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Review Article

Local bone marrow renin–angiotensin system in primitive, definitive and neoplastic haematopoiesis

Ibrahim C. HAZNEDAROGLU* and Yavuz BEYAZIT†

*Department of Hematology, Hacettepe University School of Medicine, Ankara, Turkey
†Turkiye Yuksek Ihtisas Training and Research Hospital, Ankara, Turkey

Abstract

The locally active ligand peptides, mediators, receptors and signalling pathways of the haematopoietic BM (bone marrow) autocrine/paracrine RAS (renin–angiotensin system) affect the essential steps of definitive blood cell production. Haematopoiesis, erythropoiesis, myelopoiesis, formation of monocytic and lymphocytic lineages, thrombopoiesis and other stromal cellular elements are regulated by the local BM RAS. The local BM RAS is present and active even in primitive embryonic haematopoiesis. ACE (angiotensin-converting enzyme) is expressed on the surface of the first endothelial and haematopoietic cells, forming the marrow cavity in the embryo. ACE marks early haematopoietic precursor cells and long-term blood-forming $CD34^+$ BM cells. The local autocrine tissue BM RAS may also be active in neoplastic haematopoiesis. Critical RAS mediators such as renin, ACE, AngII (angiotensin II) and angiotensinogen have been identified in leukaemic blast cells. The local tissue RAS influences tumour growth and metastases in an autocrine and paracrine fashion via the modulation of numerous carcinogenic events, such as angiogenesis, apoptosis, cellular proliferation, immune responses, cell signalling and extracellular matrix formation. The aim of the present review is to outline the known functions of the local BM RAS within the context of primitive, definitive and neoplastic haematopoiesis. Targeting the actions of local RAS molecules could represent a valuable therapeutic option for the management of neoplastic disorders.

Key words: bone marrow, cancer, haematopoiesis, neoplastic disorder, renin–angiotensin system (RAS)

INTRODUCTION

We first proposed the concept that there exists a local haematopoietic RAS (renin–angiotensin system) in the BM (bone marrow) [1,2]. The main RAS molecules, including renin, angiotensinogen, angiotensin receptors and ACE (angiotensin-converting enzyme), are all present in the BM micro-environment [3]. Locally active BM RAS affects critical steps of physiological and pathological blood cell production via autocrine, paracrine and intracrine pathways [4,5]. Haematopoietic niche [6], myelopoiesis [7], erythropoiesis [8], thrombopoiesis [9] and the development of other cellular lineages [6,10–12] are regulated by the biological actions of local BM RAS peptides. The local RAS [4,5] is also active in the BM stromal niche for crucial regulation of haematopoietic functions [3,13]. AT_1R and AT_2R (angiotensin type 1 and type 2 receptors respectively), and the inhibitory tetrapeptide AcSDKP (*N*-acetyl-Ser-Asp-Lys-Pro) are present in the BM stromal micro-environment. The major RAS effector mediator AngII (angiotensin II) exerts its haematopoietic effects by activating angiotensin receptors, primarily AT_1Rs and AT_2Rs, within the BM micro-environment [4,5]. ACE (CD143) has been implicated in enhancing the recruitment of primitive stem cells into S-phase by degrading AcSDKP [9,14–17]. Moreover, stimulation of AT_1Rs/AT_2Rs by AngII, the principle effector molecule of the RAS, exerts stimulatory/inhibitory action on the JAK/STAT (Janus kinase/signal transducer and activator of transcription)

Abbreviations: ACE, angiotensin-converting enzyme; ACEI, ACE inhibitor; AcSDKP, *N*-acetyl-Ser-Asp-Lys-Pro; AGM, aorta–gonad mesonephros; AML, acute myeloid leukaemia; Ang-(1–7), angiotensin-(1–7); AngI etc., angiotensin I etc.; AGTRAP, AngII-receptor-associated protein; APC, antigen-presenting cell; ARB, AngII receptor blocker; AT_1R etc., angiotensin type 1 receptor etc.; BM, bone marrow; C/EBPα, CCAAT/enhancer-binding protein α; CCL, CC chemokine ligand; CGRP, calcitonin gene-related peptide; D, deletion; EPO, erythropoietin; ERK, extracellular-signal-regulated kinase; GABARAP, γ-aminobutyric acid-receptor-associated protein; I, insertion; JAK, Janus kinase; JNK, c-Jun N-terminal kinase; KO, knockout; LSK, Lin^- $Sca1^+$ $c\text{-}Kit^+$; mAb, monoclonal antibody; M-CSF, macrophage colony-stimulating factor; MMP, matrix metalloproteinase; NF-κB, nuclear factor κB; NK, natural killer; NK1, neurokinin 1; NUP98, Nucleoporin 98; HOXA9, NUP98-homeobox A9; PI3K, phosphoinositide 3-kinase; PLZF, pro-myelocytic zinc-finger protein; PRR, (pro)renin receptor; PRV, polycythaemia ruba verae; PV, polycythaemia verae; RAS, renin–angiotensin system; RER, renin/prorenin receptor; RIS, retroviral integration site(s); SP, substance P; STAT, signal transducer and activator of transcription; TBI, total body irradiation; TNF, tumour necrosis factor; UCB, umbilical cord blood; UICC, Unio Internationale Contra Cancrum; VEGF, vascular endothelial growth factor.

Correspondence: Professor Yavuz Beyazit (email yavuzbeyaz@yahoo.com).

pathway, which is directly linked to the physiological functions of erythropoietin, thrombopoietin and other haematopoietic cytokines during normal haematopoiesis and in myeloproliferative neoplasms [9,18–20]. The local RAS is effective even at the stage of primitive embryonic haematopoiesis [21–24].

The RAS affects numerous biological events that are important for the formation and function of blood cells [25]. Apoptosis [26], cellular proliferative events [18,27,28], mobilization [29], angiogenesis [30], fibrosis within the cytokine network [31] and many other essential pathobiological events are affected by the critical RAS molecules [4,5,32,33]. Haematological clonal neoplastic disorders are characterized by excessive production of malignant cells, as well as disordered apoptosis, impaired differentiation, pathological signalling and cancer angiogenesis [34–39]. Neoplastic malignant blood cells are derived from leukaemic stem cells within a complex series of pathological proliferative steps. There is preliminary evidence that the local BM RAS could affect neoplastic tumoral blood cell production [34–36,40–45]. The prominent functions of the local RAS in primitive embryonic haematopoiesis [21–24] support further the hypothesis that the local autocrine BM RAS could be active in neoplastic haematopoiesis. Table 1 summarizes an overview of the current findings implying the existence of a local RAS in primitive neoplastic haematopoiesis.

The aim of the present review is to outline the situation and function of the local BM RAS within the context of primitive, definitive and neoplastic haematopoiesis. Critical RAS mediators such as renin [44], ACE [35], AngII [46] and angiotensinogen [36] have already been identified in leukaemic blast cells. RAS-modulating agents could also alter the pathological formation of neoplastic blood cells [47]. Elucidation and future dissection of the status of the local BM RAS in leukaemogenesis is not only an academic issue, but also represents a clinically relevant basic research area [4,5]. For instance, a pharmacological form of the peptide Ang-(1–7) [angiotensin-(1–7)] is already in Phase I/II clinical trials for the modulation of the local BM RAS in distinct disease states [33,48,49].

THE LOCAL BM RAS IN POST-NATAL DEFINITIVE HAEMATOPOIESIS

Haematopoietic BM gives rise to all circulating blood cells and is a highly organized complex system. HSCs (haematopoietic stem cells) and committed haematopoietic precursors interact with local BM micro-environmental stromal cells, providing the majority of essential growth factors to maintain the regulation of haematopoietic cell development. Dynamic events of post-natal haematopoiesis within the BM microenvironment are regulated by the production or degradation of numerous soluble factors, such as cytokines and bioactive peptides. The local BM RAS mediates the complicated network of BM haematopoiesis in an autocrine/paracrine/intracrine fashion. The growth, production, proliferation and differentiation of blood cells are affected by the haematopoietic RAS [1,4,5]. AT_{1a}Rs are present in $CD34^+$ haematopoietic cells and stimulate the proliferation of both BM and UCB (umbilical cord blood) haematopoietic progenitors in physiological or pathological states [32]. ACE, converting AngI (angiotensin I) into AngII, has a role in many aspects of haematopoiesis [7,15,21,50,51]. A pan-lineage proliferative effect of AngII has been demonstrated. Exogenous AngII facilitates colony formation from HSCs to CFU-GMs (colony-forming unit-granulocyte/macrophages) and CFU-GEMMs (colony-forming unit-granulocyte/erythroid/macrophage/megakaryocytes) under a pan-myeloid condition [12]. AngII specifically regulates TNF (tumour necrosis factor)-α synthesis and release from BM stromal cells, acting on monocytic lineages [52]. Richmond and co-workers [53] provided the basis for further understanding the role of AngII in the regulation of both normal and dysfunctional haematopoiesis. Locally produced AngII affects arachidonic acid release within the BM micro-environment. Arachidonic acid, upon its release by AngII, can enter the BM cellular compartment and act as a signalling molecule to influence proliferation or apoptosis of haematopoietic precursors. Arachidonic acid and its eicosanoid metabolites induced by AngII are involved in multiple pathways of the haematopoietic control mechanism [53]. Critical formation steps of distinct lineages of blood cells, such as the myeloid cells and erythroid elements, are subject to the pathobiological activities of the local BM RAS [1,4,5].

The local BM RAS regulates peptides for haematopoiesis. ACE releases AngII from AngI and degrades the bioactive SP (substance P), AcSDKP and Ang-(1–7). Moreover, SP is released from the nerve endings projecting to the BM in the vicinity of micro-environmental stroma. RAS peptides are produced from the BM tissue through the contribution of both stromal cells and haematopoietic cells. Both stromal cells and haematopoietic cells are equipped with AT_1Rs and NK1 (neurokinin 1) receptors, which mediate the action of AngII and SP respectively. Mas, the receptor for Ang-(1–7), has been detected in the BM stroma as well [12] (Figure 1).

Myelopoiesis and white blood cells

ACE, within the context of the local BM RAS, affects normal myelopoiesis. ACE-KO (knockout) mice exhibited prominent abnormalities in myelopoiesis characterized by increased BM myeloblasts and myeloid cells, as well as extramedullary myelopoiesis. AngII, through the AT_1R, also acts to boost myeloid differentiation and functional maturation. The myelopoietic effects of ACE and AngII are evident at the HSC level, extending to the committed myeloid and erythroid lineages [7]. Leucocytes expressing the angiotensinogen gene, synthesizing and releasing angiotensinogen with the capability to generate angiotensin, represent another clue for local RAS-modulating effects on myelopoiesis [54].

Lin et al. [7] demonstrated the status of ACE in myelopoiesis in a series of elegant experiments in their ACE-KO mice model. They quantified BM cell populations and found that the numbers of neutrophils (band and segmented cells) and erythroid elements are 37% reduced in ACE-KO mice in comparison with the wild-type mouse. Moreover, myeloblasts increased 2.2-fold, eosinophils 2.3-fold and immature myelocytes 2.5-fold in the ACE-KO mice. Likewise nucleated erythroid precursors decreased to 77% of control values, leading to anaemia in the animals [7]. Splenomegaly with extramedullary haematopoiesis, consisting of immature myeloid elements, further complicated the clinical

Table 1 A brief overview of the literature data implying the existence of the local RAS in primitive and neoplastic haematopoiesis

Reference	Evidence	Proposal
Wulf et al. [114] (1996); Wulf et al. [44] (1998)	Specific immunoreactive renin-like peptide of 47 kDa isolated from AML blast cells	Renin-like enzyme activity converting angiotensinogen into AngI in leukaemic blast cells
Haznedaroglu et al. [18] (2000)	AngII utilizes the JAK/STAT pathway	JAK/STAT pathway serves as a point of cross-talk between the components of the locally present RAS in the BM and haematopoiesis
Richmond et al. [53] (2004)	Arachidonic acid and its eicosanoid metabolites induced by AngII are involved in multiple pathways of the haematopoietic control mechanism	Evidence supports the idea that AngII-mediated haematopoiesis through the release of arachidonic acid partially may explain AngII-facilitated recovery of haematopoiesis in experimental myelosuppression
Aksu et al. [40] (2005)	Angiotensinogen, ACE and renin mRNA expressions are increased in PRV	Results indicate up-regulation of the local BM RAS, together with down-regulation of the cell-surface ACE receptors, in the autonomous neoplastic clonal erythropoiesis of PRV
Kato et al. [8] (2005)	AngII, through interacting with AT_1R, enhances erythroid differentiation in the BM	Results provided support the genetic evidence that activated the RAS enhances erythropoiesis through the $AT_{1a}R$ in kidney cells
Aksu et al. [35] (2006)	Overexpression of ACE (CD 143) surface antigen in leukaemic myeloid blast cells	Renin expression could have a role on leukaemia development, and angiotensin may act as an autocrine growth factor for AML cells
Goker et al. [123] (2005)	Human UCB cells expressing renin, angiotensinogen and ACE mRNAs	The local RAS could regulate cellular growth in a wide variety of tissues, including the BM; major RAS peptides can exert significant effects on primitive pluripotential HSC populations
Takeda et al. [116] (2006)	Increased renin gene activity during NUP98–HOXA9 enhanced blast formation.	Provides a comprehensive picture of alterations in proliferation, differentiation and global gene expression that causes the leukaemic transformation of human haematopoietic cells by NUP98–HOXA9
Koca et al. [45] (2007)	Renin system in K562 leukaemic cell line *in vitro* model.	K562 human erythroleukaemia cell line may serve as an *in vitro* model to clarify the function of the RAS in leukaemia
Koca et al. [118] (2007)	ACE-expressing macrophages in lymph nodes of Hodgkin's disease	ACE expression of the lymphoma-associated macrophages in the lymph nodes of Hodgkin's disease may represent the point of cross-talk between RAS and lymphomagenesis
Zambidis et al. [24] (2008); Jokubaitis et al. [21] (2008)	ACE existence in human primitive lympho-haematopoietic cells, embryonic, fetal and adult haematopoietic tissues cast attention to the effects of the RAS on neoplastic tissues	ACE and the RAS directly adjust haemangioblast expansion and differentiation via angiotensin II receptors
Jokubaitis et al. [21] (2008)	ACE existence in human HSCs throughout haematopoietic ontogeny and adulthood	Results demonstrate ACE as a newly recognized cell-surface marker of human HSCs
Heringer-Walther et al. [33] (2009)	Ang-(1–7) contributes to haematopoietic cell proliferation	Results indicate a decisive impact of Ang-(1–7) on haematopoiesis and its promising therapeutic potential in disorders necessitating progenitor stimulation
Tsubakimoto et al. [52] (2009)	AngII specifically adjusted the TNF-α synthesis and release from BM stromal cells, acting on the monocytic lineages	AngII regulates the expression of c-Fms in HSCs and monocyte lineage cells to promote M-CSF-induced differentiation/proliferation of monocyte lineage cells and takes part in the pro-atherogenic process
Lin et al. [7] (2010)	ACE regulated myeloid differentiation, proliferation, and functional maturation through AngII and SP via AT_1R, NK1 and SP receptors	Results revealed a previous unrecognized important role for ACE in myelopoiesis and imply novel perspectives for manipulating myeloid cell expansion and maturation
Oliveira et al. [17] (2010)	Decreased AcSDKP levels were observed in BM cultures from kinin-B_1-receptor-KO mice in comparison with wild-type cultures	Hyperfunction of ACE in kinin-B_1-receptor-KO mice resulted in the fast hydrolysis of AcSDKP, which in turn decreased in BM tissues that lead HSCs to enter the S-stage of the cell cycle
Rodgers et al. [55] (2012)	Ang-(1–7) increased early mixed progenitors, erythroid progenitors, megakaryocytes and BM myeloid progenitors	Ang-(1–7) in accordance with AngII seems to be a pan-haematopoietic cytokine, stimulating the critical steps of haematopoiesis and myelopoiesis
Sinka et al. [22] (2012)	ACE is expressed in all presumptive and developing blood forming tissues of the human embryo and fetus: para-aortic splanchnopleura, yolk sac, AGM, liver and BM	ACE could identify embryonic mesodermal precursors responsible for definitive haematopoiesis and is involved in the regulation of human blood formation

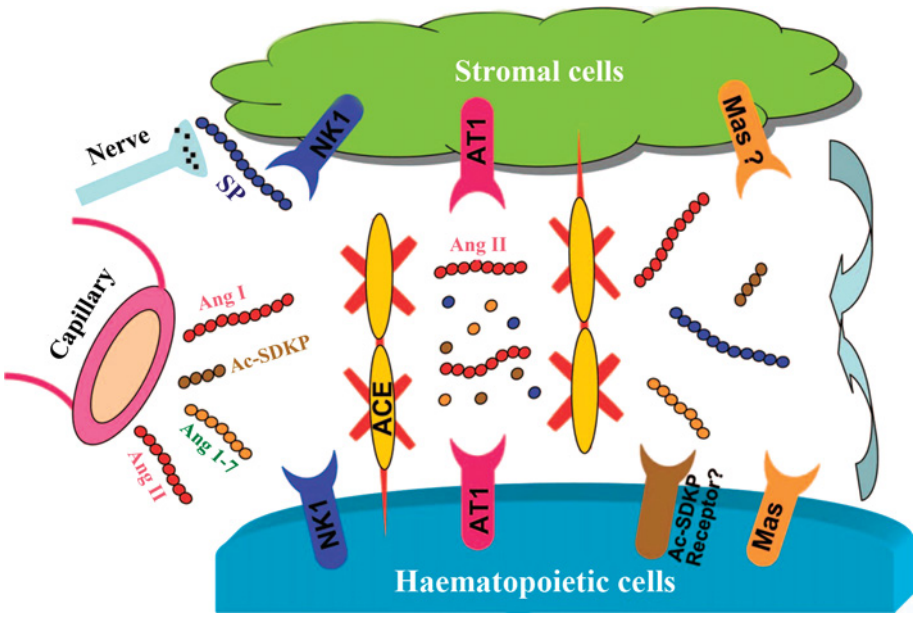

Figure 1 **The Local BM ACE regulates peptides for haematopoiesis**
Systemically, AngI, AngII, AcSDKP and Ang-(1–7) are produced in other tissues and shipped to the BM through circulation, whereas SP is released from the nerve endings projecting to the BM (in the vicinity of stroma). These peptides are also produced from BM tissue by the contribution of both stromal cells and haematopoietic cells. ACE releases AngII from AngI and degrades the bioactive SP, AcSDKP and Ang-(1–7). Both stromal cells and haematopoietic cells are equipped with AT_1Rs and NK1 receptors, which mediate the actions of AngII and SP respectively. AcSDKP is known to act on haematopoietic cells, but its receptor has not been identified. Mas, the receptor for Ang-(1–7), can been detected in BM. These peptides thus can either directly stimulate haematopoietic cells or perform their actions through stromal cells. This Figure was modified with kind permission from Xiao Z. Shen [12].

picture of the ACE-KO mice. The AngII level was 10-fold lower in these ACE-KO mice. The myeloid effects of ACE and AngII could be ascribed, in part, to the up-regulation of the central transcription factor of myelopoiesis C/EBPα (CCAAT/enhancer-binding protein α) [7]. Macrophages derived from ACE-KO mice had depressed C/EBPα expression, and AngII supplementation to ACE-KO mice rescued myeloid C/EBPα expression [12]. Therefore the local BM RAS affects haematopoiesis both by altering internal signals of transcription factors regulating gene expression and by mediating external signals from growth factors secreted from the BM micro-environmental haematopoietic and stromal cells.

ACE, as a cell-surface zinc metallopeptidase, cleaves the negative physiological haematopoietic regulator AcSDKP into its inactive form in the BM micro-environment [15]. Therefore ACE hyperfunction may lead to the acceleration of AcSDKP metabolism, which in turn would lower its levels in the BM micro-environment, finally removing the antiproliferative effects of the peptide on haematopoietic cells and blasts. The haemoregulatory tetrapeptide AcSDKP reversibly prevents the recruitment of pluripotent HSCs and normal early haematopoietic progenitors into S-phase of the cell cycle by keeping them quiescent [14,16,34].

Kinin B_1-receptor-KO mice were suggested to be an experimental model for ACE hyperfunction. Oliveira et al. [17] examined BM cell populations involved in the ACE expression and the role of AcSDKP in the ACE activity in this murine BM cell proliferation model. They demonstrated a decrease in the erythroid ($Ter119^+$), granulocytic-macrophage ($Gr1^+Mac1^+$), $CD220^+$, $CD3^+$ and primitive population LSK ($Lin^-Sca1^+c\text{-}Kit^+$) cells in the B1-KO mice. The authors used the ACE substrate Abz-YRK(Dnp)P-OH [*o*-aminobenzoic acid-Phe-Arg-Lys(DNP)-Pro-OH] and observed an increase in B1-KO long-term BM culture (LTBMC) ACE activity in relation to the wild-type cultures. However, the biological significance of those findings remain to be elucidated [17].

Ang-(1–7) is a component of the RAS, stimulating haematopoietic recovery after myelosuppression. Ang-(1–7) can be formed directly from the major RAS peptide AngII by the actions of the enzyme ACE2 and also from the AngI peptide. Mas receptors present in the BM micro-environment mediate the proliferative effect of Ang-(1–7) on HSCs [32]. Rodgers and co-workers [55] tested the ability of the Ang-(1–7) peptide to improve radiation-induced haematopoietic injury after TBI (total body irradiation) in an animal model. In their study, daily administration of subcutaneous Ang-(1–7) after radiation exposure improved survival (from 60% to 92–97%) and reduced radiation-induced thrombocytopenia (up to 2-fold) after TBI. Moreover, Ang-(1–7) increased early mixed progenitors (3–5-fold), erythroid progenitors (2–fold), megakaryocytes (2–3-fold), and BM myeloid (3–6-fold) progenitors. Therefore Ang-(1–7) seems to be a pan-haematopoietic cytokine stimulating the critical steps of haematopoiesis and myelopoiesis [27,29,33,48,49,55,56]. Ang-(1–7) is now considered as a drug candidate for the modulation of local BM RAS-mediated haematopoiesis in a variety of clinical

disorders [33,48,49]. The emerging ACE2/Ang-(1–7)/Mas-receptor-axis-oriented drugs are of particular interest [32].

AngII, through the BM cellular AT_1Rs, regulates the differentiation/proliferation of monocyte lineage cells to exert pro-atherogenic actions in the cardiac micro-environment [52,71]. AT_1R signalling is required for macrophage development. On the basis of experiments performed by Tsubakimoto et al. [52] in BM chimaeric apoE (apolipoprotein E)$^{-/-}$ mice re-populated with AT_1R-deficient (Agtr1$^{-/-}$) or wild-type (Agtr1$^{+/+}$) BM cells, AngII regulates the expression of c-Fms in HSCs and monocyte lineage cells through BM stromal cell-derived TNF-α to promote M-CSF (macrophage colony-stimulating factor)-induced differentiation/proliferation of monocyte lineage cells. M-CSF-induced differentiation from HSCs (LSK) to pro-monocytes (CD11b^{high}Ly-6G^{low}) was markedly reduced in HSCs from AT_1R-deficient Agtr1$^{-/-}$ mice in their experiments [52].

Erythropoiesis and red blood cells

Inactivation of the gene encoding mouse ACE results in anaemia in adult animals. This anaemia is corrected by AngII, demonstrating the involvement of AngII in definitive erythropoiesis [57,58]. AngII enhances erythropoiesis via the stimulation of erythroid progenitor cells and increases haematopoietic progenitor cell proliferation [8,59–61]. Kato and co-workers [8] demonstrated that transgenic mice carrying both the human renin and human angiotensinogen genes displayed persistent erythrocytosis. They introduced both transgenes into the $AT_{1a}R$-null background and found that the haematocrit level in the compound mice was restored to the normal level. They conducted BM transplantation experiments and clarified that $AT_{1a}Rs$ on BM-derived cells were dispensable for RAS-dependent erythrocytosis [8]. AngII could increase EPO (erythropoietin) levels, and EPO mediates the effects $AT_{1a}Rs$ in erythropoiesis [8]. The data on PRR [(pro)renin receptor] and erythropoiesis are very limited. Only the study by Kaneko et al. [59] disclosed the expression of PRR in BM erythroid cells and a dynamic increase in PRR expression by IFN (interferon)-γ, an inflammatory cytokine suppressing erythropoietin production and erythropoiesis. PRR is constitutively expressed in erythroid cells independently of their differentiation states. When bound to (pro)renin, PRR leads to the non-proteolytic activation of the peptide and directly activates MAPK (mitogen-activated protein kinase) ERK (extracellular-signal-regulated kinase) 1/2 signalling pathways [59]. Therefore local BM RAS-induced enhanced eythropoiesis seems to be regulated in an autocrine and intracrine manner. The PRR is a relatively new element in the RAS and thus findings are sparse [62]. The primary localization of the human RER (renin/prorenin receptor) is in the intracellular perinuclear zone. Furthermore, a transcription factor, PLZF (pro-myelocytic zinc-finger protein), was identified as a direct protein interaction partner of the renin receptor and, following the activation of RER by renin, PLZF is translocated from the cytoplasm to the nucleus, providing positive or negative regulation of target genes [63]. The regulation of c-kit expression involves transcriptional control by PLZF in CD34$^+$ cells and early erythropoiesis [64]. PLZF is also an important AT_2R-binding protein in mediating AngII-induced cellular effects through an AT_2R-dependent signalling pathway. The AngII/AT_2R/PLZF/GATA4 signalling pathway may modulate the AngII-induced pathological effects [65]. These very preliminary findings about the interactions of PRR, PLZF, AngII and erythropoiesis need to be tested in future experiments.

Savary et al. [66] indicated the presence and function of the local RAS in primitive embryonic erythropoiesis located in the yolk sac of the chicken embryo in the vicinity of blood islands. ACE, renin, angiotensinogen and angiotensin receptors are present in the yolk sac endoderm, concomitantly with the differentiation of blood islands in the adjacent yolk sac mesoderm. ACEIs (ACE inhibitors) and ARBs (AngII receptor blockers) decreased erythroblast proliferation induced by RAS in this embryonic animal model. Therefore the local autocrine RAS is active in the modulation of both primitive and definitive erythropoiesis. RAS inactivation may confer susceptibility to the haematocrit-lowering effects of ACEIs or ARBs via interfering with this BM cellular autocrine RAS network [67].

RAS can also affect pathological and neoplastic erythropoiesis. AT_1R expression in erythroid progenitors has been implicated in the pathogenesis of post-renal transplant erythrocytosis [60]. Increased local synthesis of the major RAS components has also been identified by demonstrating corresponding mRNAs in the BM in PV (polycythaemia verae). Therefore up-regulation of the local BM RAS in autonomous neoplastic clonal erythropoiesis of PV has been suggested [40].

Thrombopoiesis and platelets

The RAS member AGTRAP (AngII-receptor-associated protein) is active in haematopoietic cell proliferation and survival and points to its synergistic effect with the proliferation promoting function of the thrombopoietin receptor Mpl [9]. The Mpl proto-oncogene plays a basic role in megakaryocytic development and platelet production, as well as performing a substantial function in HSC homoeostasis and self-renewal [68].

The results on angiotensin peptides in thrombopoiesis is very limited. The only study is by Kwiatkowski et al. [9], who performed retroviral insertional mutagenesis using an MSCV (murine stem cell virus)-based vector coding for a drug-dependent dimerizable fusion protein that contains the cytoplasmic domain of Mpl. They used the vector construct to transduce the human leukaemia cell line K562, which also includes all of the RAS elements [45]. In the absence of Mpl signalling, the cells underwent erythroid differentiation and died. Cells that acquired proliferative advantage and were dependent on Mpl function were expected to point to mutations synergistic with Mpl signalling. Cloning of RIS (retroviral integration site) from the selected populations could allow the identification of over-represented clones. RIS from the over-represented clones pointed to candidate Mpl co-operating genes. AGTRAP, a member of RAS, was identified among the candidate genes. Kwiatkowski et al. [9] also tested the function of AGTRAP in haematopoietic cells. In their experiments, AGTRAP protein was overexpressed in K562 cells and in K562 cells driven by Mpl signalling. The survival of Mpl-dependent K562 cells overexpressing AGTRAP was still dependent on Mpl signalling and on JAK2 function. [9].

Platelets expressing AT_1Rs contribute to thrombogenic and inflammatory responses [69]. AngII, through its receptors, has

in vivo platelet-activating effects, and markers of platelet secretion are also significantly increased [70]. The clinicopathological relevance of these findings may be evident. AngII elicited a dose- and time-dependent increase in platelet–leucocyte–endothelial cell interactions in the cerebral venules, which included rolling platelets, adherent platelets on the leucocytes and the endothelial cells, rolling leucocytes and adherent leucocytes [69].

Formation of other blood cell lineages: dendritic cells, mast cells, T-cells, monocytes, macrophages and APCs (antigen-presenting cells)

Multiple lines of evidence indicate that the local RAS is active in haematopoietic cell proliferation and differentiation, which also leads to the production and function of distinct blood cell lineages, such as dendritic cells, mast cells, T-cells, monocytes, macrophages and antigen-presenting cells [4,5]. ACE degrades SP present in the BM micro-environment, lymphoblasts and lymphocytes by cleaving a C-terminal dipeptide or tripeptide [12].

The local RAS is also expressed in the cellular compartment of the immunohaematological system, such as on APCs. AT_1Rs are expressed on both T-cells and APCs [71]. AT_1Rs are required for the normal development and function of dendritic cells [72–74]. Dendritic cells are highly specialized APCs with a unique ability to activate resting T-cells. AngII, acting through the AT_1R, facilitates dendritic cell development, whereas signalling through the AT_2R counteracts AT_1R signalling in this haematological line [12,74]. Nahmod et al. [74] have shown the effects of AngII, AT_1Rs and AT_2Rs in dendritic cell differentiation from monocytes in the BM. Pharmacological blocking of the AT_1R during GM-CSF (granulocyte/macrophage colony-stimulating factor)-stimulated culture hampered dendritic cell maturation, as indicated by the decreased expression of surface maturation markers, the reduced efficiency of endocytosis and the decreased ability to stimulate allogeneic T-cell proliferation. In contrast, AT_2R blockade or AngII addition during culture had the opposite effect and stimulated progenitors to obtain the functional activities of the dendritic cells [74]. AT_{1a}R-deficient BM-derived dendritic cells produce higher levels of MCP-1 (monocyte chemoattractant protein-1) [73]. Another study by the same group [72] reported that the absence of AT_1Rs results in the generation of dendritic cells with major alterations in their phenotypic and functional immunohaematological properties. They suggested that AngII stimulates dendritic cell differentiation and function in an autocrine/paracrine manner by signalling via AT_1Rs to contribute to perpetuating immuno-inflammatory responses [72].

The effects of the RAS on dendritic cells and APCs seem to be clinically relevant for the pathogenesis of immuno-inflammatory disorders. Quantitative RT (reverse transcription)-PCR analyses by Stegbauer et al. [71] showed the up-regulation of renin, ACE and AT_1R in the immune system, including APCs in the myelin oligodendrocyte glycoprotein-induced experimental autoimmune encephalomyelitis. They demonstrated that the local RAS is up-regulated during autoimmune inflammation in the immune system and exerts its functions mainly via the AT_1R in an autocrine/paracrine/intracrine manner by modulating APC populations and governing the expression of chemokines. Blockade of AT_1Rs significantly reduced the number of $CD11b^+$ or $CD11c^+$ APCs in immune organs and in the inflamed spinal cord. Likewise, AT_1R blockade impaired the expression of CCL (CC chemokine ligand)2, CCL3, and CXCL (CXC chemokine ligand)10, and reduced CCL2-induced APC migration [71].

Pre-formed AngII as a potent growth factor is present and released by human mast cells. The presence of pre-formed AngII and gene expression of the RAS system were detected in human mast cells by Hara et al. [10]. The release and synthesis of AngII in mast cells was regulated by CGRP (calcitonin gene-related peptide). CGRP induced the release of AngII and increased angiotensinogen mRNA in a human mast cell line. Mast cells are, therefore, considered as a mobile RAS regulated by neural activity. As the number of mast cells increases, they may significantly contribute to the production of AngII in the target organ, such as the heart [10].

THE LOCAL RAS IN PRIMITIVE EMBRYONIC HAEMATOPOIESIS

The human embryo represents a model for the production of the first multipotent haematopoietic cells that constitute the stem cells of the fetal and post-natal blood systems. Primitive haematopoietic cells during all stages of haematopoietic ontogeny include UCB, the fetal liver, embryonic para-aortic splanchnopleura and primitive haematopoietic cells in the AGM (aorta–gonad mesonephros) region [21].

Previous studies [21–24] have focused on the status of ACE within the context of the local RAS, the earliest human embryonic HSCs [75] and the developmental sequence underlying the ontogeny of human blood cells. The local RAS regulates the genesis and function of the haematopoietic system starting from embryonic life [23]. A mAb (monoclonal antibody) named BB9, raised initially to human BM stromal cells, recognizes the somatic isoform of ACE as an HSC marker. It exhibits reactivity with primitive haematopoietic cells at all stages of haematopoietic ontogeny, including in the UCB and fetal liver [21]. Human embryonic stem-cell-derived $ACE^+ CD45^- CD34^{\pm}$ cells are the common yolk-sac-like progenitors for not only the endothelium, but also both primitive and definitive human lymphohaematopoietic stem cells (Figure 2) [24].

During embryonic development, multi-lineage HSCs/progenitor cells are derived from specialized endothelial cells, which are termed the haemogenic endothelium, within the yolk sac, placenta and aorta [76]. Jokubaitis and co-workers [21] have reported that ACE, as recognized by mAb BB9, is a marker of primitive haematopoietic cells at all stages in the ontogeny of the human haematopoietic system. In their experiments, BB9/ACE was expressed during the development of the human haematopoietic system. BB9 identified the somatic form of ACE and SCID (severe combined immunodeficiency) mouse repopulating cells, and the perturbation of RAS signalling altered the proliferation of primitive haematopoietic progenitors [21]. Zambidis et al. [24] have demonstrated that the RAS is a pivotal regulator of human haemangioblast differentiation. They showed that AT_1R and AT_2R are expressed on human haemangioblasts.

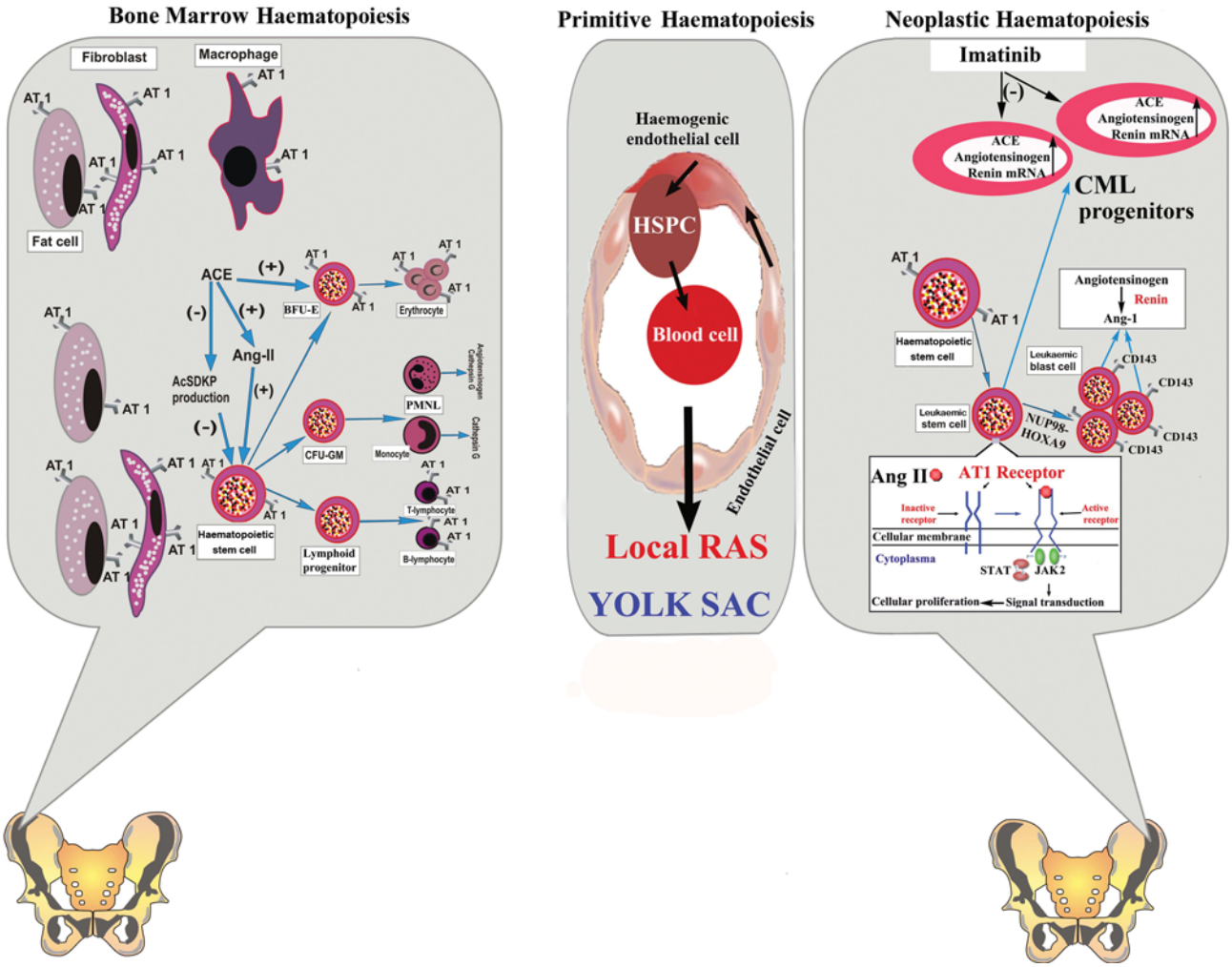

Figure 2 **The local BM RAS in association with the yolk sac embryonic haematopoiesis affects the growth, production, proliferation and differentiation of the haematopoietic progenitors in the local micro-environment**

Interactions of the angiotensin molecules, receptors, ligands and the relevant RAS elements in the cellular proliferation of haematopoietic BM are also depicted. HSPC, haematopoietic stem/progenitor cells; CFU-GM/E, colony-forming units granulocyte/macrophage/erythroid. This Figure is from Haznedaroglu, I.C. & Beyazit, Y., Journal of Renin–Angiotensin–Aldosterone System (11:4), pp. 205–213 copyright © 2010 by Landes Bioscience. Reprinted by Permission of SAGE.

Furthermore, differentiation of haemangioblasts into either haematopoietic or endothelial progenitors can be influenced by modulating the signalling through these two main AngII-binding receptors [24]. In their studies, a clonogenic human embryonic stem-cell-derived haemangioblast expressing surface ACE initiated both primitive and definitive yolk-sac-like haematopoiesis. ACE expression prospectively identified all multipotent progenitors of embryonic haematopoiesis and the RAS regulated the expansion and differentiation of human embryonic haemangioblasts. Captopril inhibition of ACE enzymatic activity severely inhibited human haemangioblast colony expansion. Therefore the authors [24] suggested that human angiohaematopoiesis initiates from an ACE^- haemangioblastic progenitor of primitive and definitive haematopoiesis under the functional activities of the local RAS [24].

Sinka et al. [22] searched for the presence of ACE in the earliest pre-AGM stages of human intra-embryonic angiohaematopoiesis. In their study, in the earliest stages of human development, the haematopoietic potential in the splanchnopleura is restricted to emerging $CD34^-ACE^+$ precursors. $ACE^+CD34^-CD45^-$ mesodermal precursors migrating from the splanchnopleura toward the ventral aorta gave rise to the $CD34^+$ intra-aortic haematopoietic clusters. ACE is expressed in all of the blood-forming tissues in the human embryo, including the yolk sac, splanchnopleura, aorta, fetal liver and BM. ACE expression has functions in the maintenance of embryonic haematopoiesis [22]. These results also confirmed our hypothesis that there is a local RAS in the BM [1,2]. Sinka and co-workers [22] examined BM ACE expression in the long bones of human embryos and fetuses by flow cytometry. On the basis of their

results, ACE is expressed at the surface of $CD34^+CD45^-$ cells, and of some $CD34^-CD45^+$ cells, which represent the first endothelial and haematopoietic (probably monocyte/macrophage) cells, forming the marrow cavity. ACE marks $CD34^-CD45^-$ early haematopoietic precursor cells. At later stages (14 weeks and over), when haematopoiesis is already active inside the medullary cavity, ACE marks $CD34^+CD45^-$ endothelial cells, but also $CD45^+CD34^+$ haematopoietic cells. At these stages, some ACE^+ cells express neither CD34 nor CD45 and probably represent mesenchymal cells in the BM micro-environment. Fetal liver and BM long-term blood-forming cells are also exclusively $CD34^+ACE^+$ [22].

Emerging haemangioblasts in the yolk sac or AGM may be directed to differentiate by the HSC niche [21–24]. Antagonistic competition between AT_2R and AT_1R for AngII binding on emerging haemangioblasts directs their development into either haematopoietic progenitors or alternatively into vascular endothelial networks. AT_1R/AT_2R-regulated stem cell proliferation may be a generalized phenomenon in primitive and definitive haematopoiesis. Therefore manipulation of angiotensin receptor signalling could be an important strategy for the expansion of multipotent haematopoietic progenitors from primitive HSCs.

There are some hypotheses regarding the local BM RAS and the cellular differentiation function of HSCs, called 'stem cell plasticity', but these lack solid data [19]. Post-natal HSCs have a tremendous capacity for cellular differentiation in distinct tissues. This HSC function mimicking embryonic haematopoiesis is dependent on, and responsive to, the specific signals present in the local tissue micro-environment. AngII and 5-azacytidine can promote the proliferation and differentiation of BM mesenchymal stem cells into cardiomyocyte-like cells [77]. There is a relationship between angiotensin and transdifferentiation of epithelial cells into fibroblasts. The local embryonic RAS also plays a fundamental role in kidney development. The process of haemovasculogenesis closely related to the functions of the local RAS highlights the capacity of the kidney to produce its own blood cells simultaneously with *in situ* blood vessel formation [78]. Further investigations should focus on testing these hypotheses in distinct experimental settings.

RAS IN THE BASIC PATHOBIOLOGICAL EVENTS OF CARCINOGENESIS AND CANCER

Carcinogenesis, the development of cancer, depends upon dysregulation in a complex series of neoplastic pathobiological events. The role of the RAS in cancer represents a model for a better understanding of the place of local BM RAS in haematological neoplastic disorders. The imbalance between cellular proliferation/differentiation and apoptosis associated with immunopathological alterations and genomic dysregulation due to somatic and/or germline mutations, neoplastic intracellular signalling due to oncogenic autocrine/paracrine/intracrine effects, tumour angiogenesis, fibrosis in the tumour microenvironment, and pathological mobilization of tumoral cells could take place in the complicated pathological network of carcinogenesis [50,79,80].

The local RAS is one of the crucial components in the tumour micro-environment and plays an important role in the tumour metabolism, survival, angiogenesis and invasion processes [81]. The local tissue RAS influences tumour growth and metastases in an autocrine and paracrine fashion, via the modulation of numerous carcinogenic events, such as angiogenesis, apoptosis, cellular proliferation, immune responses, cell signalling and extracellular matrix formation. For instance, locally formed AngII in intrahepatic cholangiocarcinoma tissues plays a role in the proliferation and activation of cancer cells [82]. Potential manipulation of the local RAS with many enzymes, peptides and feedback mechanisms can even represent a therapeutic target for the clinical management of cancer [50,79,80].

AngII induces DNA damage in the form of single- and double-strand breaks. The AT_1R-specific ARB candesartan was able to prevent all types of injury caused by AngII, proving the DNA damage to be mediated by AT_1Rs [83]. The ACEI enalapril, together with aspirin, could delay the progression of pancreatic tumours and partially inhibit the formation of invasive murine pancreatic cancer in a genetically engineered mouse cancer model [84]. Likewise, losartan improves the efficacy of systemically administered nanotherapeutics to highly fibrotic solid tumours, such as pancreatic adenocarcinomas [85].

RAS and apoptosis

The biological activities of AT_1Rs and AT_2Rs and pathological alterations in their signalling could influence whether cancer cells undergo apoptosis or survive in response to RAS activation [80]. There are conflicting reports in the literature regarding apoptosis and RAS interactions [26,28,80]. Signalling through AT_2Rs is invariably linked to the promotion of apoptosis, whereas activation of AT_1Rs by AngII in malignant cells can enhance pro-survival anti-apoptotic signalling [86]. AT_1R signalling interacting with the AT_2R and/or the Mas receptor produces multiple regulatory, biological, inflammatory, angiogenic, proliferative and apoptotic actions. Hyperactivity of the RAS axis produces apoptosis in the cardiac micro-environment [86]. The role of angiotensin receptor interactions in cellular apoptosis have been studied in cardiomyocyte physiology [87]. Staining for caspase 3, an apoptotic marker, indicated that overexpression of AT_1Rs as well as AT_2Rs resulted in cardiomyocyte apoptosis with alterations in the expression of the apoptotic molecules annexin V, Bax and Bcl2. AT_1R-mediated cardiomyocyte apoptosis may be partially mediated by the up-regulation of endogenous AT_2Rs. AT_2R overexpression induced cardiomyocyte apoptosis via iNOS (inducible NO synthase) up-regulation [87]. Ghrelin may play an antagonistic role in AngII-induced cardiomyocyte apoptosis via decreasing AT_1R expression and inhibiting the activation of the endoplasmic reticulum stress pathway [88]. RAS blockade normalized renal ACE2 expression and urinary Ang-(1–7) levels, prevented tubular apoptosis, and inhibited pro-fibrotic and pro-apoptotic gene expression in mouse models, although some other studies have shown that ACEIs and ARBs increase Ang-(1–7) levels [89]. RAS signalling has been implicated in enhanced survival and increased proliferation of cancer cells. Pro-apoptotic mechanisms of the RAS are mediated through the AT_2R or the Mas receptor, whereas anti-apoptotic actions are linked to AT_1R

signalling [80]. The RAS has been linked to the inhibition of ERK signalling and the activation of cell death receptors, which leads to apoptosis. Meanwhile, the AT_1R activates mitogenic signalling pathways, including MAPK or PI3K (phosphoinositide 3-kinase) pathways, and leads to the downstream activation of transcription factors, including NF-κB (nuclear factor κB), which is responsible for the generation of numerous anti-apoptotic proteins, such as Bcl-X_L, survivin and Bcl2 [80]. Although the effects of RAS on the apoptotic process do not seem to be homogenous, those pathobiological findings in relation to apoptosis underline the role of the local RAS in carcinogenesis.

RAS and cellular proliferation-differentiation

The RAS can enhance both the proliferation and differentiation of progenitor cells. Cellular differentiation was found to be associated with an increase in cellular renin, AngII production, AT_2R expression and a concomitant decrease in angiotensinogen and ACE expression [90]. AngII, through AT_1Rs, can induce DNA synthesis, augment the generation of superoxide and activate phosphorylation of the critical oncogenic signalling pathways JAK/STAT3 and p38 MAPK [91]. On the other hand, the AT_2R functions in the inhibition of cellular proliferation [92]. Angiotensin peptides have a role in pathological cellular proliferation during cancer development. Angiotensin-generating cascades control developmental processes and homoeostasis. For instance, AngII can stimulate the proliferation of breast cancer growth via activation of the up-regulated AT_1R [93]. The promotional effects of AngII on neoplastic proliferation are dependent upon alterations in cell-cycle machinery and downstream AT_1R signalling events. Activation of the Ras/Raf/MAPK pathway and the transcription factors NF-κB and CREB (cAMP-response-element-binding protein) takes place in malignant cellular proliferation induced by AngII. ARBs might suppress the proliferative effects of angiotensin peptides. Irbesartan, a typical ARB, significantly altered p53, PCNA (proliferating-cell nuclear antigen) and cyclin D1 expression, which was also influenced by activated the AT_1R in AT_1R^+ MCF-7 breast cancer cells *in vitro* [93].

RAS and intracellular signalling

The local tissue RAS includes an intracellular system involved in cell signalling and function. Functional intracellular or nuclear RAS may have an important role in the pathobiology of the RAS. AngII, via AT_1Rs, can induce G-protein- and non-G-protein-related signalling pathways. MAPKs [ERK 1/2, JNK (c-Jun N-terminal kinase) and p38 MAPK], receptor tyrosine kinases [PDGF (platelet-derived growth factor), EGFR (epidermal growth factor receptor), IRS-1 (insulin receptor substrate-1)] and non-tyrosine receptor kinases [Src, JAK/STAT and FAK focal adhesion kinase)] are involved in local intracellular RAS functions. AngII also promotes the association of scaffolding proteins, such as paxillin, talin and p130Cas, leading to focal adhesion and extracellular matrix formation. These signalling cascades lead to cellular growth, cell migration and disease progression [28]. For instance, the JAK/STAT pathway, an important intracellular signal transduction system for numerous haematopoietic growth factors, serves as a point of cross-talk between the components of the locally present RAS in the BM and neoplastic haematopoietic disorders [18,20,52].

Intracellular expression of all three predominant angiotensin receptor subtypes is evident in the nuclear compartment [94,95]. The AT_1R is coupled to the generation of ROS (reactive oxygen species) through the activation of PI3K signalling, whereas AT_2Rs and Ang-(1–7) receptors stimulate NO formation [94]. GABARAP [GABA (γ-aminobutyric acid) receptor-associated protein] serves as a trafficking protein for the $AT_{1a}R$. GABARAP also stimulates the AngII-mediated phospho-ERK1/2 signalling pathway induction. Cell-penetrating peptides, which were designed to block the AT_1R–GABARAP interaction, effectively reduced intracellular accumulation of the AT_1R, cell-surface expression and signalling [95].

Likewise, the biological activities of the endogenous RAS peptide Ang-(1–7) is mediated through inactivation of the PI3K/Akt, p38 MAPK and JNK signalling pathways [81]. Ang-(1–7) and its receptor Mas have antiproliferative properties. For instance, Ang-(1–7) inhibits both the growth of human lung cancer cells *in vitro* and tumour angiogenesis *in vivo* through activation of the Mas receptor. Antimetastatic, antimigration and anti-invasion effects of the Ang-(1–7) peptide in A549 human lung adenocarcinoma cells has been demonstrated [81]. Ang-(1–7) also has a role in the mobilization of haematopoietic cells [29,32].

Ang-(1–7) is a pleiotropic heptapeptide hormone with dual actions. Ang-(1–7) has antiproliferative effects on cancer cells, but paradoxically has a pro-mobilization effect on haematopoietic cells. Ang-(1–7) stimulates haematopoietic recovery after irradiation, as, for example, *in vivo* studies have shown that angiotensin peptides increase haematopoietic recovery after myelosuppression [48,49,96]. The myelosuppressive therapies evaluated included TBI and intravenous administration of two different chemotherapeutic agents. The increases were most profound and longest lasting in the BM, consistent with the observed effects on early progenitors and effects on blood cell lineages, including observed increases in red blood cell [BFU-E (burst-forming unit erythroid)] and platelet [CFU-Meg (colony-forming cells megakaryocyte)] progenitors after Ang-(1–7) treatment. In addition, Ang-(1–7) stimulates dendritic cell function and plays a role in maturation of human dendritic cells [48,49,96]. The exact mechanism for the contradictory cellular effects of Ang-(1–7) is unknown. However, Ang-(1–7) alters the concentration and the balance between proliferative and antiproliferative prostaglandins [97]. Since AngII also utilizes eicosanoid pathways, the paradoxical cellular events might be ascribed to the dual effects on prostanoids.

RAS and other essential pathobiological events of oncogenesis: angiogenesis, inflammation, genomic aberrations and immunological dysregulation

Angiogenesis plays a pivotal role in tumour development, and the local RAS contributes to the promotion of tumour angiogenesis. AngII can play an augmentory role in the expression of VEGF (vascular endothelial growth factor), the main stimulator of pathological vessel formation [98]. The inhibitory effect of the ACEI benazepril on the growth of oesophageal carcinoma

xenografts has been shown. The potential mechanism of benazepril action may be the suppression of tumour vessel formation via inversely affecting VEGF expression [99]. Likewise, the blockade of the RAS by ACEIs or ARBs markedly attenuated HCC (hepatocellular carcinoma) along with suppression of angiogenesis [100]. Similar effects regarding the RAS and tumour angiogenesis have been described in breast cancer and other malignant diseases [79,98,101,102]. Inflammation, together with neo-angiogenesis, represents the point of cross-talk between the local RAS and carcinogenesis. AngII increases vascular permeability via the release of prostaglandins and VEGF or the re-arrangement of cytoskeletal proteins [103]. Thus AngII is involved in the key initiative events of the cancer-associated inflammatory process, cell growth and matrix synthesis. Furthermore, AngII could contribute to the recruitment of inflammatory cells into the cancer tissue through the regulation of adhesion molecules and chemokines by resident cells. RAS activation also plays a role in the immunologically induced inflammation and transcriptional regulation, predominantly via NF-κB and AP-1 (activator protein-1) activation [80,103].

The influence of ACE gene polymorphism on gastric cancer progression has been investigated [104]. ACE is expressed locally in gastric cancer and the gene polymorphism is associated with metastatic behaviour. ACE genotypes correlate with the number of lymph node metastases and the UICC (Unio Internationale Contra Cancrum) tumour stage. In that study, gastric cancer patients with the I/I (insertion/insertion) genotype had a highly significant smaller number of lymph node metastases and a significantly lower UICC tumour stage ($P = 0.01$) than patients with the D/D (deletion/deletion) genotype [104]. Germline and somatic mutations leading to dysregulation of the local RAS may promote tumour progression in solid tumours such as breast cancer, lung cancer and cancer of the stomach [80,105–107]. AT_1Rs and AT_2Rs are expressed locally in gastric cancer and the combination of AT_1R expression and the ACE I/D gene polymorphism correlates with nodal spread in intestinal type gastric cancer [108]. The D/D genotype of ACE may contribute to a higher risk of developing squamous cell lung carcinoma [109]. AngII may trigger activating signals that contribute to cancer cell extravasation and metastasis [110].

Pre-treatment of cancer cells with AngII increases the number of mice with metastases, as well as the number and size of metastases per mouse. *In vitro*, AngII contributes to each sequential step of cancer metastasis by promoting cancer cell adhesion to endothelial cells, transendothelial migration and tumour cell migration across the extracellular matrix. At the molecular level, a total of 102 genes differentially expressed following AngII pre-treatment were identified by comparative DNA microarray [110]. AngII regulates two groups of connected genes related to its precursor angiotensinogen. Among those, up-regulated MMP (matrix metalloproteinase) 2/MMP9 and ICAM-1 (intercellular adhesion molecule-1) stand at the crossroads of a network of genes involved in cell adhesion, migration and invasion [110].

Immunological dysregulation can cause tumour development and progression. T- and NK (natural killer) cells are fully equipped with local RAS elements and are potentially capable of producing and delivering AngII to tissue sites [111]. NK and T-cells express renin, the renin receptor, angiotensinogen and ACE by mRNA analysis. The co-stimulatory effect of AngII and anti-CD3-stimulated T- and NK cell proliferation with AngII treatment were reported. AT_1R and AT_2R expression was found in monocytes, NK cells and T-cells. These receptors were functional in immunological events such as calcium signalling, chemotaxis and proliferation [111]. Dendritic cell differentiation is controlled by AngII [74]. Impaired functions of dendritic cells deficient in AT_1Rs have been shown [72]. AT_1Rs control the differentiation and functionality of dendritic cells and have a crucial impact on cellular immune processes where local angiotensinergic systems are activated.

Bernstein's group has described a unique mouse model called ACE 10/10, expressing high levels of ACE in macrophages, but lacking ACE in blood vessels and kidney [112]. They studied six groups of mice implanted with B16-F10 melanoma, an aggressive and commonly used tumour model: ACE 10/10 mice, wild-type mice, both ACE 10/10- and wild-type mice treated with the ACEI captopril, and both ACE 10/10 and wild-type mice treated with the ARB losartan. They observed that ACE 10/10 mice have a marked resistance to the growth of B16 melanoma. ACE 10/10 mice respond to melanoma challenge with an enhanced inflammatory response, including increased tumour-specific $CD8^+$ T-cells. ACE 10/10 mice treated with captopril had a more rapid growth of the melanoma compared with untreated ACE 10/10 mice. Likewise, the angiotensinogen/ACE 10/10-double-KO mice resisted B16-F10 tumours much more effectively than angiotensinogen/ACE wild-type mice ($P < 0.0005$). Direct transfer of macrophages or the engraftment of wild-type mice with BM from ACE 10/10 mice transferred B16 tumour resistance [112]. Thus their experiments suggested that it is the presence of ACE, and not its absence, that is important in the resistance of ACE 10/10 mice to melanoma growth. They concluded that the resistance to melanoma in ACE 10/10 mice is mediated by immunological mechanisms and not by aberrant AngII production [112]. The same group also reported that ACE is a major peptidase involved in MHC class I antigen processing [113]. On the basis of their outstanding experiments, it was shown that (i) ACE expression is increased with APC maturation, (ii) ACE affects surface MHC class I expression, (iii) ACE edits the MHC class I peptide repertoire, (iv) ACE edits self-antigens, (v) ACE affects the presentation of viral antigens, and (vi) ACE works as a carboxyl dipeptidase on proteasome products, and thus, ACE depletion or ACE overexpression changes immunogenicity [113]. AngII is the most important product of ACE and most of the effects of AngII are mediated by the AT_1R.

THE LOCAL BM RAS IN NEOPLASTIC HAEMATOPOIESIS

Renin

The relevance of renin expression as an aberrant leukaemic marker in acute leukaemia has been proposed by real-time PCR analyses. Renin-like enzyme activity converting angiotensinogen into AngI has been detected in leukaemic blast cells [44,114,115].

A specific immunoreactive renin-like peptide of 47 kDa was isolated from AML (acute myeloid leukaemia) blast cells. Renin is expressed in some myeloid human leukaemia cell lines, such as K562, KU812 and MEG-01 [41]. Multipotential haematopoietic malignant K562 leukaemic blast cells also exhibit significant expression of angiotensinogen and ACE [45]. When K562 cells were treated with inducers of growth inhibition and/or differentiation, renin expression did not disappear, indicating that its expression is associated with a blastic phenotype, rather than with cell proliferation [41]. Casares et al. [41] demonstrated that some myeloid blast cells express renin, but normal BM does not display this expression. They observed renin expression in cells from AML, CML (chronic myeloid leukaemia) and ALL (acute lymphoid leukaemia). The highest frequency was observed in AML patients (47.2% of the cases). Renin expression disappeared during the complete remission of AML [41]. Inigo and co-workers [42] analysed 76 samples from patients with AML, with follow-up of positive patients. A total of 31 patients (41%) were positive for renin gene expression at diagnosis. All renin-positive patients at diagnosis showed no expression during complete remission, but expression recurred in those experiencing relapse and persisted when the disease was refractory to treatment [42].

The chimaeric protein NUP98 (Nucleoporin 98)–HOXA9 (NUP98-homeobox A9) is a prototype of several NUP98 fusions that occur in AML and myelodysplastic syndromes. NUP98–HOXA9 affects the differentiation, proliferation and gene expression of primary human $CD34^+$ haematopoietic cells. NUP98–HOXA9 can increase the numbers of erythroid precursors and impairs both myeloid and erythroid differentiation. Increased renin gene activity was detected during NUP98–HOXA9 enhanced blast formation [116,117].

ACE

The presence of ACE in human haematopoietic stem cells throughout haematopoietic ontogeny indicates that primitive haematopoiesis [21–24] represents the role of the ACE molecule in neoplastic haematological disorders involving neoplastic haematopoiesis. The existence of ACE in human primitive lymphohaematopoietic cells, and embryonic, fetal and adult haematopoietic tissues [21–24] draws attention to the effects of RAS on neoplastic tissues. Immunohistochemical studies have shown the possible role of ACE/RAS in BM by evaluating ACE expression in normal BM, several myeloproliferative disorders and myelodysplasia [51]. ACE and p53 expression were detected in $CD34^+$ cells in patients with acute leukaemia during and after the induction of chemotherapy [43]. ACE-expressing macrophages in the lymph nodes of patients Hodgkin's disease have been detected [118]. ACE activity was also linked to multiple myeloma [119,120]. ACE hyperfunction results in faster hydrolysis of the AcSDKP peptide, which in turn decreases in BM tissues, allowing HSCs to enter the S-stage of the cell cycle [4,16,34]. The plasma concentration of AcSDKP, a reversible negative regulator of the proliferation of normal haematopoietic stem cells, is physiologically regulated by ACE [14–16,121]. *In vitro* incubation of AML cells with an ACEI decreased the growth and colony-forming ability of AML cells in a dose-dependent manner. Addition of the AngII peptide to AML cells partially rescued their colony-forming ability [121]. Overexpression of ACE-surface antigen in leukaemic myeloid blast cells has been detected by flow cytometric analyses. Moreover, a positive correlation has been found between ACE and BM blast count [35]. We investigated ACE I/D gene polymorphisms in patients with haematological malignancies, including acute and chronic leukaemia, myelodysplastic syndrome and multiple myeloma. Our results showed that 80.4% of the patients had a ID/II genotype compared with 55.9% in the control group, with a 3.2-fold increased disease risk in the presence of the insertion allele (ID/II) [122].

Angiotensinogen

We found that human UCB cells express renin, angiotensinogen, and ACE mRNAs [123]. Angiotensinogen, ACE and renin mRNA expression are increased in PRV [40]. Leucocytes also express the angiotensinogen gene, synthesizing and releasing angiotensinogen, with the capability of generating angiotensin [54]. CML patients had increased ACE, angiotensinogen and renin mRNA levels and these expression levels decreased following the administration of imatinib. The expression of RAS components were studied in patients with CML at the time of diagnosis and at 3, 6 and 12 months after diagnosis by quantitative real-time PCR. *De novo* CML patients had increased ACE, angiotensinogen and renin mRNA levels, and these expression levels decreased following administration of imatinib. The RAS activities were significantly different among Sokal risk groups of CML, highlighting the altered biological activity of RAS in neoplastic disorders. Therefore the haematopoietic RAS affects neoplastic cell production, which may be altered via administration of tyrosine kinase inhibitors such as imatinib [124].

Angiotensin peptides

$AT_{1a}Rs$ are present on human $CD34^+CD38^-$ cells, $CD34^+CD38^+$ cells, lymphocytes and stromal cells. AngII, the main effector peptide of the RAS, increased haematopoietic progenitor cell proliferation by acting on $AT_{1a}Rs$ present on $CD34^+$ HSCs [27]. AngII utilizes the JAK/STAT pathway [18]. Ang-(1–7) contributes to haematopoietic cell proliferation as well [48,49,55,56]. AngII, through interaction with AT_1Rs, enhances erythroid differentiation in the BM [61]. AngII was found to be acting as an autocrine growth factor for AML [46]. The RAS member AGTRAP is important in haematopoietic cell proliferation and survival and has a synergistic effect with the proliferation-promoting function of thrombopoietin receptor Mpl [9]. BM AT_1R expression levels of myeloma patients had a positive correlation with their BM infiltration pattern and tumour load, indicated by serum β_2-microglobulin levels [120]. $AT_{1a}Rs$ in BM cells participates in the pathogenesis of atherosclerosis, as shown by analysing several BM chimaeric mice whose BM cells were positive or negative for $AT_{1a}Rs$. $AT_{1a}Rs$ expressed on BM-derived cells plays a role in the pathogenesis of atherosclerosis by accelerating infiltration of BM-derived inflammatory cells in the vessel wall. Blockade of AT_1Rs not only in vascular cells, but also in BM, can be an important strategy to prevent progression and destabilization of atherosclerotic plaques. Therefore the effects of local RAS on the pathobiology of atherosclerosis give

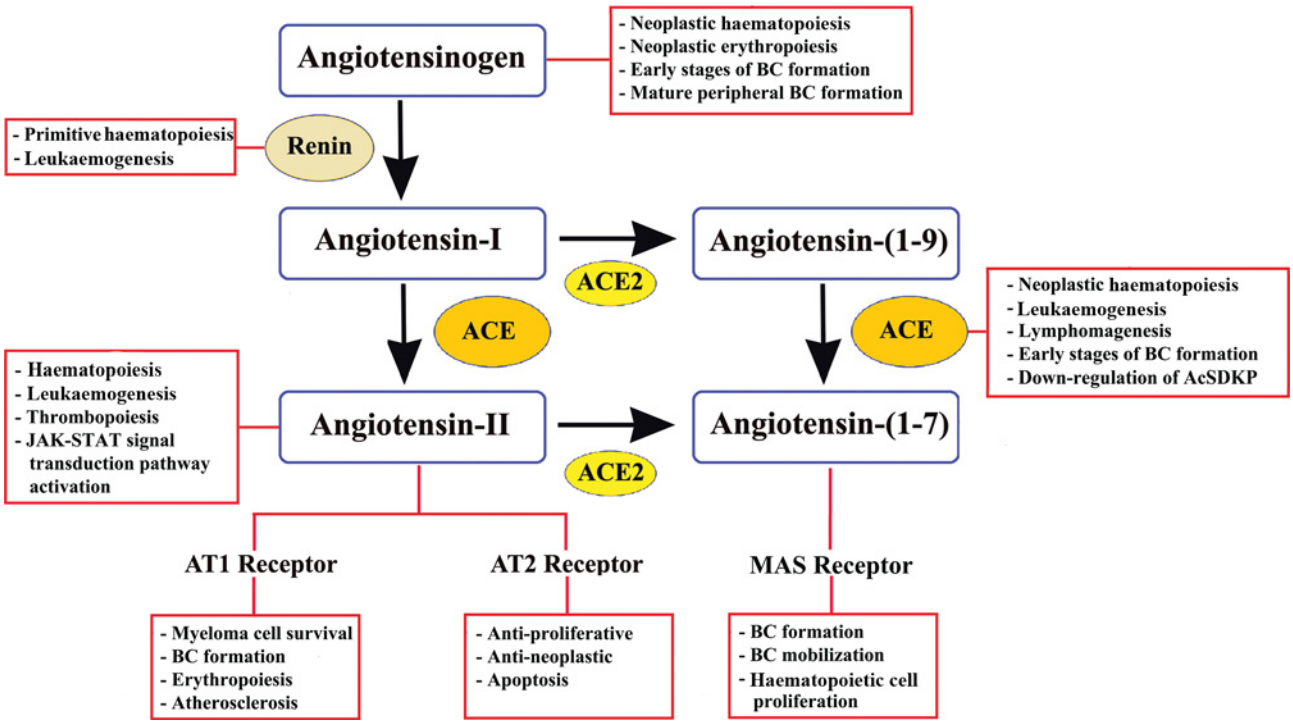

Figure 3 Potential role(s) of the essential peptides of the RAS in primitive and neoplastic haematopoiesis

very important clues to better understand the effects of the local haematopoietic RAS on the development of neoplastic disorders [52,125–133]. The comparable proliferative functions of the local RAS throughout the human body (including the myocardium, pancreas, pituitary gland, ovary and kidney) in health and disease represent the true basis for the design of future experimental studies to test this hypothesis [4]. Figure 3 gives an overview of the contribution of the RAS components to primitive and neoplastic haematopoiesis.

FUTURE PERSPECTIVES

The local tissue RAS, including the local haematopoietic BM RAS [4], is active from embryogenesis [22] to aging [134]. Therefore targeting the actions of the local RAS may represent a valuable therapeutic option for the management of neoplastic disorders [135]. Current medical practice includes RAS-modulating drugs for the management of cardiovascular disorders. The effects of RAS blockers on circulating RAS are well known, but their effects on the genesis and progression of cancer are conflicting [50,136–139]. Future therapeutic designs for the alteration of the local BM RAS should focus on locally active molecules. For instance, the tetrapeptide AcSDKP acts as an inhibitor of primitive haematopoietic cell proliferation and induces angiogenesis in BM stroma. Therefore goralatide can be suggested as a possible therapeutic application in a variety of states of haematopoiesis [140]. AT_1R antagonists impair the *in vivo* AngII-related angiogenesis of BM mesenchymal stem cells by inactivation of the $AT_{1a}R$/PI3K/Akt-related pathway [141]. The pharmacologically developed drug Ang-(1–7) is already in Phase I/II clinical trials for the modulation of the BM RAS in distinct disease states [33,48,49]. However, since most of the cellular effects of the local RAS are via autocrine, paracrine and intracrine pathways, future drugs intended to modulate local RAS functions should be prepared to have local targeted actions in the tissue micro-environment, such as inside the BM. For instance, Kawabata et al. [142] recently formulated a nanoparticle vector (dTAT NP) to leverage the efficiency of this cell-penetrating strategy for tumour-targeted gene delivery in the setting of intratracheal administration. They found that bolus administration of dTAT NP-encapsulated AT_2Rs or TRAIL (TNF-related apoptosis-inducing ligand) cDNA markedly attenuated tumour growth. Thus they suggested a novel gene delivery system that offers an effective intratracheal strategy for administering lung cancer gene therapy [142].

Future experimental and clinical studies are needed to elucidate the puzzling functions of the local tissue RAS, including the local BM RAS. These efforts should focus on dissecting local RAS interactions with the complicated pathobiological characteristics of neoplastic disorders and on manipulating autocrine/paracrine/intracrine systems for the better clinical management of patients with cancer.

REFERENCES

1 Haznedaroglu, I. C., Tuncer, S. and Gursoy, M. (1996) A local renin-angiotensin system in the bone marrow. Med. Hypotheses **46**, 507–510

2 Haznedaroglu, I. C. (2004) A local renin-angiotensin system in the bone marrow still awaits its Christopher Columbus. Exp. Hematol. **26**, 279
3 Strawn, W. B., Richmond, R. S., Tallant, E. A., Gallagher, P. E. and Ferrario, C. M. (2004) Renin-angiotensin system expression in rat bone marrow haematopoietic and stromal cells. Br. J. Haematol. **126**, 120–126
4 Haznedaroglu, I. C. and Beyazit, Y. (2010) Pathobiological aspects of the local bone marrow renin-angiotensin system: a review. JRAAS **11**, 205–213
5 Haznedaroglu, I. C. and Ozturk, M. A. (2003) Towards the understanding of the local hematopoietic bone marrow renin-angiotensin system. Int. J. Biochem. Cell. Biol. **35**, 867–880
6 Hubert, C., Savary, K., Gasc, J. M. and Corvol, P. (2006) The hematopoietic system: a new niche for the renin-angiotensin system. Nat. Clin. Cardiovasc. Med. **3**, 80–85
7 Lin, C., Datta, V., Okwan-Duodu, D., Chen, X., Fuchs, S., Alsabeh, R., Billet, S., Bernstein, K. E. and Shen, X. Z. (2010) Angiotensin-converting enzyme is required for normal myelopoiesis. FASEB J. **25**, 1145–1155
8 Kato, H., Ishida, J., Imagawa, S., Saito, T., Suzuki, N., Matsuoka, T., Sugaya, T., Tanimoto, K., Yokoo, T., Ohneda, O. et al. (2005) Enhanced erythropoiesis mediated by activation of the renin-angiotensin system via angiotensin II type 1a receptor. FASEB J. **19**, 2023–2025
9 Kwiatkowski, B. A. and Richard, R. E. (2009) Angiotensin II receptor-associated protein (AGTRAP) synergizes with Mpl signaling to promote survival and to increase proliferation rate of hematopoietic cells. ASH Annual Meeting Abstracts **114**, 3606
10 Hara, M., Ono, K., Wada, H., Sasayama, S. and Matsumori, A. (2004) Preformed angiotensin II is present in human mast cells. Cardiovasc. Drugs Ther. **18**, 415–420
11 Park, T. S. and Zambidis, E. T. (2009) A role for the renin-angiotensin system in hematopoiesis. Haematologica **94**, 745–747
12 Shen, X. Z. and Bernstein, K. E. (2011) The peptide network regulated by angiotensin converting enzyme (ACE) in hematopoiesis. Cell Cycle **10**, 1363–1369
13 Huang, Y. L., Kuang, J., Hu, Y. Z., Song, Y. B., Qiu, R. F. and Mai, W. Y. (2012) Bone marrow stromal cell transplantation combined with angiotensin-converting enzyme inhibitor treatment in rat with acute myocardial infarction and the role of insulin-like growth factor-1. Cytotherapy **14**, 563–569
14 Comte, L., Lorgeot, V., Bignon, J., Volkov, L., Dupuis, F., Wdzieczak-Bakala, J. and Praloran, V. (1998) *In vivo* modifications of AcSDKP metabolism and haematopoiesis in mice treated with 5-fluorouracil and Goralatide. Eur. J. Clin. Invest. **28**, 856–863
15 Comte, L., Lorgeot, V., Volkov, L., Allegraud, A., Aldigier, J. C. and Praloran, V. (1997) Effects of the angiotensin-converting enzyme inhibitor enalapril on blood haematopoietic progenitors and acetyl-*N*-Ser-Asp-Lys-Pro concentrations. Eur. J. Clin. Invest. **27**, 788–790
16 Li, J., Volkov, L., Comte, L., Herve, P., Praloran, V. and Charbord, P. (1997) Production and consumption of the tetrapeptide AcSDKP, a negative regulator of hematopoietic stem cells, by hematopoietic microenvironmental cells. Exp. Hematol. **25**, 140–146
17 Oliveira, C. R., Paredes-Gamero, E.J., Barbosa, C. M., Nascimento, F. D., Batista, E. C., Reis, F. C., Martins, A. H., Ferreira, A. T., Carmona, A. K., Pesquero, J. B. et al. (2010) Myelopoiesis modulation by ACE hyperfunction in kinin B_1 receptor knockout mice: relationship with AcSDKP levels. Chem.–Biol. Interact. **184**, 388–395
18 Haznedaroglu, I. C., Arici, M. and Buyukasik, Y. (2000) A unifying hypothesis for the renin-angiotensin system and hematopoiesis: sticking the pieces together with the JAK-STAT pathway. Med. Hypotheses **54**, 80–83
19 Ozturk, M. A., Guven, G. S. and Haznedaroglu, I. C. (2004) How hematopoietic stem cells know and act in cardiac microenvironment for stem cell plasticity? Impact of local renin-angiotensin systems. Med. Hypotheses **63**, 866–874
20 Vrsalovic, M. M., Pejsa, V., Veic, T. S., Kolonic, S. O., Ajdukovic, R., Haris, V., Jaksic, O. and Kusec, R. (2007) Bone marrow renin-angiotensin system expression in polycythemia vera and essential thrombocythemia depends on JAK2 mutational status. Cancer Biol. Ther. **6**, 1434–1436
21 Jokubaitis, V. J., Sinka, L., Driessen, R., Whitty, G., Haylock, D. N., Bertoncello, I., Smith, I., Péault, B., Tavian, M. and Simmons, P. J. (2008) Angiotensin-converting enzyme (CD143) marks hematopoietic stem cells in human embryonic, fetal, and adult hematopoietic tissues. Blood **111**, 4055–4063
22 Sinka, L., Biasch, K., Khazaal, I., Péault, B. and Tavian, M. (2012) Angiotensin-converting enzyme (CD143) specifies emerging lympho-hematopoietic progenitors in the human embryo. Blood **119**, 3712–3723
23 Tavian, M., Biasch, K., Sinka, L., Vallet, J. and Péault, B. (2010) Embryonic origin of human hematopoiesis. Int. J. Dev. Biol. **54**, 1061–1065
24 Zambidis, E. T., Soon Park, T., Yu, W., Tam, A., Levine, M., Yuan, X., Pryzhkova, M. and Péault, B. (2008) Expression of angiotensin-converting enzyme (CD143) identifies and regulates primitive hemangioblasts derived from human pluripotent stem cells. Blood **112**, 3601–3614
25 Paul, M., Poyan Mehr, A. and Kreutz, R. (2006) Physiology of local renin-angiotensin systems. Physiol. Rev. **86**, 747–803
26 Leung, P. S. (2004) The peptide hormone angiotensin II: Its new functions in tissues and organs. Curr. Protein Pept. Sci. **5**, 267–273
27 Rodgers, K. E., Xiong, S., Steer, R. and diZerega, G. S. (2000) Effect of angiotensin II on hematopoietic progenitor cell proliferation. Stem Cells **18**, 287–294
28 Mehta, P. K. and Griendling, K. K. (2007) Angiotensin II cell signaling: physiological and pathological effects in the cardiovascular system. Am. J. Physiol. Cell. Physiol. **292**, C82–C97
29 Ellefson, D. D., diZerega, G. S., Espinoza, T., Roda, N., Maldonado, S. and Rodgers, K. E. (2004) Synergistic effects of co-administration of angiotensin 1–7 and Neupogen on hematopoietic recovery in mice. Cancer Chemother. Pharmacol. **53**, 15–24
30 Shi, R. Z., Wang, J. C., Huang, S. H., Wang, X. J. and Li, Q. P. (2009) Angiotensin II induces vascular endothelial growth factor synthesis in mesenchymal stem cells. Exp. Cell Res. **315**, 10–15
31 Li, Y., Zhang, C., Wu, Y., Han, Y., Cui, W., Jia, L., Cai, L., Cheng, J., Li, H. and Du, J. (2012) Interleukin-12p35 deletion promotes CD4 T-cell-dependent macrophage differentiation and enhances angiotensin ii-induced cardiac fibrosis. Arterioscler. Thromb. Vasc. Biol. **32**, 1662–1674
32 Durik, M., Seva Pessoa, B. and Roks, A. J. (2012) The renin-angiotensin system, bone marrow and progenitor cells. Clin. Sci. **123**, 205–223
33 Heringer-Walther, S., Eckert, K., Schumacher, S. M., Uharek, L., Wulf-Goldenberg, A., Gembardt, F., Fichtner, I., Schultheiss, H. P., Rodgers, K. and Walther, T. (2009) Angiotensin-(1–7) stimulates hematopoietic progenitor cells *in vitro* and *in vivo*. Haematologica **94**, 857–860
34 Abali, H., Haznedaroglu, I. C., Goker, H., Celik, I., Ozatli, D., Koray, Z. and Caglar, M. (2002) Circulating and local bone marrow renin-angiotensin system in leukemic hematopoiesis: preliminary evidences. Hematology **7**, 75–82
35 Aksu, S., Beyazit, Y., Haznedaroglu, I. C., Canpinar, H., Kekilli, M., Uner, A., Sayinalp, N., Büyükaşik, Y., Goker, H. and Ozcebe, O. I. (2006) Over-expression of angiotensin-converting enzyme (CD 143) on leukemic blasts as a clue for the activated local bone marrow RAS in AML. Leuk. Lymphoma **47**, 891–896

36 Beyazit, Y., Aksu, S., Haznedaroglu, I. C., Kekilli, M., Misirlioglu, M., Tuncer, S., Karakaya, J., Koca, E., Buyukasik, Y., Sayinalp, N. and Goker, H. (2007) Overexpression of the local bone marrow renin-angiotensin system in acute myeloid leukemia. J. Natl. Med. Assoc. **99**, 57–63

37 Tufan, A., Unal, N., Koca, E., Onal, I., Aksu, S. and Haznedaroglu, I. (2006) Spontaneous tumor lysis syndrome in a patient with diffuse large B cell lymphoma and Richter syndrome. Ann. Hematol. **85**, 183–184

38 Sayinalp, N., Cinar, H., Uner, A., Haznedaroğlu, I. C., Büyükaşik, Y., Göker, H., Aksu, S., Ozcebe, O. I., Karakuş, S., Kirazli, S. and Dündar, S. V. (2004) Plasma basic fibroblast growth factor and bone marrow fibrosis in clonal myeloproliferative disorders. Clin. Lab. Haematol. **26**, 265–268

39 Dizdar, O., Yurekli, B. S., Purnak, T., Aksu, S. and Haznedaroglu, I. C. (2004) Tumor lysis syndrome associated with fludarabine treatment in chronic lymphocytic leukemia. Ann. Pharmacother. **38**, 1319–1320

40 Aksu, S., Beyazit, Y., Haznedaroglu, I. C., Kekilli, M., Canpinar, H., Misirlioğlu, M., Uner, A., Tuncer, S., Sayinalp, N., Büyükaşik, Y., Goker, H. and Ozcebe, I. H. (2005) Enhanced expression of the local haematopoietic bone marrow renin-angiotensin system in polycythemia Rubra vera. J. Int. Med. Res. **33**, 661–667

41 Teresa Gomez Casares, M., de la Iglesia, S., Perera, M., Lemes, A., Campo, C., Gonzalez San Miguel, J. D., Bosch, J. M., Suarez, A., Guerra, L., Rodriguez-Peréz, J. C. and Molero, T. (2002) Renin expression in hematological malignancies and its role in the regulation of hematopoiesis. Leuk. Lymphoma **43**, 2377–2381

42 Iñigo Sde, L., Casares, M. T., Jorge, C. E., Leiza, S. M., Santana, G. S., Bravo de Laguna, S. J., San Miguel, J. D., Caballero, A., Alvarez Mdel, M., Castellano, A. L. et al. (2006) Relevance of renin expression by real-time PCR in acute myeloid leukemia. Leuk. Lymphoma **47**, 409–416

43 Khodunova, E. E., Parovichnikova, E. N., Gal'tseval, V., Kulikov, S. M., Isaev, V. G. and Savchenko, V. G. (2011) Dynamic study of Bcl-2, Bax, p53, and ACE expression in $CD34^+$ cells of peripheral blood and bone marrow in acute leukemia patients in the course of induction chemotherapy. Ter. Arkh. **83**, 32–37

44 Wulf, G. G., Jahns-Streubel, G., Nobiling, R., Strutz, F., Hemmerlein, B., Hiddemann, W. and Wörmann, B. (1998) Renin in acute myeloid leukaemia blasts. Br. J. Haematol. **100**, 335–337

45 Koca, E., Haznedaroglu, I. C., Acar, K., Beyazit, Y., Aksu, S., Misirlioglu, M., Tuncer, S., Sayinalp, N., Ozcebe, O. I. and Uner, A. (2007) Renin-angiotensin system expression in the K562 human erythroleukaemic cell line. JRAAS **8**, 145–147

46 Pinto, R. P., Wang, K. K., Khoury, H., Schimmer, A. D. and Minden, M. D. (2004) aberrant expression of angiotensin in acute myeloid leukemia. Blood **102**, 2124A

47 De la Iglesia Iñigo, S., López-Jorge, C. E., Gómez-Casares, M. T., Lemes Castellano, A., Martín Cabrera, P., López Brito, J., Suárez Cabrera, A. and Molero Labarta, T. (2009) Induction of apoptosis in leukemic cell lines treated with captopril, trandolapril and losartan: a new role in the treatment of leukaemia for these agents. Leuk. Res. **33**, 810–816

48 Rodgers, K., Xiong, S. and DiZerega, G. S. (2003) Effect of angiotensin II and angiotensin(1–7) on hematopoietic recovery after intravenous chemotherapy. Cancer Chemother. Pharmacol. **51**, 97–106

49 Rodgers, K. E., Oliver, J. and diZerega, G. S. (2006) Phase I/II dose escalation study of angiotensin 1–7 [A(1–7)] administered before and after chemotherapy in patients with newly diagnosed breast cancer. Cancer Chemother. Pharmacol. **57**, 559–568

50 Abali, H., Gullu, I. H., Engin, H., Haznedaroğlu, I. C., Erman, M. and Tekuzman, G. (2002) Old antihypertensives as novel antineoplastics: angiotensin-I-converting enzyme inhibitors and angiotensin II type 1 receptor antagonists. Med. Hypotheses **59**, 344–348

51 Marusic-Vrsalovic, M., Dominis, M., Jaksic, B. and Kusec, R. (2003) Angiotensin I-converting enzyme is expressed by erythropoietic cells of normal and myeloproliferative bone marrow. Br. J. Haematol. **123**, 539–541

52 Tsubakimoto, Y., Yamada, H., Yokoi, H., Kishida, S., Takata, H., Kawahito, H., Matsui, A., Urao, N., Nozawa, Y., Hirai, H. et al. (2009) Bone marrow angiotensin AT_1 receptor regulates differentiation of monocyte lineage progenitors from hematopoietic stem cells. Arterioscler. Thromb. Vasc. Biol. **29**, 1529–1536

53 Richmond, R. S., Tallant, E. A., Gallagher, P. E., Ferrario, C. M. and Strawn, W. B. (2004) Angiotensin II stimulates arachidonic acid release from bone marrow stromal cells. JRAAS **5**, 176–182

54 Gomez, R. A., Norling, L. L., Wilfong, N., Isakson, P., Lynch, K. R., Hock, R. and Quesenberry, P. (1993) Leukocytes synthesize angiotensinogen. Hypertension **21**, 470–475

55 Rodgers, K. E., Espinoza, T., Roda, N., Meeks, C. J., Hill, C., Louie, S. G. and Dizerega, G. S. (2012) Accelerated hematopoietic recovery with angiotensin-(1–7) after total body radiation. Int. J. Radiat. Biol. **88**, 466–476

56 Rodgers, K. E., Xiong, S. and diZerega, G. S. (2002) Accelerated recovery from irradiation injury by angiotensin peptides. Cancer Chemother. Pharmacol. **49**, 403–411

57 Cole, J., Ertoy, D. and Bernstein, K. E. (2000) Insights derived from ACE knockout mice. JRAAS **1**, 137–141

58 Cole, J., Ertoy, D., Lin, H., Sutliff, R. L., Ezan, E., Guyene, T. T., Capecchi, M., Corvol, P. and Bernstein, K. E. (2000) Lack of angiotensin II-facilitated erythropoiesis causes anemia in angiotensin-converting enzyme-deficient mice. J. Clin. Invest. **106**, 1391–1398

59 Kaneko, K., Nishiyama, H., Ohba, K., Shibasaki, A., Hirose, T., Totsune, K., Furuyama, K. and Takahashi, K. (2012), Expression of (pro)renin receptor in human erythroid cell lines and its increased protein accumulation by interferon-γ. Peptides **37**, 285–289

60 Mrug, M., Julian, B. A. and Prchal, J. T. (2004) Angiotensin II receptor type 1 expression in erythroid progenitors: Implications for the pathogenesis of postrenal transplant erythrocytosis. Semin. Nephrol. **24**, 120–130

61 Mrug, M., Stopka, T., Julian, B. A., Prchal, J. F. and Prchal, J. T. (1997) Angiotensin II stimulates proliferation of normal early erythroid progenitors. J. Clin. Invest. **100**, 2310–2314

62 Batenburg, W. W. and Danser, A. H. (2012) (Pro)renin and its receptors: pathophysiological implications. Clin. Sci. **123**, 121–133

63 De Mello, W. C. (2006) On the pathophysiological implications of an intracellular renin receptor. Circ. Res. **99**, 1285–1286

64 Spinello, I., Quaranta, M. T., Pasquini, L., Pelosi, E., Petrucci, E., Pagliuca, A., Castelli, G., Mariani, G., Diverio, D., Foà, R. et al. (2009) PLZF-mediated control on c-kit expression in $CD34^+$ cells and early erythropoiesis. Oncogene **28**, 2276–2288

65 Wang, N., Frank, G. D., Ding, R., Tan, Z., Rachakonda, A., Pandolfi, P. P., Senbonmatsu, T., Landon, E. J. and Inagami, T. (2012) Promyelocytic leukemia zinc finger protein activates GATA4 transcription and mediates cardiac hypertrophic signaling from angiotensin ii receptor 2. PLoS ONE **7**, e35632

66 Savary, K., Michaud, A., Favier, J., Larger, E., Corvol, P. and Gasc, J. M. (2005) Role of the renin-angiotensin system in primitive erythropoiesis in the chick embryo. Blood **105**, 103–110

67 Marathias, K. P., Agroyannis, B., Mavromoustakos, T., Matsoukas, J. and Vlahakos, D. V. (2004) Hematocrit-lowering effect following inactivation of renin-angiotensin system with angiotensin converting enzyme inhibitors and angiotensin receptor blockers. Curr. Top. Med. Chem. **4**, 483–486

68 Haznedaroglu, I. C., Goker, H., Turgut, M., Buyukasik, Y. and Benekli, M. (2002) Thrombopoietin as a drug: biologic expectations, clinical realities, and future directions. Clin. Appl. Thromb. Hemostasis **8**, 193–212

69 Ishikawa, M., Sekizuka, E., Yamaguchi, N., Nakadate, H., Terao, S., Granger, D. N. and Minamitani, H. (2007) Angiotensin II type 1 receptor signaling contributes to platelet-leukocyte-endothelial cell interactions in the cerebral microvasculature. Am. J. Physiol. Heart Circ. Physiol. **292**, H2306–H2315
70 Larsson, P. T., Schwieler, J. H. and Wallen, N. H. (2000) Platelet activation during angiotensin II infusion in healthy volunteers. Blood. Coagul. Fibrinolysis **11**, 61–69
71 Stegbauer, J., Lee, D. H., Seubert, S., Ellrichmann, G., Manzel, A., Kvakan, H., Muller, D. N., Gaupp, S., Rump, L. C., Gold, R. and Linker, R. A. (2009) Role of the renin-angiotensin system in autoimmune inflammation of the central nervous system. Proc. Natl. Acad. Sci. U.S.A. **106**, 14942–14947
72 Nahmod, K., Gentilini, C., Vermeulen, M., Uharek, L., Wang, Y., Zhang, J., Schultheiss, H. P., Geffner, J. and Walther, T. (2010) Impaired function of dendritic cells deficient in angiotensin II type 1 receptors. J. Pharmacol. Exp. Ther. **334**, 854–862
73 Nahmod, K. A., Geffner, J. R. and Walther, T. (2010) Angiotensin II type 1a-deficient bone marrow-derived dendritic cells produce higher levels of monocyte chemoattractant protein 1. Hypertension **56**, e6–7
74 Nahmod, K. A., Vermeulen, M. E., Raiden, S., Salamone, G., Gamberale, R., Fernández-Calotti, P., Alvarez, A., Nahmod, V., Giordano, M. and Geffner, J. R. (2003) Control of dendritic cell differentiation by angiotensin II. FASEB J. **17**, 491–493
75 Tavian, M. and Peault, B. (2005) The changing cellular environments of hematopoiesis in human development in utero. Exp. Hematol. **33**, 1062–1069
76 Hirschi, K. K. (2012) Hemogenic endothelium during development and beyond. Blood **119**, 4823–4827
77 Xing, Y., Lv, A. and Wang, L. (2011) The combination of angiotensin II and 5-azacytidine promotes cardiomyocyte differentiation of rat bone marrow mesenchymal stem cells. Mol. Cell. Biochem. **360**, 279–287
78 Sequeira Lopez, M. L. and Gomez, R. A. (2004) The role of angiotensin II in kidney embryogenesis and kidney abnormalities. Curr. Opin. Nephrol. Hypertens. **13**, 117–122
79 Ager, E. I., Neo, J. and Christophi, C. (2008) The renin-angiotensin system and malignancy. Carcinogenesis **29**, 1675–1684
80 George, A. J., Thomas, W. G. and Hannan, R. D. (2012) The renin-angiotensin system and cancer: old dog, new tricks. Nat. Rev. Cancer **10**, 745–759
81 Ni, L., Feng, Y., Wan, H., Ma, Q., Fan, L., Qian, Y., Li, Q., Xiang, Y. and Gao, B. (2012) Angiotensin-(1–7) inhibits the migration and invasion of A549 human lung adenocarcinoma cells through inactivation of the PI3K/Akt and MAPK signaling pathways. Oncol. Rep. **27**, 783–790
82 Okamoto, K., Tajima, H., Ohta, T., Nakanuma, S., Hayashi, H., Nakagawara, H., Onishi, I., Takamura, H., Ninomiya, I., Kitagawa, H. et al. (2012) Angiotensin II induces tumor progression and fibrosis in intrahepatic cholangiocarcinoma through an interaction with hepatic stellate cells. Int. J. Oncol. **37**, 1251–1259
83 Schmid, U., Stopper, H., Schweda, F., Queisser, N. and Schupp, N. (2008) Angiotensin II induces dna damage in the kidney. Cancer Res. **68**, 9239–9246
84 Fendrich, V., Chen, N. M., Neef, M., Waldmann, J., Buchholz, M., Feldmann, G., Slater, E. P., Maitra, A. and Bartsch, D. K. (2010) The angiotensin-I-converting enzyme inhibitor enalapril and aspirin delay progression of pancreatic intraepithelial neoplasia and cancer formation in a genetically engineered mouse model of pancreatic cancer. Gut **59**, 630–637
85 Diop-Frimpong, B., Chauhan, V. P., Krane, S., Boucher, Y. and Jain, R. K. (2011) Losartan inhibits collagen I synthesis and improves the distribution and efficacy of nanotherapeutics in tumors. Proc. Natl. Acad. Sci. U.S.A. **108**, 2909–2914
86 Velez Rueda, J. O., Palomeque, J. and Mattiazzi, A. (2012) Early apoptosis in different models of cardiac hypertrophy induced by high renin-angiotensin system activity involves CaMKII. J. Appl. Physiol. **112**, 2110–2120
87 Wang, X., Lu, J., Khaidakov, M., Mitra, S., Ding, Z., Goyal, T. and Mehta, J. L. (2012) Delineation of the effects of angiotensin type 1 and 2 receptors on HL-1 cardiomyocyte apoptosis. Apoptosis **17**, 908–915
88 Yang, C., Wang, Y., Liu, H., Li, N., Sun, Y., Liu, Z. and Yang, P. (2012) Ghrelin protects H9c2 cardiomyocytes from angiotensin II-induced apoptosis through the endoplasmic reticulum stress pathway. J. Cardiovasc. Pharmacol. **59**, 465–471
89 Lo, C. S., Liu, F., Shi, Y., Maachi, H., Chenier, I., Godin, N., Filep, J. G., Ingelfinger, J. R., Zhang, S. L. and Chan, J. S. (2012) Dual RAS blockade normalizes angiotensin-converting enzyme-2 expression and prevents hypertension and tubular apoptosis in Akita angiotensinogen-transgenic mice. Am. J. Physiol. Renal Physiol. **302**, F840–F852
90 Matsushita, K., Wu, Y. J., Okamoto, Y., Pratt, R. E. and Dzau, V. J. (2006) Local renin angiotensin expression regulates human mesenchymal stem cell differentiation to adipocytes. Hypertension **48**, 1095–1102
91 Ishizuka, T., Goshima, H., Ozawa, A. and Watanabe, Y. (2012) Effect of angiotensin II on proliferation and differentiation of mouse induced pluripotent stem cells into mesodermal progenitor cells. Biochem. Biophys. Res. Commun. **420**, 148–155
92 Peters, B., Podlich, D., Ritter, M., Müller, A., Wanka, H., Maser-Gluth, C., Seitz, C., de Boni, L., Maier, E., Gretz, N., Peters, J. and Hoffmann, S. C. (2012) A new transgenic rat model overexpressing the angiotensin II type 2 receptor provides evidence for inhibition of cell proliferation in the outer adrenal cortex. Am. J. Physiol. Endocrinol. Metab. **15**, E1044–E1054
93 Du, N., Feng, J., Hu, L. J., Sun, X., Sun, H. B., Zhao, Y., Yang, Y. P. and Ren, H. (2012) Angiotensin II receptor type 1 blockers suppress the cell proliferation effects of angiotensin II in breast cancer cells by inhibiting AT_1R signaling. Oncol. Rep. **27**, 1893–1903
94 Gwathmey, T. M., Alzayadneh, E. M., Pendergrass, K. D. and Chappell, M. C. (2011) Novel roles of nuclear angiotensin receptors and signaling mechanisms. Am. J. Physiol. Regul. Integr. Comp. Physiol. **302**, R518–R530
95 Vitko, J. R., Re, R. N., Alam, J. and Cook, J. L (2012) Cell-penetrating peptides corresponding to the angiotensin II Type 1 receptor reduce receptor accumulation and cell surface expression and signaling. Am. J. Hypertens. **25**, 24–28
96 Rodgers, K. E., Xiong, S., Steer, R. and diZerega, G. S. (2000) Effect of angiotensin II on hematopoietic progenitor cell proliferation. Stem Cells **18**, 287–294
97 McCollum, L. T., Gallagher, P. E. and Tallant, E. A. (2012) Angiotensin-(1–7) abrogates mitogen-stimulated proliferation of cardiac fibroblasts. Peptides **34**, 380–388
98 Carbajo-Lozoya, J., Lutz, S., Feng, Y., Kroll, J., Hammes, H. P. and Wieland, T. (2012) Angiotensin II modulates VEGF-driven angiogenesis by opposing effects of type 1 and type 2 receptor stimulation in the microvascular endothelium. Cell. Signalling **24**, 1261–1269
99 Wang, Q., Lei, X., Zhu, S. and Zhang, S. (2012) Angiotensin-I converting enzyme inhibitors suppress angiogenesis and growth of esophageal carcinoma xenografts. Dis. Esophagus, doi:10.1111/j.1442-2050.2011.01320.x
100 Kaji, K., Yoshiji, H., Ikenaka, Y., Noguchi, R., Aihara, Y., Shirai, Y., Douhara, A. and Fukui, H. (2012) Possible involvement of angiogenesis in chronic liver diseases: interaction among renin-angiotensin-aldosterone system, insulin resistance and oxidative stress. Curr. Med. Chem. **19**, 1889–1898

101 Jethon, A., Pula, B., Piotrowska, A., Wojnar, A., Rys, J., Dziegiel, P. and Podhorska-Okolow, M. (2012) Angiotensin II type 1 receptor (AT-1R) expression correlates with VEGF-A and VEGF-D expression in invasive ductal breast cancer. Pathol. Oncol. Res., **18**, 867–873

102 Vinson, G. P., Barker, S. and Puddefoot, J. R. (2011) The renin-angiotensin system in the breast and breast cancer. Endocr. Relat. Cancer **19**, R1–R19

103 Suzuki, Y., Ruiz-Ortega, M., Lorenzo, O., Ruperez, M., Esteban, V. and Egido, J. (2003) Inflammation and angiotensin II. Int. J. Biochem. Cell. Biol. **35**, 881–900

104 Röcken, C., Lendel, U., Dierkes, J., Westphal, S., Carl-McGrath, S., Peters, B., Krüger, S., Malfertheiner, P., Roessner, A. and Ebert, M. P. (2005) The number of lymph node metastases in gastric cancer correlates with the angiotensin I-converting enzyme gene insertion/deletion polymorphism. Clin. Cancer Res. **11**, 2526–2530

105 Yaren, A., Oztop, I., Turgut, S., Turgut, G., Degirmencioglu, S. and Demirpence, M. (2008) Angiotensin-converting enzyme gene polymorphism is associated with anemia in non-small-cell lung cancer. Exp. Biol. Med. **233**, 32–37

106 Yaren, A., Turgut, S., Kursunluoglu, R., Oztop, I., Turgut, G., Degirmencioglu, S., Kelten, C. and Erdem, E. (2007) Insertion/deletion polymorphism of the angiotensin I-converting enzyme gene in patients with breast cancer and effects on prognostic factors. J. Investig. Med. **55**, 255–261

107 Yigit, B., Bozkurt, N., Narter, F., Yilmaz, H., Yucebas, E. and Isbir, T. (2007) Effects of ACE I/D polymorphism on prostate cancer risk, tumor grade and metastatis. Anticancer Res. **27**, 933–936

108 Rocken, C., Rohl, F. W., Diebler, E., Lendeckel, U., Pross, M., Carl-McGrath, S. and Ebert, M. P. (2007) The angiotensin II/angiotensin II receptor system correlates with nodal spread in intestinal type gastric cancer. Cancer Epidemiol. Biomarkers Prev. **16**, 1206–1212

109 Dević Pavlić, S., Ristić, S., Flego, V., Kapović, M. and Radojčić Badovinac, A. (2012) Angiotensin-converting enzyme insertion/deletion gene polymorphism in lung cancer patients. Genet. Test. Mol. Biomarkers **16**, 722–725

110 Rodrigues-Ferreira, S., Abdelkarim, M., Dillenburg-Pilla, P., Luissint, A. C., di-Tommaso, A., Deshayes, F., Pontes, C. L., Molina, A., Cagnard, N., Letourneur, F. et al. (2012) Angiotensin II facilitates breast cancer cell migration and metastasis. PLoS ONE **7**, e35667

111 Jurewicz, M., McDermott, D. H., Sechler, J. M., Tinckam, K., Takakura, A., Carpenter, C. B., Milford, E. and Abdi, R. (2007) Human T and natural killer cells possess a functional renin-angiotensin system: further mechanisms of angiotensin II-induced inflammation. J. Am. Soc. Nephrol. **18**, 1093–1102

112 Shen, X. Z., Li, P., Weiss, D., Fuchs, S., Xiao, H. D., Adams, J. A., Williams, I. R., Capecchi, M. R., Taylor, W. R. and Bernstein, K. E. (2007) Mice with enhanced macrophage angiotensin-converting enzyme are resistant to melanoma. Am. J. Pathol. **170**, 2122–2134

113 Shen, X. Z., Billet, S., Lin, C., Okwan-Duodu, D., Chen, X., Lukacher, A. E. and Bernstein, K. E. (2012) The carboxypeptidase angiotensin converting enzyme (ACE) shapes the MHC class I peptide repertoire. Nat. Immunol. **12**, 1078–1085

114 Wulf, G. G., Jahns-Streubel, G., Strutz, F., Basenau, D., Hüfner, M., Buske, C., Wörmann, B. and Hiddemann, W. (1996) Paraneoplastic hypokalemia in acute myeloid leukemia: A case of renin activity in AML blast cells. Ann. Hematol. **73**, 139–141

115 Haznedaroglu, I. C., Savas, M. C. and Benekli, M. (1997) Renin-like activity in leukemic blast cells: an initial clue to a local renin-angiotensin system in the bone marrow. Ann. Hematol. **75**, 69–70

116 Takeda, A., Goolsby, C. and Yaseen, N. R. (2006) NUP98-HOXA9 induces long-term proliferation and blocks differentiation of primary human CD34$^+$ hematopoietic cells. Cancer Res. **66**, 6628–6637

117 Yassin, E. R., Abdul-Nabi, A. M., Takeda, A. and Yaseen, N. R. (2010) Effects of the NUP98-DDX10 oncogene on primary human CD34$^+$ cells: role of a conserved helicase motif. Leukemia **24**, 1001–1011

118 Koca, E., Haznedaroglu, I. C., Uner, A., Sayinalp, N., Saglam, A. E., Goker, H. and Ozcebe, O. I. (2007) Angiotensin-converting enzyme expression of the lymphoma-associated macrophages in the lymph nodes of Hodgkin's disease. J. Natl. Med. Assoc. **99**, 1243–1244, 1246–1247

119 Albayrak, M., Celebi, H., Albayrak, A., Sayilir, A., Yesil, Y., Balcik, O. S., Yokus, O. and Celik, T. (2012) Elevated serum angiotensin converting enzyme levels as a reflection of bone marrow renin-angiotensin system activation in multiple myeloma. JRAAS **13**, 259–64

120 Saka, B., Dogan, O., Tascioglu, C., Besisik, S. K., Karan, M. A., Sayitoglu, M., Erten, N. and Genc, S. (2009) Bone marrow renin-angiotensin system in multiple myeloma. Clin. Lymphoma Myeloma Leuk. **9** (Suppl. 1), S10

121 Brunet de la Grange, P., Ivanovic, Z., Leprivey-Lorgeot, V. and Praloran, V. (2002) Angiotensin II that reduces the colony-forming ability of hematopoietic progenitors in serum free medium has an inverse effect in serum-supplemented medium. Stem Cells **20**, 269–271

122 Akalin, I., Koca, E., Karabulut, H. G., Haznedaroglu, I. C., Cetiner, D., Hayran, M., Onal, I. K., Ozcebe, O. I. and Tukun, A. (2011) Angiotensin-converting enzyme (ACE) insertion/deletion (I/D) gene polymorphisms in leukemic hematopoiesis. Int. J. Hematol. Oncol. **21**, 1–9

123 Goker, H., Haznedaroglu, I. C., Beyazit, Y., Aksu, S., Tuncer, S., Misirlioglu, M., Bayramoglu, F., Kekilli, M., Büyükasik, Y., Sayinalp, N. et al. (2005) Local umbilical cord blood renin-angiotensin system. Ann. Hematol. **84**, 277–81

124 Sayitoglu, M., Haznedaroğlu, I. C., Hatirnaz, O., Erbilgin, Y., Aksu, S., Koca, E., Adiguzel, C., Bayik, M., Akalin, I., Gülbas, Z. et al. (2009) Effects of imatinib mesylate on renin-angiotensin system (RAS) activity during the clinical course of chronic myeloid leukaemia. J. Int. Med. Res. **37**, 1018–1028

125 Beyazit, Y., Purnak, T., Guven, G. S. and Haznedaroglu, I. C. (2010) Local bone marrow renin-angiotensin system and atherosclerosis. Cardiol. Res. Pract. **2011**, 714515

126 Ferrario, C. M., Richmond, R. S., Smith, R., Levy, P., Strawn, W. B. and Kivlighn, S. (2004) Renin-angiotensin system as a therapeutic target in managing atherosclerosis. Am. J. Ther. **11**, 44–53

127 Fukuda, D. and Sata, M. (2008) Role of bone marrow renin-angiotensin system in the pathogenesis of atherosclerosis. Pharmacol. Ther. **118**, 268–276

128 Fukuda, D., Sata, M., Ishizaka, N. and Nagai, R. (2008) Critical role of bone marrow angiotensin II type 1 receptor in the pathogenesis of atherosclerosis in apolipoprotein E-deficient mice. Arterioscler. Thromb. Vasc. Biol. **28**, 90–96

129 Kato, H., Ishida, J., Nagano, K., Honjo, K., Sugaya, T., Takeda, N., Sugiyama, Y., Yagami, K., Fujita, T., Nangaku, M. and Fukamizu, A. (2008) Deterioration of atherosclerosis in mice lacking angiotensin II type 1A receptor in bone marrow-derived cells. Lab. Invest. **88**, 731–739

130 Sata, M. and Fukuda, D. (2010) Crucial role of renin-angiotensin system in the pathogenesis of atherosclerosis. J. Med. Invest. **57**, 12–25

131 Matsubara, H. (2007) Clinical pathology and treatment of renin-angiotensin system – 3. Atherosclerosis and the renin-angiotensin system. Intern. Med. **46**, 1299–1301

132 Strawn, W., Richmond, R. and Ferrario, C. (2003) A new understanding of atherosclerosis: the bone marrow response-to-lipid hypothesis. In: Heart Disease: Pathogenesis, Diagnosis and Treatment, pp. 183–188, Third World Congress on Heart Disease, Washington, DC

133 Strawn, W. B. and Ferrario, C. M. (2008) Angiotensin II AT(1) receptor blockade normalizes CD11b$^+$ monocyte production in bone marrow of hypercholesterolemic monkeys. Atherosclerosis **196**, 624–632

134 Stegbauer, J. and Coffman, T. M. (2011) New insights into angiotensin receptor actions: from blood pressure to aging. Curr. Opin Nephrol. Hypertens. **20**, 84–88

135 Purclutepe, O., Iskender, G., Kiper, H. D., Tezcanli, B., Selvi, N., Avci, C. B., Kosova, B., Gokbulut, A. A., Sahin, F., Baran, Y. and Saydam, G. (2012) Enalapril-induced apoptosis of acute promyelocytic leukaemia cells involves STAT5A. Anticancer Res. **32**, 2885–2893

136 Bhaskaran, K., Douglas, I., Evans, S., van Staa, T. and Smeeth, L. (2012) Angiotensin receptor blockers and risk of cancer: cohort study among people receiving antihypertensive drugs in UK General Practice Research Database. BMJ **344**, e2697

137 Gao, M., Wang, Y., Shi, Y., Liu, D., Liang, Y., Yu, Y., Zhaobin, J., Zhu, L. and Jin, S. (2012) The relationship between three well-characterized polymorphisms of the angiotensin converting enzyme gene and lung cancer risk: a case-control study and a meta-analysis. JRAAS, doi: 10.1177/1470320312443912

138 Hallas, J., Depont, C., Christensen, R., Andersen, M., Friis, S. and Bjerrum, L. (2012) Long-term use of drugs affecting the renin-angiotensin system and the risk of cancer. A population-based case-control study. Br. J. Clin. Pharmacol. **74**, 180–188

139 McMenamin, U. C., Murray, L. J., Cantwell, M. M. and Hughes, C. M. (2011) Angiotensin-converting enzyme inhibitors and angiotensin receptor blockers in cancer progression and survival: a systematic review. Cancer Causes Control **23**, 221–230

140 Liu, J. M., Lawrence, F., Kovacevic, M., Bignon, J., Papadimitriou, E., Lallemand, J. Y., Katsoris, P., Potier, P., Fromes, Y. and Wdzieczak-Bakala, J. (2003) The tetrapeptide AcSDKP, an inhibitor of primitive hematopoietic cell proliferation, induces angiogenesis *in vitro* and *in vivo*. Blood **101**, 3014–3020

141 Cheng, C. I., Hsiao, C. C., Wu, S. C., Peng, S. Y., Yip, H. K., Fu, M. and Wang, W. S. (2012) Valsartan impairs angiogenesis of mesenchymal stem cells through Akt pathway. Int. J. Cardiol., doi:10.1016/j.ijcard.2012.06.128

142 Kawabata, A., Baoum, A., Ohta, N., Jacquez, S., Seo, G. M., Berkland, C. and Tamura, M. (2012) Intratracheal administration of a nanoparticle-based therapy with the angiotensin II type 2 receptor gene attenuates lung cancer growth. Cancer Res. **72**, 2057–2067

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