor CTGF in the soleus muscle was blocked by administration of the heptapeptide hormone prior to irradiation. These results suggest that Ang-(1–7) may serve as an effective prophylactic to prevent or recover tissue or organ damage caused by radiotherapy.

Many chemotherapeutic drugs also cause multi-lineage cytopenias and most hematopoietic agents do not provide protection for the various cell types found in the blood. Adjuvant treatment of Ang-(1–7) significantly increased early lineage bone marrow progenitor cells in mice following treatment with the myelosuppressive drugs 5-fluoruracil or cyclophosphamide [42, 43] or gemcitabine [44]. The combination drug treatment not only enhanced the number of bone marrow progenitor cells, circulating blood cells and platelets in myelosuppressive mice greater than the concentration observed with either agent alone but reduced concentrations of Neupogen or Epogen was required when in combination with Ang-(1–7). Collectively, these studies indicated that Ang-(1–7) may enhance recovery of bone marrow hematopoietic precursors and circulating blood cells and elements in patients following myelosuppressive chemotherapy.

Ager et al. [30, 31] published the first study showing that Ang-(1–7) effectively reduced liver metastasis induced by intrasplenic injection of colorectal cancer into mice. Combination therapy of the heptapeptide hormone with an ACE inhibitor or AT1 receptor blocker did not result in enhanced tumor inhibition. A subsequent study supported the attenuation of liver metastasis in mice by Ang-(1–7) or the ACE inhibitor Captopril and further showed that both treatments also caused a marked increase in hepatic macrophages at the tumor margins [30, 31]. This suggests that

one mechanism whereby the heptapeptide hormone or ACE inhibitors reduce liver metastasis is through the immunomodulation of Kupffer cells, which secrete anti-proliferative factors.

Doxorubicin (Dox) is an effective, anti-cancer drug used to treat patients with breast, lung, ovarian, bladder, and pediatric cancers. Unfortunately, clinical administration is limited due to potential cumulative, dose-dependent drug toxicity, which may be transient or ultimately lead to heart failure. Clinically equivalent doses of Dox induced alterations in cardiac morphometry as well as global and diastolic dysfunction in male and female juvenile rats, similar to the pathologies observed in pediatric patients administered anthracycline therapy [45]. The Dox-mediated cardiac toxicity was blocked by Ang-(1–7) co-administration to the rats. The heptapeptide hormone attenuated the increase in NADPH oxidase 4 (Nox4), by-products of lipid peroxidation, malondialdehyde and 4-hydroxynonenal and the decrease in the antioxidant enzymes superoxide dismutase and catalase caused by Dox administration. Further, the heptapeptide hormone prevented the enhanced interstitial and coronary vessel fibrosis with an associated increase in inflammatory cardiac TGF-β1 and pSMAD2 in the hearts of juvenile rats of both sexes following Dox administration. Dox caused a significant increase in pulse wave velocity, a measure of arterial stiffness in rats of both sexes but the mechanism was distinct [46]. An increase in lumen diameter, wall thickness, media hypertrophy and reduced elasticity was observed in the aortic arches of male rats administered Dox, while in the juvenile female rats the anthracycline increased fibrosis. The Dox-mediated damage to the juvenile rat aortic arches was prevented by co-administrations of Ang-(1–7). The results of these two studies suggest that the heptapeptide hormone may serve as an effective preventative agent to reduce cardiovascular damage caused by anthracycline administration to cancer patients (Fig. 24.4).

Administration of Ang-(1–7) or sunitinib (a multi-targeted receptor tyrosine kinase inhibitor) reduced human clear cell renal cell (RCC) cancer growth in mice; however, treatment with both drugs in combination more effectively diminished tumor proliferation [47]. The mas1 receptor antagonist A779 blocked the heptapeptide hormone inhibition of RCC growth in mice, demonstrating a receptor-mediated process. Similar results were obtained with co-administration of Ang-(1–7) with axitinib, a combined PD-L1 (immune checkpoint inhibitor of the programmed death-ligand 1) and VEGF-TKI (VEGF receptor-tyrosine kinase inhibitor), a chemotherapy regimen used to treat resistant RCC. Monotherapy using Ang-(1–7) or the dual combination of VEGF-TKI and PD-L1 inhibitor significantly inhibited RCC tumor growth in mice compared to tumors in the control animals, while combination of the two drugs enhanced the reduction in tumor proliferation. These results suggest that this multiple drug regimen including Ang-(1–7) may be more efficacious in patients with RCC cancer than standard of care therapy.

Taken together, the result of these combination studies suggest that Ang-(1–7) may enhance the efficacy as well as prevent the toxicity of standard of care therapies, as no adverse outcomes were reported in the animals receiving combination therapy.

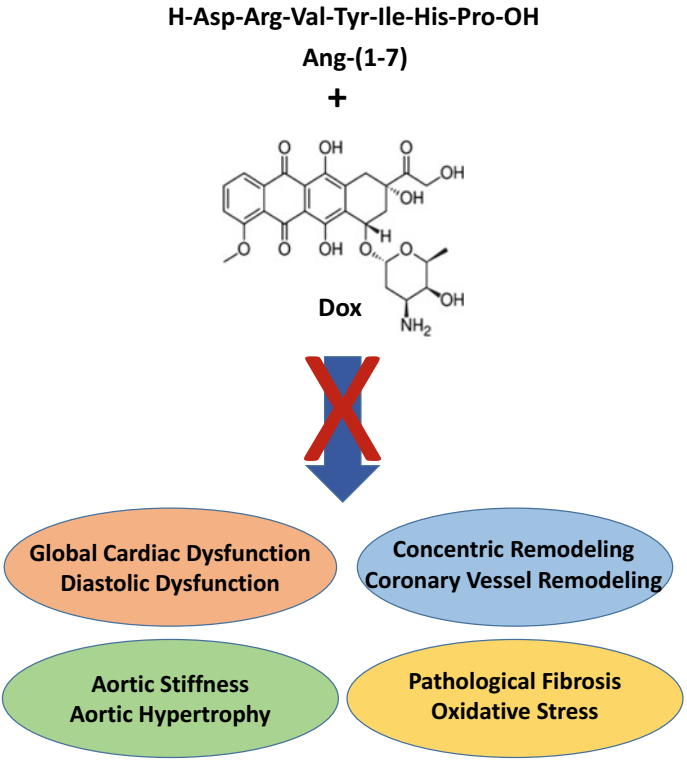

Fig. 24.4 Dox-induced cardiac damage that is prevented by Ang-(1–7) administration

Clinical Trials in Cancer Patients Administered Ang-(1–7) With Standard of Care Therapy

Rodgers et al. [34] assessed the use of Ang-(1–7) as an adjuvant to Dox and cyclophosphamide chemotherapy administered in newly diagnosed breast cancer patients. This Phase I/II trial evaluated the toxicity and optimal biologic dose of Ang-(1–7) administered to breast cancer patients after surgery as well as before and during chemotherapy to mitigate multiple types of cytopenia. Two days after chemotherapy and at least 10 consecutive days in three consecutive cycles, patients received escalating doses of Ang-(1–7) by subcutaneous injection or filgrastim, an approved synthetic drug that stimulates bone marrow production of granulocyte colony-stimulating factor. Ang-(1–7) was administered daily two days after surgery for seven consecutive days followed by a 1 week drug holiday before the first cycle of chemotherapy to assess toxicity. No dose limiting toxicity was found for Ang-(1–7) and no patients administered with the heptapeptide hormone experienced a treatment-related SAE. While the dose required to mitigate cytopenia varied by hematological lineages, patients treated with the heptapeptide hormone

following chemotherapy showed stabilized platelet concentration, reduced incidence of anemia, lymphopenia, and mucositis, as well as an accelerated recovery of leukocytes, lymphocytes, neutrophils and hemoglobin. This study suggests that Ang-(1–7) is safe and mitigated the multilineage cytopenias caused by chemotherapy, demonstrating the potential of Ang-(1–7) as an adjuvant for chemotherapy.

Similar results were obtained in a clinical trial evaluating the efficacy of Ang-(1–7) as an adjuvant therapy for patients with recurrent ovarian, Fallopian tube, or peritoneal carcinoma [48]. In this randomized, double-blind, placebo-controlled Phase II trial, patients received placebo or Ang-(1–7) with either intravenous cisplatin followed by gemcitabine or gemcitabine with carboplatin. The heptapeptide hormone in combination with myelosuppressive chemotherapy markedly enhanced platelet count and reduced Grade 4 thrombocytopenia in patients. Importantly, Ang-(1–7) was evaluated as a safe, tolerable drug with limited side effects. Unfortunately, the study was terminated early, due to low enrollment, lower than expected grade 3–4 thrombocytopenia in the placebo group, and a change in clinical practices to taxane-based chemotherapies. Nevertheless, the results of this trial support the use of Ang-(1–7) as a therapeutic to prevent cumulative myelotoxicity induced by chemotherapeutic agents.

Conclusions

The research data summarized above indicate that Ang-(1–7) attenuates the growth of multiple tumor types in mice by pleiotropic mechanisms, including altered or activated cellular signaling, reduced angiogenesis, tumor-associated fibrosis, and inflammation as well as increased apoptosis and autophagy. Ang-(1–7) activates mas1, a unique angiotensin receptor to mediate these anti-cancer properties, indicating that the heptapeptide hormone is a targeted therapy. The heptapeptide hormone has a broader spectrum of anti-cancer properties than current standard-of-care therapies with limited toxicity, indicating enhanced quality-of-life for patients. Thus, Ang-(1–7) may effectively serve as a first-in-class, targeted drug for the treatment of cancer and cancer-related pathologies or as an adjuvant to mitigate toxicity caused by existing treatment regimens. However, before Ang-(1–7) could be considered a marketable drug it will be necessary to overcome the limited stability of the heptapeptide hormone in the circulation as well as the lack of oral bioavailability and a patent-protected structure needed for drug development to proceed.

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