

The renin–angiotensin system, bone marrow and progenitor cells

Matej DURIK, Bruno SEVÁ PESSÔA and Anton J. M. ROKS

Division of Vascular Medicine and Pharmacology, Department of Internal Medicine, Erasmus Medical Center, Dr. Molewaterplein 50, 3015 GE Rotterdam, The Netherlands

A B S T R A C T

Modulation of the RAS (renin–angiotensin system), in particular of the function of the hormones AngII (angiotensin II) and Ang-(1–7) [angiotensin-(1–7)], is an important target for pharmacotherapy in the cardiovascular system. In the classical view, such modulation affects cardiovascular cells to decrease hypertrophy, fibrosis and endothelial dysfunction, and improves diuresis. In this view, excessive stimulation of AT₁ receptors (AngII type 1 receptors) fulfils a detrimental role, as it promotes cardiovascular pathogenesis, and this is opposed by stimulation of the AT₂ receptor (angiotensin II type 2 receptor) and the Ang-(1–7) receptor encoded by the Mas proto-oncogene. In recent years, this view has been broadened with the observation that the RAS regulates bone marrow stromal cells and stem cells, thus involving haematopoiesis and tissue regeneration by progenitor cells. This change of paradigm has enlarged the field of perspectives for therapeutic application of existing as well as newly developed medicines that alter angiotensin signalling, which now stretches beyond cardiovascular therapy. In the present article, we review the role of AngII and Ang-(1–7) and their respective receptors in haematopoietic and mesenchymal stem cells, and discuss possible pharmacotherapeutical implications.

INTRODUCTION

AngII (angiotensin II) compared with Ang-(1–7) [angiotensin-(1–7)]

The RAS (renin–angiotensin system) is a major regulator of renal and cardiovascular function, and plays a central

role in the homeostasis of the cardiovascular system and of the hydro-electrolyte balance [1]. For a long period, research was focused on production and signalling of AngII, highlighting ACE (angiotensin-converting enzyme) and renin with respect to production, and the AT₁ receptor (AngII type 1 receptor) with respect to

Key words: angiotensin, angiotensin II receptor, bone marrow, Mas receptor, haematopoietic stem cell, mesenchymal stem cell, renin–angiotensin system.

Abbreviations: ACE, angiotensin-converting enzyme; ACE2, angiotensin-converting enzyme 2; ACEi, ACE inhibitor; Ac-LDL, acetylated low-density lipoprotein; Ang-(1–7), angiotensin-(1–7); AngII, angiotensin II; ApoE, apolipoprotein E; AT₁ receptor, AngII type 1 receptor; ARB, AT₁ receptor blocker; AT₂ receptor, AngII type 2 receptor; BFU-E, erythroid burst-forming units; BM, bone marrow; BP, blood pressure; C21, Compound 21; CCR2, CC chemokine receptor 2; CFU, colony-forming units; CSF, colony-stimulating factor; CXCR, CXC chemokine receptor; EC, endothelial cell; eNOS, endothelial NO synthase; EPC, endothelial progenitor cell; EPO, erythropoietin; GEMM-CFU, granulocyte erythroid megakaryocyte macrophage CFU; GFP, green fluorescent protein; GM-CFU, granulocyte macrophage CFU; KDR, kinase domain receptor; HPC, haematopoietic progenitor cell; HSC, haematopoietic stem cell; HUVEC, human umbilical vein EC; IL, interleukin; KDR, kinase domain receptor; KO, knockout; MCP-1, monocyte chemoattractant protein-1; M-CSF, monocyte CSF; MNC, mononuclear cell; MSC, mesenchymal stem cell; NF- κ B, nuclear factor κ B; PPAR, peroxisome-proliferator-activated receptor; RAS, renin–angiotensin system; ROS, reactive oxygen species; SDF-1, stromal-derived factor-1; α -SMA, α -smooth muscle actin; TNF, tumour necrosis factor; VEGF, vascular endothelial growth factor; VSMC, vascular smooth muscle cell; WBC, white blood cell; WT, wild-type.

Correspondence: Dr Anton J. M. Roks (email a.roks@erasmusmc.nl).

signalling of AngII. Since AngII is an important player in unfavourable remodelling of cardiovascular tissue, ACEis (ACE inhibitors), ARBs (AT₁ receptor blockers) and renin inhibitors are successfully used in the treatment of cardiovascular disease, and novel tools to further optimize intervention in the RAS are being developed [1].

In the past two decades, these optimization efforts have resulted in the identification of novel therapeutic targets within the RAS, including the AT₂ receptor (AngII type 2 receptor) [2], Ang-(1-7) and its G-protein-coupled receptor Mas [3], and ACE2 (angiotensin-converting enzyme 2) [4,5], which is important for Ang-(1-7) formation. An increasing amount of evidence shows that Ang-(1-7) is one of the most important hormones of the RAS [6–8]. In the cardiovascular system, Ang-(1-7) mainly has opposing actions compared with AngII, leading to a dichotomy in the RAS, namely the proliferative, pro-thrombotic and vasoconstrictor actions of AngII against the anti-proliferative, anti-thrombotic and vasodilator actions of Ang-(1-7) [6]. Ang-(1-7) can be formed directly from AngII by ACE2, but also from AngI (angiotensin I). As ACE metabolizes Ang-(1-7), ACEi treatment increases Ang-(1-7) levels, and this is believed to play an important role in the beneficial cardiovascular effects of ACEis [6,7]. As an alternative, improvement in Ang-(1-7) formation by ACE2 is now under consideration as a novel cardiovascular therapy [8]. Similarly, stimulation of the AT₂ receptor, a receptor for both AngII and Ang-(1-7), often opposes AT₁ receptor signalling, and this principle is being explored for cardiovascular therapy with specific AT₂ receptor agonists [9].

Stem cells: general features and clinical use

At the same time that novel pharmacological tools within the RAS are being found for cardiovascular therapy, a novel target tissue for RAS intervention has been identified, namely the BM (bone marrow) and other sources of stem cells and progenitor cells. These cells can either be HSCs (haematopoietic stem cells) or HPCs (haematopoietic progenitor cells), or can be MSCs (mesenchymal stem cells), also called multipotent stromal cells. Many articles use the term HSC for cells that are in a very early stage of phenotypic development, still allowing differentiation towards diverse lineages, and that may have a potential for self-renewal. HPC is then used for cells that are further differentiated and have lost the potential for self-renewal. HSCs and HPCs form cells of the erythroid, myeloid or lymphoid lines (see Figure 1). Since the boundaries between these two haematopoietic populations are not always clear, in the present review we will use the term HSC for both populations. EPCs (endothelial progenitor cells) and fibrocytes are special HPC types that are involved

in angiogenesis and fibrosis respectively. MSCs have been identified as cells that can form a plethora of non-haematopoietic cells, including cardiomyocytes, smooth-muscle-like cells, renal cell types, connective tissue cells, neural cells etc. (see Figure 2). MSCs are most abundantly present in BM, fat tissue and dental pulp, but can also be present in tissues such as the myocardium and the subintima or adventitia of large arteries. Accordingly, these cells are under investigation as a tool for tissue regeneration of any imaginable kind. Since both HSCs, MSCs and isolated whole BM are interesting for tissue repair, all three sources have been used for cardiac and vascular repair. Other precursor cells that may potentially be used for this purpose are embryonal stem cells or induced pluripotent stem cells [10]. These cells can either serve as sources for novel cardiomyocytes or angiogenic cells, or act as paracrine cells that favourably affect cardiac remodelling or vascular repair. Several clinical studies have been performed with autologous BM-derived stem cells showing moderately improved cardiac performance in ischaemic cardiac diseases [11,12]. As yet, it is not clear which stem cell type and which preparation and infusion method gives the best results, and this is an important research goal. A second important goal is upgrading of the stem cells' abilities to perform their reparative function. For this purpose, diverse strategies are being studied [10], and, as will become clear from the present review, pharmacological intervention in the RAS may be one of them.

RAS and stem cells: general implications

The notion that the RAS is involved in regulation of BM and stem cells, and in particular haematopoiesis and erythropoiesis, was generated already shortly after the introduction of ACEis in the clinic when, in 1982, two independent groups observed that a high dose of ACE inhibition caused anaemia and leucopenia [13,14]. Later, the presence of all major RAS components in BM cells, including stromal cells, HSCs/HPCs and MSCs, was confirmed. This led to the concept of a potential autocrine–paracrine mechanism for local RAS-mediated regulation of haematopoiesis [15]. Another important reason to study the effects of angiotensins and RAS-modulating medicines on BM-derived cells is that, in the case of cardiovascular disease where RAS modulation is indicated as a treatment, stem or progenitor cells are under investigation for application in tissue regeneration, in particular of the vascular bed and the myocardium [16–26]. Furthermore, inflammation and fibrosis have been identified as important targets for RAS modulation, and these processes may be related to regulation of HSCs and MSCs, as will be explained later in the present review. Dedicated studies have now provided clear evidence that ACE, AngII/AT₁ receptor and Ang-(1-7)/Mas receptor activity are involved in haematopoiesis, as well as in the formation of cardiovascular cells and other somatic cell types from progenitor cells [27].

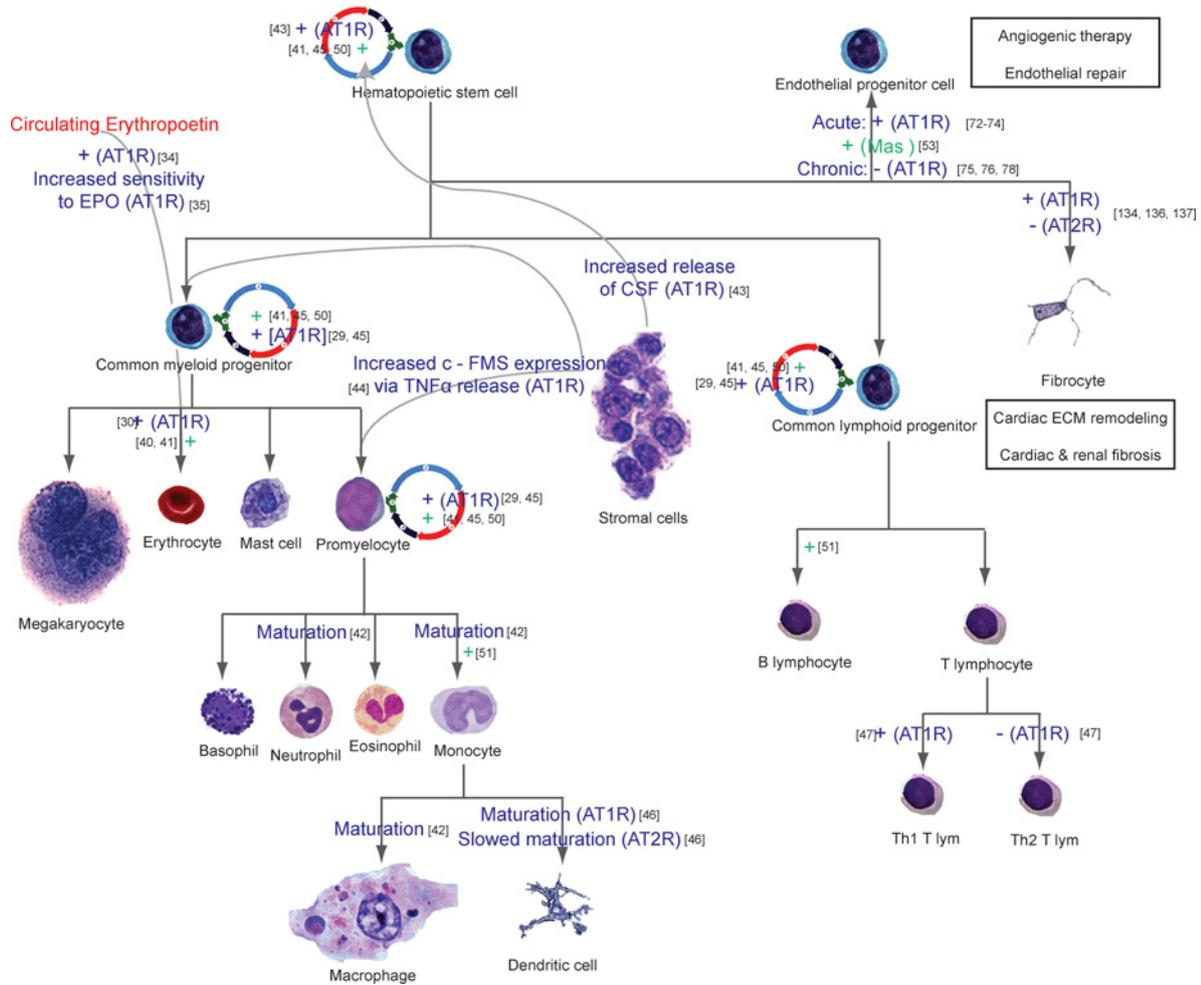


Figure 1 Schematic representation of the effects of AngII (blue lettering) and Ang-(1–7) (green lettering) on haematopoietic cells

The direction of the effect of AngII, Ang-(1–7) or angiotensin receptor stimulation is indicated, with ‘+’ being stimulatory and ‘–’ being inhibitory. The receptor involved, if known, is indicated in parentheses (AT1R, AT₁ receptor signalling; AT2R, AT₂ receptor signalling). Straight black arrows indicate effects on differentiation or, when specifically indicated, on maturation. Cell-cycle diagrams indicate a proliferative effect. Curved arrows originating from stromal cells represent paracrine effects. Reference numbers are to reports that directly show an effect of the angiotensin. Th T lymphocyte, T-helper cell.

In the present article, we will review the role of AngII, Ang-(1–7) and their respective receptor/signalling pathways in the propagation and differentiation of HSCs and MSCs, and discuss possible clinical implications. However, for more extensive discussions regarding ACE substrates and metabolites other than AngII and Ang-(1–7), we refer to previous reviews [28,29].

THE ROLE OF ANGIOII AND ANG-(1–7) IN HAEMATOPOIESIS

In the subsequent sections, we outline the effects of the RAS in haematopoiesis, and these are summarized in Figure 1 and Table 1.

Erythropoiesis

Soon after the initial studies that uncovered that manipulation of ACE activity interfered with erythropoiesis, it became clear that the role of the RAS in erythropoiesis is very complex, being involved in virtually every step between the haematopoietic stem cell and the fully differentiated erythrocyte. When looked at in further detail, it was shown that the stimulation of the AT₁ receptor increased the formation of early erythroid progenitors, an effect that requires the presence of EPO (erythropoietin) [30]. In addition, genetic manipulation leading to overactivity of AT_{1a} receptors in mice results in an increase in haematocrit [31]. Conversely, AT₁-receptor-KO (knockout) mice show a decrease in haematocrit

Table 1 Summary of the beneficial or detrimental effects of angiotensin receptor signalling effects in BM stem cells

Receptor signalling	HSC		MSC	
	Beneficial	Detrimental	Beneficial	Detrimental
AT ₁ receptor	Improves HSC proliferation under haematopoietic stress [45] Pro-angiogenic EPC stimulation [73,74]	EPC apoptosis and senescence [76–78] Fibrocyte-related fibrosis [134,136–137]		Neointima formation or inflammation by VSMC-like progenitor cells [168–170] Promotes adipocyte formation [160] Inhibits cardiomyocyte formation [142] Inhibits neural repair by MSCs after brain ischaemia [145]
AT ₂ receptor	Counteracts fibrocytes [134]		Promotes cardiomyocyte formation [141,142] Inhibits adipocyte formation [160] Improves neural repair by MSCs after brain ischaemia [145]	
Ang-(1–7)/Mas receptor	Improves HSC proliferation under haematopoietic stress [40,41,45,50] Increases early EPCs [53]			

values when compared with WT (wild-type) animals [32]. The stimulatory role of AT₁ receptors in erythropoiesis has clinical implications: as with ACEis, ARB treatment was reported to reduced erythropoiesis in healthy individuals and also in patients undergoing haemodialysis [33].

The mechanism of AngII-mediated regulation of erythropoiesis is largely unclear. Most of its effects are exerted in the early phases of erythropoiesis [30,33]. Some authors imply that AngII acts indirectly via its influence on EPO levels [34] or EPO sensitivity [35], whereas others do not observe a link between EPO and AngII in erythropoiesis [36,37]. A possible second messenger system via which AngII could be affecting erythropoiesis is the JAK (Janus kinase)/STAT (signal transducer and activator of transcription) pathway, which is known to be stimulated by AngII [38] and to be vital in the erythrocyte-stimulating action of EPO [39].

Since Ang-(1–7) is degraded by ACE, the anaemic effect of ACEis might be due to changed Ang-(1–7) levels. However, it was shown that Ang-(1–7) stimulated BFU-E (erythroid burst-forming units) cultured from mice that were treated with the myelosuppressive agent 5-fluorouracil and reduced anaemia in breast cancer patients after chemotherapy [40,41]. These findings suggest that Ang-(1–7) stimulates erythropoiesis and should counteract the anaemic effect of ACEis. Apparently, this does not happen and the question remains how important Ang-(1–7) is for erythropoiesis.

General effects of AngII on haematopoiesis of WBCs (white blood cells)

Leucopenia induced by a high dose of ACEis, as observed early after introduction of ACEis [14], was an indication that the RAS may be involved in the formation of WBCs by HSCs. In agreement, genetic ablation of ACE in mice leads to perturbations in myelopoiesis, which can be reliably recapitulated with ACE inhibition [42]. When focusing the attention on AngII, it has been shown that AngII induces the proliferation of mouse BM and human cord blood HSCs *in vitro*. This effect of AngII is elicited, in part, through stimulation of Lin⁻ BM stromal cells and is partly mediated by the direct stimulation of HSCs in the presence of CSF (colony-stimulating factor) [43]. AT₁ receptors mediate this effect of AngII because losartan blocks it, and the presence of the AT₁ receptor in both HSCs and stromal cells is compatible with the dual pathway [43]. Furthermore, AngII/AT₁ receptor signalling promotes M-CSF (monocyte CSF)-mediated differentiation/proliferation of BM monocyte lineage cells.

Through these general mechanisms, AngII can contribute to the regulation of WBC haematopoiesis. Strictly speaking, however, AT₁ receptor signalling does not seem to be of great importance for haematopoiesis under normal physiological conditions: ACE-KO mice show a block in terminal granulopoiesis, which leads to a reduction in segmented neutrophils. Monocytes and macrophages are at normal levels in ACE-KO and AT₁-receptor-KO mice, although they are functionally

immature [29,42,44]. This would correspond to the fact that ARB treatment does not reduce WBC levels. However, as observed recently [29], under haematopoietic stress AngII/AT₁ receptor signalling has readily visible effects. This is observed for instance after chemotherapy or irradiation, where AngII infusion improves the re-population of BM with HSCs, and thus accelerates restoration of WBC counts. It is with these circumstances in mind that the next section, in which we deliberate specific HSC subtypes, has to be read.

Role of AngII in specific subtypes of WBC precursors

As already indicated, important findings on the role of AngII in the regulation of HSC comes from studies exploring the restoration of WBC populations after irradiation or chemotherapy in animal models. Under such circumstances, *in vivo* AngII infusion leads to an accelerated restoration of total blood leucocytes, lymphocytes and platelets, and to increased GM-CFU [granulocyte macrophage CFU (colony-forming units)], GEMM-CFU (granulocyte erythroid megakaryocyte macrophage CFU) and BFU-E in *in vitro*-expanded BM cells [45]. Therefore AngII has a rather broad spectrum when it comes to stimulation of HSCs. This is in accordance with the observation that, in the early development of HSCs to WBCs, i.e. in CFU cultured under pan-lineage conditions, AngII has a proliferative effect [29].

This proliferative effect is lost when cells are further differentiated due to culturing in lineage-specific medium, containing GM-CSF (granulocyte/macrophage CSF), with M-CSF or G-CSF (granulocyte CSF) [42]. When the HSC has passed the stage of myeloblast, pharmacological or genetically induced interruption of the AT₁ receptor or ACE activity delays myeloid differentiation rather than proliferation, as observed by an increase in the myeloblast/early myelocyte marker CD11b and a decrease in the development of macrophage and neutrophil markers [42]. This was observed *in vivo* in the absence of haematopoietic stress. Furthermore, AT₁ receptor blockade also leads to the decrease in the differentiation towards dendritic cells of human monocyte or murine BM cell cultures [46]. AT₂ receptor stimulation counteracts this AT₁ receptor effect. Accordingly, the sum effect of these AngII functions is an accelerated general increase in leucocytes during haematopoietic stress and more subtle qualitative changes in populations of mature leucocytes, which can be seen in the absence of haematopoietic stress.

Similar to erythropoiesis, AngII does not seem to have a stand-alone effect in the development of WBCs, but rather plays a facilitating role, as observed during GM-CFU and GEMM-CFU formation. In the absence

of colony-stimulation factors [SCF (stem cell factor), GM-CSF, IL (interleukin)-3 and EPO], no growth effect of AngII on these colonies was observed, but, in their presence, AngII dose-dependently increased GM-CFU and GEMM-CFU formation [43]. The factors that are needed to allow an AngII effect are released by Lin⁻ BM stromal cells [43]. In addition, in the absence of M-CSF, cultured BM cells of AT₁-receptor-KO and WT mice showed no difference in myeloid progenitors and pro-monocytes [44]. However, the increase in these cell types that occurs upon stimulation with M-CSF was attenuated in AT₁-receptor-KO compared with WT mice. The facilitating role of AngII on M-CSF was shown to be due to TNF (tumour necrosis factor)- α release from stromal cells, which increases the expression of C-Fms, the receptor for M-CSF, in HSCs [44].

Apart from stimulating the myeloid pathway of haematopoiesis, AngII affects lymphoid development. AT₁ receptor stimulation appears to promote inflammatory activation of lymphocytes. In addition, AngII infusion leads to a shift of CD4⁺ T-cells (T helper) from Th2 to Th1, which leads to increased production of pro-inflammatory cytokines [IFN (interferon)- γ , IL-2, and TNF- β]. Blockade of AT₁ receptors decreases this shift and leads to reduction in the infiltration of tissues by activated macrophages and T-cells [47].

On the basis of the weak effects of ACE/AngII/AT₁ receptors on the WBC population in the absence of haematopoietic stress, it seems that the major part of the effect of RAS modulation on inflammation is mainly caused by its effects that do not directly relate to haematopoiesis. AngII is known to induce monocyte recruitment to the vascular wall and to stimulate these monocytes to release various inflammatory cytokines. It also increases production of ROS (reactive oxygen species) which in turn stimulate NF- κ B (nuclear factor κ B) signalling, leading to a pro-inflammatory phenotypes in various cell types. These processes are reviewed in detail elsewhere [48,49].

Role of Ang-(1–7) in HSC regulation

The effects of Ang-(1–7) on haematopoiesis are documented in several reports and are, in short, similar to the effects of AngII. Ang-(1–7) stimulates the recovery from irradiation and chemotherapy by increasing the proliferation of HSCs and multi-lineage haematopoietic progenitors [41,45,50]. In NOD (non-obese diabetic)/SCID (severe combined immunodeficiency) mice, the engraftment and proliferation of human MNCs was increased in mice receiving Ang-(1–7) treatment, which also increased the numbers of differentiated cells of myelomonocytic and B-cell lineages [51]. As with AngII, the effects of Ang-(1–7) are readily visible during haematopoietic stress, but not so evidently under normal physiological circumstances.

This is reflected in a recent study in which toxicological studies with Ang-(1–7) infusion did not show apparent effects on blood variables [52].

The role of angiotensin receptor subtypes in the effects of Ang-(1–7) on haematopoiesis has not been investigated. However, our own studies in which we used isolated BM-MNCs (mononuclear cells) from rats and mice suggest that Mas receptors mediate the proliferative effect of Ang-(1–7) on HSCs [28,53]. As these tests were done under conditions that promote the development of EPCs (endothelial progenitor cells), they do not provide conclusive evidence.

EPCs

Although a modest number of studies have addressed the relationship between HSCs and the RAS, some more intensive research has been done on EPCs [27,28]. EPCs are a special type of HSC- or MSC-derived progenitor cells that develop endothelial-like features under specific culturing conditions and have been implicated in endothelial repair and vasculogenesis [54,55]. Vasculogenesis is the formation of new blood vessels from progenitor cells, such as haemangioblasts, as opposed to angiogenesis, in which new vessels originate from sprouting of pre-existing ones. Vasculogenesis takes place during embryogenesis, but it has been proposed that it can also occur during adulthood, which would involve EPCs [56].

EPCs starting out as HSCs in the BM are being studied intensively. Their recruitment as HSCs from the BM to the circulation involves stress-induced SDF-1 (stromal-derived factor-1), which activates proteases that degrade the adherence proteins that bind HSC to endosteal cells. Subsequently, HSCs can migrate into the circulation. Once they arrive in the vascular lumen, they can home as angiogenic monocytes and macrophages that pre-process the tissue that requires neovascularization or endothelial repair, or as EPCs that will form the new endothelium [57]. Thus the process of vasculogenesis involves various haematopoietic cell types with various progenitor, myeloid and EC (endothelial cell) markers, complicating the identification of 'true EPCs' [27]. This identification can either be based on immunohistological staining for stem cell and endothelial membrane markers, followed by flow cytometry, or by culturing BM-MNCs or blood-derived MNCs in specialized endothelial culture medium and subsequent colony observation or (immuno)histochemical staining. A simple histochemical staining for cultured angiogenic cells is combined Ac-LDL (acetylated low-density lipoprotein) uptake (a feature of phagocytotic monocytes, macrophages and ECs) with binding to lectin from *Bandeiraea* (*Griffonia simplicifolia* {BS-1 [*B. simplicifolia*-1]} or *Ulex europaeus* [UEA-1 (*U. europaeus* agglutinin-1)]) [54]. More specific

EPC markers, for cultured as well as freshly obtained cells processed for flow cytometry, combine the stem-cell-surface markers c-Kit, Sca-1 and CD133 with an EC surface marker {e.g. CD34 or Flk-1 (mouse equivalent for human KDR (kinase domain receptor) or VEGFR2 [VEGF (vascular endothelial growth factor) receptor 2]}. With respect to culturing methods, *in vitro* propagation of blood-derived MNCs distinguishes early EPCs and CFU-ECs (CFU-Hill) on the one hand from late EPCs [comprising OEC (outgrowth ECs) and ECFCs (endothelial colony-forming cells)] on the other hand [56,58–62]. Early EPCs and CFU-Hill are found from 2 days until 3 weeks of culture and might be more related to angiogenic monocytes and macrophage, as they show low proliferation and tube-formation capacity. Late EPCs, appearing from 3 weeks up until 12 weeks of culture, could represent true EPCs, displaying highly proliferative and tube-formation capacity. For further critical reviews on EPC selection criteria, we refer readers to previous publications [27,63,64].

Although the identity of true EPCs and their permanent incorporation into repaired and newly formed vessels is still a matter of debate, it is evident that the various 'EPCs' contribute to the vascular regeneration and repair processes, and thus have clinical relevance [27,28]. In addition, plasma levels of EPCs have been used as a biological marker of vascular function and cumulative cardiovascular risk. It has been shown that the number of CFU-Hill correlate with brachial endothelial function, measured as reactive hyperaemia, arterial calcification, Framingham risk score and several cardiovascular risk factors [60,65,66]. However, quantification of cultured EPCs might be a rather laborious method for prognostic purposes. Instead, circulating EPCs can be measured by flow cytometry, using CD34 alone in combination with a marker expressed by ECs, often KDR. As such, CD34⁺KDR⁺ cell levels have been shown to be associated with coronary artery disease, outcome after angioplasty and traumatic brain injury, although peripheral arterial calcification only correlated with colony number of cultured EPCs [66–69]. In a direct comparison between cultured EPCs and quantification by flow cytometry, both methods showed an association of EPC levels with coronary artery disease, but only cultured EPCs are predictive for progression of the disease [70]. In addition, an increase in these markers is associated with beneficial effects of RAS intervention, as reviewed previously [27]. The use of EPCs as a standard risk marker in cardiovascular disease is, however, still remote for daily practice. Hence EPCs remain under investigation as a regenerative angiogenic therapy in organs after ischaemic events and as a prognostic marker during pharmacotherapy directed against vascular disease. As RAS intervention is often used in those cases, it is important to characterize the effects of angiotensins on EPCs.

Role of AngII, AT₁ receptors and AT₂ receptors in EPCs

Although the identity of true EPCs is still unresolved [27], many studies have confirmed the role of angiogenesis-associated progenitor cells, whether they are mobilized from BM to peripheral blood or isolated, cultured and re-injected, in endothelial regeneration and neovascularization [71]. The fundamental parts of these processes bear relationship with AngII signalling through AT₁ and AT₂ receptors (Figure 1). AT₁ receptor stimulation can lead to pro-angiogenic effects and recruitment of EPCs, but on the other hand stimulation can reduce EPC proliferation and function. These paradoxical effects can be explained from acute compared with chronic AT₁ receptor signalling. Acute AngII signalling is pro-angiogenic. In EPCs, this pro-angiogenic effect depends on NADPH oxidase activation and enhanced VEGF anti-apoptotic function through up-regulation of VEGFR2 and improved NO release, as well as on PI3K (phosphoinositide 3-kinase)/Akt signalling [72–74]. The deleterious effects of AngII arise from chronic stimulation and consist of two consecutive phases [75]. In the first phase, taking place between days 2 and 5 of stimulation of EPCs with AngII, an AT₁-receptor-mediated increase in NADPH oxidase activity leads to ASK-1 (apoptosis signal-regulating kinase-1)/JNK (C-Jun N-terminal kinase)/p38 MAPK (mitogen-activated protein kinase)/Bax/Bcl2-signalling-induced apoptosis involving caspase 3 [76]. The second phase, which is observed from day 5 and onward, involves the production of cytotoxic levels of ROS, leading to cellular senescence [77,78]. As a result, chronic treatment with AngII decreases human and mouse EPC numbers and function [67,76–79]. Employing AT₁-receptor-KO MNCs and BM transplants in WT and ApoE (apolipoprotein E)-KO mice, it was shown that AT₁ receptor signalling affects vascular repair function and thus promotes atherosclerosis [76]. As commented in detail above, it is still unknown whether these *in vivo* vascular effects solely depend on EPCs or involve an interplay with inflammatory cells or even BM stromal cells [75].

Role of Ang-(1–7)/Mas receptor signalling in EPCs

Ang-(1–7) has been shown to improve endothelial vasodilator and eNOS (endothelial NO synthase) function in various studies [80–93]. Although some of these effects depend on the acute activation of eNOS or inhibition of NADPH oxidase [94–96], others may point to a potential role of Ang-(1–7) in endothelial regeneration. Such a role was suggested by the observation that Ang-(1–7) improved the recovery of HSCs [40,41,45], from which EPCs are derived. Therefore dedicated studies to explore the effects of Ang-(1–7) on EPCs have been performed [53]. In adherent rat

or mice BM-MNC cultures, which most likely resemble early EPCs, 7 days of treatment with Ang-(1–7) increased Ac-LDL/lectin-positive cells, which were also found to be positive for VEGFR2. Mas receptor signalling is an important mediator of the stimulatory effect of Ang-(1–7) on BM-MNCs and EPCs, because the effect disappeared when Mas receptor signalling was prohibited by genetic ablation of Mas or treatment with A779. AT₂ receptors did not seem to play a role in this effect [28]. The *in vitro*-stimulatory effect of Ang-(1–7) on EPCs can explain why we found that *in vivo* infusion of the peptide in mice with myocardial infarction led to an increase in VEGF⁺ and c-kit⁺ cells in the heart [53]. As the local cardiac overexpression of Ang-(1–7) did not lead to such an effect, we hypothesized that BM-derived angiogenesis-related progenitor cells were recruited to the heart. This potential mechanism, as well as the consequences for myocardial angiogenesis, remains to be characterized.

Several other questions regarding angiogenesis need to be clarified as well. Ang-(1–7) can also inhibit angiogenesis *in vivo* [97–100] and tends to inhibit *in vitro* tube formation by HUVECs (human umbilical vein ECs) [101]. This principle is now under investigation in the context of the application of Ang-(1–7) as an anti-cancer therapy [99,102]. There can be several explanations for such a discrepancy with our findings on EPCs. First, it can relate to differences in cell types, i.e. EPC compared with adult ECs. Secondly, the inhibitory effect of Ang-(1–7) on tube formation by HUVECs was not dose-dependent and involved Mas receptors as well as AT₁ receptors [101]. Since these were acute responses, the inhibitory effect of Mas receptors on AT₁ receptor signalling [103] might have inhibited angiogenesis. However, this explanation only works if one presumes the presence of spontaneous AT₁ receptor signalling or a paracrine/autocrine RAS. Thirdly, in the sponge model of angiogenesis and after tumour implantation, the anti-angiogenic response might involve effects on surrounding cells that produce angiogenic factors. The myocardium in our experiments may respond differently to Ang-(1–7) with respect to the release of angiogenic factors as compared with the tissue that surrounds sponges or tumours. In fact, there is evidence that tumour angiogenesis markedly differs from that in normal tissues, as extensively reviewed elsewhere [104]. Fourthly, the concentration of Ang-(1–7) and its relationship with the duration of the stimulus may be important. In acute studies, such as the study in HUVECs [101], Ang-(1–7) might simply display the usual opposite effect AngII. Instead, our studies in cultured MNCs and EPCs employed chronic stimulation and again Ang-(1–7) appears to induce an effect that is opposite to the effect of AngII, i.e. stimulatory compared with inhibitory. Looking in more detail, we found a bell-shaped concentration-dependent effect of a 7-day treatment with Ang-(1–7), given every 2 days, with a maximal response between 10^{−9} and 10^{−8} mol/l

[53]. Similarly, pre-treatment with 10^{-8} mol/l Ang-(1-7) stimulated an increase in tube formation in porcine BM-MNCs, whereas higher concentrations seem to reduce this ability [105]. This observation prompts another explanation. Recently, it was discovered that Ang-(1-7) stimulated Mas receptor internalization [106]. The continuous presence of an Ang-(1-7) concentration of 1 nmol/l and higher might induce permanent Mas receptor internalization, thus abolishing Mas receptor signalling. This could, in turn, even promote chronic AT_1 receptor signalling, thus reducing EPC levels. Alternatively, Ang-(1-7) might be diverted to AT_2 receptors, which would suppress angiogenesis [107]. As Ang-(1-7) is rapidly degraded in the presence of serum [108], the presence of Mas receptors at the cell membrane might be warranted when intermittent administration of the peptide is used, even at concentrations that are slightly higher than 1 nmol/l.

Regardless of the reasons mentioned above for the paradoxical effects of Ang-(1-7) on EPCs compared with angiogenesis, it is clear that, in the development of pharmacotherapy based on EPC stimulation by Ang-(1-7), it will be important to dissect the diverse signalling pathways. This concerns both the exploration of the differential function of these pathways, as well as the development of optimal pharmacotherapy.

Improvement of EPC recruitment with ARBs and ACEis

The *in vivo* effects of ARBs on EPCs generally correspond well with the effects that would be predicted from the functions of AngII and Ang-(1-7) as observed in animal or cell culture studies. ARB treatment in animal studies increases EPC levels during hypertension [109–111], after nephrectomy [112] or myocardial infarction [113], in atherosclerosis models [114], and in the ischaemic hindlimb model [115,116]. Although the situation in animal models may be quite different from the clinical presentation of patients, the effects of ARBs on EPCs from animals and patients correlate rather well. In patients with Type 2 diabetes, ARB treatment alone or as a part of multiple drug therapy was proposed to exert its beneficial cardiovascular effects by increasing the number of regenerative EPCs [117,118]. In accordance with this idea, ARB treatment increased EPC counts, angiogenic factors and endothelial vasodilator function in normotensive patients with coronary artery disease [119]. Similarly, ARBs increase EPC levels in patients with acute coronary syndrome [120]. ARB treatment also promotes an increase in EPC levels in kidney transplant patients [121]. A special role might be performed by telmisartan, a combined ARB/PPAR (peroxisome-proliferator-activated receptor)- γ agonist. PPAR- γ might have additional value when stimulation

of EPCs is concerned [122,123], as has been discussed in detail in previous reviews [27,28]. Taken together, the findings suggest that in animal models, as well as in patients, ARB treatment increases EPC levels, thus preserving the endothelium. It seems logical to ascribe these beneficial effects to a decrease in EPC apoptosis and senescence caused by excessive AngII/ AT_1 receptor signalling.

ACEis have been shown to have beneficial effects in cardiovascular disease that are believed to be related to EPC function [60,67,124–129]. However, the actual number of patient studies that have explored the effects of ACEis on EPCs is very limited. ACEis increased the levels, proliferation, migration, adhesiveness and tube formation of EPCs that were cultured from the blood of patients with coronary artery disease [79]. In patients with acute coronary syndrome, ramipril increased circulating EPCs as measured by flow cytometry, and its effect on EPCs was nearly identical with that of the ARB telmisartan [120]. Very recently, two studies have appeared that claim a stimulatory effect of ACEis on EPCs of patients with hypertension and acute coronary syndrome, although the characterization of EPCs in these studies is limited [130,131]. In agreement with clinical studies, ACEis increase EPC levels in several animal models [113,115,116,132,133]. These effects may involve various factors other than angiotensin metabolism, as amply discussed elsewhere [27,28].

Differentiation of HSCs into fibrocytes

Fibrocytes are $CD34^+$, $CD45^+$ or $CD133^+$ BM-derived circulating cells that co-express collagen I or smooth muscle actin [134]. They are derived from haematopoietic cells and are related to monocytes, and infiltrate organs to establish fibrosis. In models of renal fibrosis, the AT_1 receptor mediates increased BM fibrocyte levels, increased renal infiltration and stimulates collagen I production in cultured fibrocytes. The AT_2 receptor counteracts these effects. Recruitment of fibrocytes to the kidney involves CXCL16 (CXC chemokine ligand 16), a ligand for the receptor CXCR6 (CXC chemokine receptor 6) [135]. However, it is not certain whether this relates to AngII signalling.

With respect to cardiac remodelling, it has been shown that AngII infusion promotes cardiac fibrosis through the recruitment of fibrocytes in mice [136,137]. Since CCR2 (CC chemokine receptor2) KO abrogated the effect of AngII, CCL2 (CC chemokine ligand 2), also known as MCP-1 (monocyte chemoattractant protein-1), is apparently required for AngII-induced fibrocyte recruitment. BP (blood pressure) is not involved, since CCR2 KO did not change this variable. MCP-1 is an important stimulator of monocytes, dendritic cells and T-cells, and therefore these findings show an important relationship between the inflammatory response and AngII-induced fibrosis. It should be noted, however, that the recruitment of

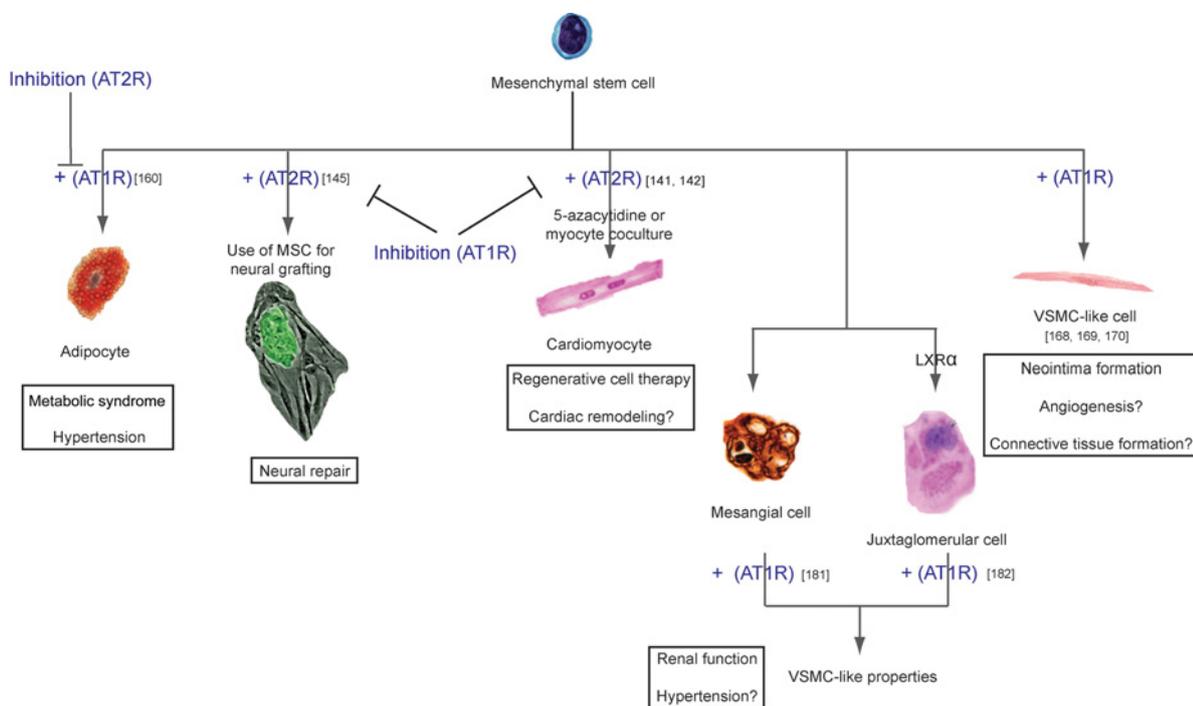


Figure 2 Effects of AngII (blue lettering) on MSCs

The direction of the effect of AngII or angiotensin receptor stimulation is indicated, with '+' being stimulatory on differentiation, proliferation or use in regenerative therapy. The receptor involved, if known, is indicated in parentheses (AT1R, AT₁ receptor signalling; AT2R, AT₂ receptor signalling). Reference numbers are to reports that directly show an effect of AngII. Clinical implications or possible applications are indicated in the boxes.

fibrocytes by AngII to the myocardium is not necessarily detrimental. CCR2 KO, which ablates this effect of AngII, leads to less fibrosis, but also to an exaggerated ventricular dilation and a worsened systolic function. The fibrotic response seems to provide the necessary matrix to support the hypertrophied myocardium [136]. Therefore, to obtain clinical benefit from suppression of AngII-induced fibrosis, myocardial hypertrophy should also be prevented to avoid myocardial dilation, which is detrimental.

MSCs FROM BM

MSCs are non-haematopoietic progenitor cells that are able to form many diverse cell types, including cells with features of adipocytes, cardiomyocytes, fibroblasts, ECs, VSMCs (vascular smooth muscle cells) and renal cells, which all have relevance for cardiovascular disease and therapy. MSCs are found in many different tissues, including the BM. Given the pluripotency of MSCs, these cells may play a role in diverse physiological and therapeutical processes. In the subsequent sections, we outline the role of the RAS in a number of these processes, which are summarized in Figure 2 and Table 1.

Cardiomyocyte formation from MSCs

BM-MSCs have been shown to develop cardiomyocyte features through reprogramming of the genetic programme by *in vitro* treatment with 5-azacytidine, a DNA-demethylating agent [138]. This finding was an important step towards the principle that autologous MSC transplantation could be used for cardiac repair and has led to clinical trials. Meta-analyses have shown that the small trials conducted thus far show only limited benefit, which has prompted novel research on the optimization of stem cell therapy [139,140]. Optimization studies include issues such as the cell type that is used, the number of grafted cells and the timing of injection, but also the effect of pharmacological modulation, including components of the RAS. AngII has been shown to promote differentiation of rat BM-MSCs into cardiomyocytes *in vitro* and has an additive effect to 5-azacytidine [141]. Thus AngII treatment might reduce the culture time of BM-MSCs, allowing earlier grafting of the differentiated cultures.

Further characterization of the receptor subtypes involved came from a study in which human BM-MSCs (Yub623) were stimulated to differentiate into cardiomyocytes by co-culturing on murine cardiomyocytes for 2 weeks [142]. Various ARBs were shown to equally improve BM-MSC differentiation, apart from

telmisartan, which had additional effectiveness because of its pleiotropic actions as a PPAR- γ agonist. The effect was independent of the co-cultured murine cells. The AT₂ receptor antagonist PD123319 alone was without effect, but it reduced the effect of candesartan. As AngII alone also did not stimulate differentiation in that study, unless given together with candesartan, it was concluded that the differentiation of BM-MSCs into cardiomyocytes was mediated through AT₂ receptors. Because ACE inhibition mimicked the effects of ARBs, a local RAS in BM-MSCs was proposed, as was to be expected from previous PCR studies on rat BM-MSCs [15]. However, the fact that the renin inhibitor aliskiren was without effect in human BM-MSCs suggests that not all of the RAS components may be present in relevant amounts in those cells. Therefore it cannot be excluded that ARBs, when given in the absence of exogenously applied AngII, might have unknown pleiotropic effects, especially when applying at the high concentration (3 μ mol/l) used in that particular study.

The AngII-induced differentiation of BM-MSCs into cardiomyocytes is theoretically in accordance with the observation that AngII stimulates cardiac extracellular matrix remodelling through recruitment of fibrocytes [136]. In the context of physiological remodelling, these effects of AngII would be complementary to each other if there is a need for increased myocardial mass, for instance to improve physical performance, because they would provide a balanced increase in myocytes and extracellular matrix. Such a remodelling would beneficially influence cardiac performance. Whether AngII-induced BM-MSC differentiation into cardiomyocytes occurs, as well if this is in balance with fibrocyte recruitment, remains to be investigated in models for physiological cardiac remodelling. An exaggerated AT₁-receptor-mediated response may, however, cause stiffening of the myocardium and relative ischaemia, a concept that fits with the general paradigm of pathological remodelling due to high BP or ischaemia. Blockade of AT₁ receptor binding of AngII and concurrent redirection of AngII towards AT₂ receptors would inhibit this detrimental response, because it restores the equilibrium between the differentiation of MSCs into fibrocytes and cardiomyocytes.

ARB treatment can also be combined with BM-MSC grafting. Indeed, in a rat myocardial infarction model, it has been shown that pre-treatment of BM-MSCs with an ARB improved the therapeutic effect of BM-MSC grafting on systolic heart function [142]. This was improved further by oral administration of an ARB. However, oral ARB intake combined with non-pre-treated BM-MSC grafting did not have an additive effect compared with the single ARB treatment. Therefore pre-treatment of BM-MSCs with ARBs or AT₂ receptor agonists may be the most optimal condition for cardiac repair. It should be noted, however, that BM-MSCs that are injected for cell therapy into the myocardium are only

transiently present, as observed in mice, and the fusion with resident cardiomyocytes might be poor [143]. This has favoured the concept that BM-MSCs are therapeutic primarily due to a paracrine function [144]. Therefore it is important to explore these paracrine factors and the impact of RAS modulation upon them.

Neural repair

Similar to cardiac ischaemia, BM-MSC grafting has been tested for brain ischaemia [145]. In the mouse MCA (mid-cerebral artery) occlusion model, BM-MSC grafting improved survival, ischaemic lesion volume, neurological score and cerebral oedema. Cerebral blood flow and TNF- α measurements suggested that a reduction in inflammation, rather than a pro-angiogenic effect, was responsible for the effect of BM-MSC grafting. BM-MSCs from AT₂-receptor-KO mice, however, did not improve neurological variables and even showed a trend to worsen survival. Pre-treatment with the ARB valsartan restored the survival effect of BM-MSC grafting. These results imply that AT₁ receptor signalling, when not counteracted by the AT₂ receptor, is detrimental for BM-MSC repair function. ROS-mediated pro-inflammatory AT₁ receptor signalling could be the provocative stimulus in the case where AT₂-receptor-KO cells seem to worsen the outcome. Since AT₂ receptor signalling results in NF- κ B-mediated neural repair and neural differentiation of embryonic stem cells through stimulation of MMS2 (methyl methanesulfonate-sensitive 2) release [146,147], it would be worthwhile to study whether these mechanisms are also involved in BM-MSC grafting.

BM-MSC grafting is believed to represent a new interventional technique for patients with stroke [148,149], and AT₁ receptor blockade might provide further improvement. Pre-treatment of mice with valsartan at a dose without an effect on BP led to a comparable improvement in neurological score and better blood flow than BM-MSC grafting [145,150]. Other ARBs show comparable results to valsartan [151]. Further neuroprotective effects of ARBs were shown in hypertensive patients, who show less stroke-related events when ARB treatment was applied, leading to a better prognosis [152]. In acute stroke, animal models also show improved recovery with ARB treatment [153–155]. However, in patient studies of acute stroke, ARB treatment was not at all effective, showing even a tendency towards adverse effects, which might relate to a hypersensitivity of the patients towards BP-lowering [155,156]. Therefore optimization of RAS treatment in acute ischaemia might benefit from (pre)clinical research on the interaction with endogenous as well as grafted BM-MSCs.

Adipogenesis

Adipogenesis, the formation of fat tissue, is importantly implicated in the metabolic syndrome and cardiovascular

risk, as extensively reviewed elsewhere [157,158]. Adipose tissue contains a complete RAS needed for AngII production and signalling. The physiological importance of this local RAS extends to the entire organism, contributing to both the local and systemic regulation of the RAS. This is believed to contribute to obesity, diabetes, the metabolic syndrome and cardiovascular disease. Adipose tissue can be approximately divided in two compartments, the visceral and the subcutaneous adipose tissue. Adipose tissue can form part of normal physiology as an energy storage compartment. This healthy fat tissue contains small insulin-sensitive adipocytes that store fat until use as an energy source. These cells produce anti-inflammatory effectors, such as adiponectin. Adipose tissue can also be related to diabetes and the metabolic syndrome, and in this case has a feature of the occurrence of large insulin-resistant adipocytes [159]. These large adipocytes release pro-inflammatory factors, among which is TNF- α , a key player in insulin resistance, diabetes and the metabolic syndrome. Obesity is associated with large adipocytes and an activated adipose tissue RAS, and it is believed that obesity thus contributes to cardiovascular disease [157].

Adult adipocytes may arise from two sources: pre-adipocytes in the fat tissue and MSCs from various sources, including the BM. In pre-adipocytes, the role of AngII and its respective receptors is a matter of debate that involves culture conditions, the type of adipogenic stimulus, the phase of differentiation to name a few [160,161]. A feasible concept of AngII-mediated dysregulation of adipogenesis in diabetes and the metabolic syndrome is that AT₁ receptor stimulation prevents insulin-induced differentiation of pre-adipocytes into adult small adipocytes and that, as a consequence, the pool of large adipocytes increases [162]. Compared with research in pre-adipocytes, research in MSC differentiation into adipocytes is relatively young. It has been shown that all key AngII-related RAS components are expressed in human BM-MSCs cultured under adipocyte-inducing conditions. Thus AngII seems to enhance the differentiation into adipocytes through AT₁ receptors in an autocrine fashion [160]. This effect is inhibited by the AT₂ receptor. Since expression of the AT₂ receptor is rather high in MSCs that are cultured under the given conditions, the blockade of adipogenesis by AT₂ receptors was readily visible when the antagonist PD123319 was added. However, since exogenously given AngII mimicked the AT₂-receptor-mediated blockade of differentiation, AT₁ receptor signalling is dominant when only the locally produced AngII is present.

Given the complicated manner in which AT₁ receptor and AT₂ receptor signalling is involved in the differentiation of MSCs and pre-adipocytes, as well as the question as to what fate will fall upon MSCs with respect to the formation of small compared with large

adipocytes, at this moment it is difficult to establish the importance of BM-MSCs differentiation in the therapeutic effects of RAS modulation. Further studies will require BM-MSCs-specific RAS component KO cell culture or mouse models, as has also been suggested previously for adipose tissue [157]. Culturing conditions and metabolic status will be important issues in such studies.

The importance of the Ang-(1–7)/Mas axis in adipose tissue is only beginning to be uncovered. Ang-(1–7) and ACE2 are present in adipose tissue [163,164], and ACE2 is regulated by dietary fat [164]. In addition, Ang-(1–7) and the Mas receptor have been reported to have an impact on fat and glucose metabolism, adiponectin levels and on the insulin sensitivity of adipocytes [165,166]. Moreover, peri-aortic fat may play a role in Ang-(1–7)-induced vasodilation [167]. Therefore research into the role of Ang-(1–7) in BM-MSCs differentiation into adipocytes is warranted.

MSCs with VSMC traits

VSMC-like cells can be derived from BM stem cell pools, as well as MSC populations present in the adipose tissue. In mouse studies, it has been shown that AngII stimulates the expression of typical smooth muscle cell markers in cultured adherent BM-MNCs and MSCs from adipose tissue through AT₁ receptors [168,169]. Similar results were obtained with MNC derived from peripheral blood of rabbits [170]. In adipose tissue MSCs, it has been shown that this effect is mediated by TGF (transforming growth factor)- β receptor stimulation and subsequent Smad2 and ERK (extracellular-signal-regulated kinase) activation [169]. Replacement of WT with GFP (green fluorescent protein)-labelled BM in mice showed that GFP-positive α -SMA (α -smooth muscle actin)-positive cells incorporated in the neointima of damaged femoral arteries of WT C57bl/6 mice [168]. The recruitment of such cells was stimulated by AngII infusion and was inhibited by AT₁ receptor blockade. Since neointima formation followed a similar pattern, it was concluded that AngII-induced differentiation of BM progenitors to VSMCs contributes to neointima formation. Indeed, BM-derived cells that express typical VSMC markers have been implicated previously in neointima formation [171,172]. In ApoE-KO mice with LacZ-positive BM, it was shown that local vascular production of SDF-1 α , a ligand for CXCR4 that is important for the chemoattraction of various BM-derived cells, plays a central role in homing of the VSMC-like cells to the neointima. Recently, a study that explored the effect of replacing normal BM with AT₁-receptor-KO BM in WT mice showed a connection between AngII and SDF-1 α signalling [173]. AT₁ receptor KO has led to diminished plasma levels and neointimal incorporation of Lin[−] BM-derived progenitor cells, and decreased neointima formation. Although no specific VSMC markers were used in that study, it was proposed that the lack of AT₁

receptor signalling leads to decreased VSMC progenitor recruitment. This effect appeared to depend on decreased SDF-1 α release by local platelets at the site of injury due to the absence of AT₁ receptor signalling in those platelets. Therefore AT₁ receptor signalling may affect neointimal homing of VSMC-like cells from BM in two ways: (i) through increased differentiation of progenitor cells into VSMC-like cells, and (ii) attraction of progenitor cells through stimulation of platelet-derived SDF-1 α .

It should be noted, however, that VSMC-like progenitor cells may not undergo complete differentiation into adult VSMCs. Some studies show that, when injected into the ischaemic myocardium, BM stem cells/BM-MSCs with VSMC traits are not incorporated in vessels [174,175]. Indeed, in earlier studies, it was suggested that MSCs develop a smooth-muscle-cell-like contractile apparatus to form myoid cells that use this apparatus in their function to support haematopoiesis of inflammatory cells [176]. Interestingly, it was shown recently that BM-derived cells that incorporate into the neointima and atherosclerotic lesions and express α -SMA also express monocyte/macrophage markers [177]. Therefore VSMC-like cells originating from the BM, and perhaps also from adipose tissue, may play a pro-inflammatory role, rather than representing a pool of progenitor cells that will permanently differentiate into adult VSMCs. This might explain why we found that all BM-derived cells in the neointima after stenting expressed inflammatory cell markers [178]. The importance of such cells may of course be significant: the effect of AngII on stimulating the development of the VSMC-like cells that support or possess traits of inflammatory cells may be another example of the versatility of this peptide as a pro-inflammatory factor. Exploration of the role of AT₂ and Mas receptors on these cells would be of great interest. Of additional importance is the further exploration of the link with SDF-1 α /CXCR4 signalling in pro-atherogenic environments, since blockade of this pathway in inflammatory cells leads to increased plaque formation in ApoE-KO mice [179], which is in contrast with the decrease in neointima formation in injured arteries of WT mice discussed above [173]. It is essential to discover how these contrasting findings translate to the effects of AT₁ receptor blockade in a clinical setting.

Finally, α -SMA expression in MSCs might be part of a transition towards the development of connective tissue [180].

Renal progenitor cells

BM-derived progenitor cells may play a role in the remodelling of renal tissue. Approximately a decade ago, it was shown, with the help of replacing WT BM with GFP-positive BM in mice, that BM-derived cells incorporate into the glomerular mesangium of the kidney [181]. GFP-positive mesangial cells increased over time after BM replacement, suggesting a long lasting, if not

permanent, residence of these cells. Although some of these cells express macrophage and lymphocyte markers, a large proportion of these cells do not appear to be leucocytes. As with MSCs, these cells express α -SMA and therefore might represent pools of mesangial cells that contribute to glomerular vasomotor control. In accordance with this idea, isolated and cultured GFP-positive cells contracted upon exposure to AngII [181].

Another α -SMA-expressing cell type that is associated with the glomerulus is the juxtaglomerular cell, present at the intersection of the distal convoluted tubule and the afferent arteriole of the glomerulus. These cells contain renin granules that can be released in response to low BP or low filtrate osmolarity. The resulting increase in AngII production causes normalization of the BP and filtrate osmolarity. It was shown recently that BM-MSCs can develop into renin-expressing granular cells that resemble juxtaglomerular cells, and that they subsequently start to increase expressing α -SMA [182]. This process is under the control of LXR α (liver X receptor α) stimulation. This observation has shifted the paradigm of the origin of juxtaglomerular cells from being a VSMC-derived cell towards a MSC-derived cell that actually develops VSMC-like features in a later phase of differentiation. Perhaps even a portion of the BM-derived GFP-positive glomerular cells that were observed in the fate-tracking experiment in mice might have developed in juxtaglomerular cells, but this was not explicitly explored [181]. As the VSMC-like features develop relatively late during MSC differentiation, it is tempting to speculate that this phenotype is a result of auto-crine AngII signalling following the up-regulation of renin. Thus AngII might contribute to the development of cell types that release renin, control glomerular blood flow or both. This in turn would implicate an involvement in hypertension and renal disease.

GENERAL CONCLUSIONS AND FUTURE PERSPECTIVES

There is a growing body of evidence suggesting that AngII and Ang-(1-7) can affect proliferation and differentiation of BM-HSCs and BM-MSCs. The observed effects imply that RAS modulation in these cells can be used to inhibit cardiac, vascular and renal fibrosis, as well as neointima formation, to improve renal function, to improve BP control and organ perfusion, and to induce cardiac and renal repair. Beyond the cardiovascular system, modulation of the RAS in these cells can beneficially influence adipogenesis, neural repair, connective tissue formation and wound healing, but, foremost, stimulation of the Ang-(1-7)/Mas signalling axis is now under evaluation as an anti-cancer therapy with the combined benefit of improving haematological recovery after chemotherapy or irradiation. For a

large part, the signalling mechanisms remain to be investigated, especially in relation to the stage of differentiation of HSCs and MSCs, to the culture conditions (*in vitro*) or surrounding tissue (*in vivo*), and to the pathophysiological context. Such information might lead to more refined pharmacotherapy, and novel drugs for the stimulation of specific angiotensin receptor subtypes are already being developed.

With respect to these novel drugs, the emerging ACE2/Ang-(1–7)/Mas receptor-axis-oriented drugs are of particular interest. As Ang-(1–7) promotes post-chemotherapy or -irradiation haematopoiesis in the absence of a pressor effect, the peptide is now under evaluation for the haematological recovery of cancer patients. This already involves clinical trials with TXA127, an Ang-(1–7)-containing drug formulation (see <http://www.tarixpharma.com/clinical-pipeline/> for more information). The paradoxical effect of Ang-(1–7) on angiogenesis and its potential dependency on a delicate balance in the stimulation of specific receptor subtypes has been discussed. Several attempts have been made to specifically improve Ang-(1–7) signalling, for example through infusion of cyclodextrin-enveloped Ang-(1–7) [183] or increased ACE2 expression [184]. Even more specific is the non-peptide analogue CGEN-856S [185] or the thioether-bridged Ang-(1–7) analogue called cyclic Ang-(1–7) [186,187], which are specific Mas receptor agonists. Infusion of cyclic Ang-(1–7) has shown promise in rat models as an intervention for acute respiratory syndrome or after myocardial infarction [188,189]. It will be of major interest to perform comparative studies on native Ang-(1–7) with these specific Mas receptor agonists and to unravel their effects on EPCs and other progenitor cells.

As discussed, the AT₂ receptor is importantly involved in the effects of AngII on progenitor cells. Through its effect on HSCs and MSCs, AT₂ receptor stimulation may have beneficial effects on fibrosis by inhibiting fibrocyte development, prevention of heart failure by promoting myocardial regeneration, and improvement of neural repair. Therefore the design of AT₂ receptor agonists may be an important development. Currently, two agonists are being explored: the non-peptide drug C21 (Compound 21) and AngII with single β -amino-acid substitutions [9,190]. C21 has shown promise in a rat model of myocardial infarction by improving systolic and diastolic function, and by anti-apoptotic and anti-inflammatory effects [191]. In addition, it was found that C21 alone or in combination with losartan may improve endothelial function and vascular composition by reducing oxidative stress, collagen content, fibronectin and inflammatory cell infiltration in stroke-prone spontaneously hypertensive rats [192]. The development of β -amino-acid AngII analogues awaits further *in vivo* characterization with respect to pharmacokinetic aspects, as well as therapeutic effects.

Interaction with stem cell therapy is certainly one of the most relevant issues that can be addressed in further studies with these AT₂ receptor agonists.

Another relevant goal for future research is the further characterization of α -SMA-expressing cells types formed by MSCs in response to AngII. It will be important to study the relationship between tissue localization and culture conditions and the final phenotype and function that such cells will assume, which comprises VSMCs, connective tissue and neointimal cell types, pro-inflammatory cell types, mesangial cells or juxtaglomerular cells. Without any doubt this feature of AngII/AT₁ receptor signalling will play a role in the clinical effects of modulating the RAS on arterial and renal remodelling, and perhaps on BP control. Furthermore, the formation of connective tissue cells from MSCs is being explored for an application in tissue engineering related to wound healing and repair of cartilage [193]. This may represent a novel research field in which to explore the role of the RAS. It will be a major challenge to chart the versatile effects of AngII and possibly also other angiotensins, such as Ang-(1–7), on this route of MSC differentiation.

FUNDING

B.S.P. and A.J.M.R. thank the Netherlands Heart Foundation [grant number 2010B009] for supporting their research into the effects of Ang-(1–7) on angiogenic progenitor cells.

REFERENCES

- 1 Paulis, L. and Unger, T. (2010) Novel therapeutic targets for hypertension. *Nat. Rev. Cardiol.* **7**, 431–441
- 2 Jones, E. S., Vinh, A., McCarthy, C. A., Gaspari, T. A. and Widdop, R. E. (2008) AT₂ receptors: functional relevance in cardiovascular disease. *Pharmacol. Ther.* **120**, 292–316
- 3 Santos, R. A., Simoes e Silva, A. C., Maric, C., Silva, D. M., Machado, R. P., de Buhr, I., Heringer-Walther, S., Pinheiro, S. V., Lopes, M. T., Bader, M. et al. (2003) Angiotensin-(1–7) is an endogenous ligand for the G protein-coupled receptor Mas. *Proc. Natl. Acad. Sci. U.S.A.* **100**, 8258–8263
- 4 Tipnis, S. R., Hooper, N. M., Hyde, R., Karran, E., Christie, G. and Turner, A. J. (2000) A human homolog of angiotensin-converting enzyme. Cloning and functional expression as a captopril-insensitive carboxypeptidase. *J. Biol. Chem.* **275**, 33238–33243
- 5 Donoghue, M., Hsieh, F., Baronas, E., Godbout, K., Gosselin, M., Stagliano, N., Donovan, M., Woolf, B., Robison, K., Jeyaseelan, R., Breitbart, R. E. and Acton, S. (2000) A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1–9. *Circ. Res.* **87**, E1–E9
- 6 Iusuf, D., Henning, R. H., van Gilst, W. H. and Roks, A. J. (2008) Angiotensin-(1–7): pharmacological properties and pharmacotherapeutic perspectives. *Eur. J. Pharmacol.* **585**, 303–312
- 7 Luque, M., Martin, P., Martell, N., Fernandez, C., Brosnihan, K. B. and Ferrario, C. M. (1996) Effects of captopril related to increased levels of prostacyclin and angiotensin-(1–7) in essential hypertension. *J. Hypertens.* **14**, 799–805

- 8 Rabelo, L. A., Alenina, N. and Bader, M. (2011) ACE2-angiotensin-(1-7)-Mas axis and oxidative stress in cardiovascular disease. *Hypertens. Res.* **34**, 154–160
- 9 Steckelings, U. M., Larhed, M., Hallberg, A., Widdop, R. E., Jones, E. S., Wallinder, C., Namsolleck, P., Dahlof, B. and Unger, T. (2011) Non-peptide AT2-receptor agonists. *Curr. Opin. Pharmacol.* **11**, 187–192
- 10 Mohsin, S., Siddiqi, S., Collins, B. and Sussman, M. A. (2011) Empowering adult stem cells for myocardial regeneration. *Circ. Res.* **109**, 1415–1428
- 11 Strauer, B. E. and Steinhoff, G. (2011) 10 years of intracoronary and intramyocardial bone marrow stem cell therapy of the heart: from the methodological origin to clinical practice. *J. Am. Coll. Cardiol.* **58**, 1095–1104
- 12 Donndorf, P., Strauer, B. E. and Steinhoff, G. (2012) Update on cardiac stem cell therapy in heart failure. *Curr. Opin. Cardiol.* **27**, 154–160
- 13 Griffing, G. T. and Melby, J. C. (1982) Enalapril (MK-421) and the white cell count and haematocrit. *Lancet* **i**, 1361
- 14 Studer, A. and Vetter, W. (1982) Reversible leucopenia associated with angiotensin-converting-enzyme inhibitor MK 421. *Lancet* **i**, 458
- 15 Strawn, W. B., Richmond, R. S., Ann Tallant, E., 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
- 16 Agbulut, O., Menot, M. L., Li, Z., Marotte, F., Paulin, D., Hagege, A. A., Chomienne, C., Samuel, J. L. and Menasche, P. (2003) Temporal patterns of bone marrow cell differentiation following transplantation in doxorubicin-induced cardiomyopathy. *Cardiovasc. Res.* **58**, 451–459
- 17 Balsam, L. B., Wagers, A. J., Christensen, J. L., Kofidis, T., Weissman, I. L. and Robbins, R. C. (2004) Haematopoietic stem cells adopt mature haematopoietic fates in ischaemic myocardium. *Nature* **428**, 668–673
- 18 Davani, S., Marandin, A., Mersin, N., Royer, B., Kantelip, B., Herve, P., Etievent, J. P. and Kantelip, J. P. (2003) Mesenchymal progenitor cells differentiate into an endothelial phenotype, enhance vascular density, and improve heart function in a rat cellular cardiomyoplasty model. *Circulation* **108** (Suppl. 1), II253–II258
- 19 Jackson, K. A., Majka, S. M., Wang, H., Pocius, J., Hartley, C. J., Majesky, M. W., Entman, M. L., Michael, L. H., Hirschi, K. K. and Goodell, M. A. (2001) Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells. *J. Clin. Invest.* **107**, 1395–1402
- 20 Kajstura, J., Rota, M., Whang, B., Cascapera, S., Hosoda, T., Bearzi, C., Nurzynska, D., Kasahara, H., Zias, E., Bonafe, M. et al. (2005) Bone marrow cells differentiate in cardiac cell lineages after infarction independently of cell fusion. *Circ. Res.* **96**, 127–137
- 21 Kudo, M., Wang, Y., Wani, M. A., Xu, M., Ayub, A. and Ashraf, M. (2003) Implantation of bone marrow stem cells reduces the infarction and fibrosis in ischemic mouse heart. *J. Mol. Cell. Cardiol.* **35**, 1113–1119
- 22 Nygren, J. M., Jovinge, S., Breitbach, M., Sawen, P., Roll, W., Hescheler, J., Taneera, J., Fleischmann, B. K. and Jacobsen, S. E. (2004) Bone marrow-derived hematopoietic cells generate cardiomyocytes at a low frequency through cell fusion, but not transdifferentiation. *Nat. Med.* **10**, 494–501
- 23 Orlic, D., Kajstura, J., Chimenti, S., Jakoniuk, I., Anderson, S. M., Li, B., Pickel, J., McKay, R., Nadal-Ginard, B., Bodine, D. M. et al. (2001) Bone marrow cells regenerate infarcted myocardium. *Nature* **410**, 701–705
- 24 Toma, C., Pittenger, M. F., Cahill, K. S., Byrne, B. J. and Kessler, P. D. (2002) Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart. *Circulation* **105**, 93–98
- 25 Uemura, R., Xu, M., Ahmad, N. and Ashraf, M. (2006) Bone marrow stem cells prevent left ventricular remodeling of ischemic heart through paracrine signaling. *Circ. Res.* **98**, 1414–1421
- 26 Yang, J., Zhou, W., Zheng, W., Ma, Y., Lin, L., Tang, T., Liu, J., Yu, J., Zhou, X. and Hu, J. (2007) Effects of myocardial transplantation of marrow mesenchymal stem cells transfected with vascular endothelial growth factor for the improvement of heart function and angiogenesis after myocardial infarction. *Cardiology* **107**, 17–29
- 27 Roks, A. J., Rodgers, K. and Walther, T. (2011) Effects of the renin angiotensin system on vasculogenesis-related progenitor cells. *Curr. Opin. Pharmacol.* **11**, 162–174
- 28 Qian, C., Schoemaker, R. G., van Gilst, W. H. and Roks, A. J. (2009) The role of the renin-angiotensin-aldosterone system in cardiovascular progenitor cell function. *Clin. Sci.* **116**, 301–314
- 29 Shen, X. Z. and Bernstein, K. E. (2011) The peptide network regulated by angiotensin converting enzyme (ACE) in hematopoiesis. *Cell Cycle* **10**, 1363–1369
- 30 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
- 31 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
- 32 Doan, T. N., Gletsu, N., Cole, J. and Bernstein, K. E. (2001) Genetic manipulation of the renin-angiotensin system. *Curr. Opin. Nephrol. Hypertens.* **10**, 483–491
- 33 Naito, M., Kawashima, A., Akiba, T., Takanashi, M. and Nihei, H. (2003) Effects of an angiotensin II receptor antagonist and angiotensin-converting enzyme inhibitors on burst forming units-erythroid in chronic hemodialysis patients. *Am. J. Nephrol.* **23**, 287–293
- 34 Freudenthaler, S. M., Schreeb, K., Korner, T. and Gleiter, C. H. (1999) Angiotensin II increases erythropoietin production in healthy human volunteers. *Eur. J. Clin. Invest.* **29**, 816–823
- 35 Nakamoto, H., Kanno, Y., Okada, H. and Suzuki, H. (2004) Erythropoietin resistance in patients on continuous ambulatory peritoneal dialysis. *Adv. Perit. Dial.* **20**, 111–116
- 36 Chew, C. G., Weise, M. D. and Disney, A. P. (1999) The effect of angiotensin II receptor antagonist on the exogenous erythropoietin requirement of haemodialysis patients. *Nephrol. Dial. Transplant.* **14**, 2047–2049
- 37 Julian, B. A., Gaston, R. S., Barker, C. V., Krystal, G., Diethelm, A. G. and Curtis, J. J. (1994) Erythropoiesis after withdrawal of enalapril in post-transplant erythrocytosis. *Kidney Int.* **46**, 1397–1403
- 38 Marrero, M. B., Schieffer, B., Paxton, W. G., Heerdt, L., Berk, B. C., Delafontaine, P. and Bernstein, K. E. (1995) Direct stimulation of Jak/STAT pathway by the angiotensin II AT1 receptor. *Nature* **375**, 247–250
- 39 Remy, I., Wilson, I. A. and Michnick, S. W. (1999) Erythropoietin receptor activation by a ligand-induced conformation change. *Science* **283**, 990–993
- 40 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
- 41 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
- 42 Lin, C., Datta, V., Okwan-Duodu, D., Chen, X., Fuchs, S., Alsabeh, R., Billet, S., Bernstein, K. E. and Shen, X. Z. (2011) Angiotensin-converting enzyme is required for normal myelopoiesis. *FASEB J.* **25**, 1145–1155
- 43 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
- 44 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 AT1 receptor regulates differentiation of monocyte lineage progenitors from hematopoietic stem cells. *Arterioscler. Thromb., Vasc. Biol.* **29**, 1529–1536

- 45 Rodgers, K. E., Xiong, S. and diZerega, G. S. (2002) Accelerated recovery from irradiation injury by angiotensin. *Peptides Cancer Chemother. Pharmacol.* **49**, 403–411
- 46 Nahmod, K. A., Vermeulen, M. E., Raiden, S., Salamone, G., Gamberale, R., Fernandez-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
- 47 Shao, J., Nangaku, M., Miyata, T., Inagi, R., Yamada, K., Kurokawa, K. and Fujita, T. (2003) Imbalance of T-cell subsets in angiotensin II-infused hypertensive rats with kidney injury. *Hypertension* **42**, 31–38
- 48 Benigni, A., Cassis, P. and Remuzzi, G. (2010) Angiotensin II revisited: new roles in inflammation, immunology and aging. *EMBO Mol. Med.* **2**, 247–257
- 49 Marchesi, C., Paradis, P. and Schiffrin, E. L. (2008) Role of the renin-angiotensin system in vascular inflammation. *Trends Pharmacol. Sci.* **29**, 367–374
- 50 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
- 51 Heringer-Walther, S., Eckert, K., Schumacher, S. M., Uharek, L., Wulf-Goldenberg, A., Gemhardt, 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
- 52 Mordwinkin, N. M., Russell, J. R., Burke, A. S., Dizerega, G. S., Louie, S. G. and Rodgers, K. E. (2011) Toxicological and toxicokinetic analysis of angiotensin (1–7) in two species. *J. Pharm. Sci.* **101**, 373–380
- 53 Wang, Y., Qian, C., Roks, A. J., Westermann, D., Schumacher, S. M., Escher, F., Schoemaker, R. G., Reudelhuber, T. L., van Gilst, W. H., Schultheiss, H. P. et al. (2010) Circulating rather than cardiac angiotensin-(1–7) stimulates cardioprotection after myocardial infarction. *Circ. Heart Fail.* **3**, 286–293
- 54 Qian, C., Schoemaker, R. G., van Gilst, W. H., Yu, B. and Roks, A. J. (2008) Regenerative cell therapy and pharmacotherapeutic intervention in heart failure. Part 1: cardiovascular progenitor cells, their functions and sources. *Neth. Heart J.* **16**, 305–309
- 55 Qian, C., Schoemaker, R. G., van Gilst, W. H., Yu, B. and Roks, A. J. (2008) Regenerative cell therapy and pharmacotherapeutic intervention in heart failure. Part 2: pharmacological targets, agents and intervention perspectives. *Neth. Heart J.* **16**, 337–343
- 56 Asahara, T., Murohara, T., Sullivan, A., Silver, M., van der Zee, R., Li, T., Witztzenbichler, B., Schatteman, G. and Isner, J. M. (1997) Isolation of putative progenitor endothelial cells for angiogenesis. *Science* **275**, 964–967
- 57 Li Calzi, S., Neu, M. B., Shaw, L. C., Kielczewski, J. L., Moldovan, N. I. and Grant, M. B. (2010) EPCs and pathological angiogenesis: when good cells go bad. *Microvasc. Res.* **79**, 207–216
- 58 Hur, J., Yoon, C. H., Kim, H. S., Choi, J. H., Kang, H. J., Hwang, K. K., Oh, B. H., Lee, M. M. and Park, Y. B. (2004) Characterization of two types of endothelial progenitor cells and their different contributions to neovascularization. *Arterioscler., Thromb., Vasc. Biol.* **24**, 288–293
- 59 Gulati, R., Jevremovic, D., Peterson, T. E., Chatterjee, S., Shah, V., Vile, R. G. and Simari, R. D. (2003) Diverse origin and function of cells with endothelial phenotype obtained from adult human blood. *Circ. Res.* **93**, 1023–1025
- 60 Hill, J. M., Zalos, G., Halcox, J. P., Schenke, W. H., Waclawiw, M. A., Quyyumi, A. A. and Finkel, T. (2003) Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. *N. Engl. J. Med.* **348**, 593–600
- 61 Lin, Y., Weisdorf, D. J., Solovey, A. and Hebbel, R. P. (2000) Origins of circulating endothelial cells and endothelial outgrowth from blood. *J. Clin. Invest.* **105**, 71–77
- 62 Yoder, M. C., Mead, L. E., Prater, D., Krier, T. R., Mroueh, K. N., Li, F., Krasich, R., Temm, C. J., Prchal, J. T. and Ingram, D. A. (2007) Redefining endothelial progenitor cells via clonal analysis and hematopoietic stem/progenitor cell principals. *Blood* **109**, 1801–1809
- 63 Sirker, A. A., Astroulakis, Z. M. and Hill, J. M. (2009) Vascular progenitor cells and translational research: the role of endothelial and smooth muscle progenitor cells in endogenous arterial remodelling in the adult. *Clin. Sci.* **116**, 283–299
- 64 Richardson, M. R. and Yoder, M. C. (2011) Endothelial progenitor cells: quo vadis? *J. Mol. Cell. Cardiol.* **50**, 266–272
- 65 Shaw, S. Y., Cheng, S., Cupples, L. A., Larson, M. G., McCabe, E. L., Ngwa, J. S., Wang, Y. A., Martin, R. P., Klein, R. J., Hashmi, B. et al. (2011) Genetic and clinical correlates of early-outgrowth colony-forming units. *Circ. Cardiovasc. Genet.* **4**, 296–304
- 66 Cheng, S., Cohen, K. S., Shaw, S. Y., Larson, M. G., Hwang, S. J., McCabe, E. L., Martin, R. P., Klein, R. J., Hashmi, B., Hoffmann, U. et al. (2010) Association of colony-forming units with coronary artery and abdominal aortic calcification. *Circulation* **122**, 1176–1182
- 67 Vasa, M., Fichtlscherer, S., Aicher, A., Adler, K., Urbich, C., Martin, H., Zeiher, A. M. and Dimmeler, S. (2001) Number and migratory activity of circulating endothelial progenitor cells inversely correlate with risk factors for coronary artery disease. *Circ. Res.* **89**, E1–E7
- 68 Liu, L., Wei, H., Chen, F., Wang, J., Dong, J. F. and Zhang, J. (2011) Endothelial progenitor cells correlate with clinical outcome of traumatic brain injury. *Crit. Care Med.* **39**, 1760–1765
- 69 Surdacki, A., Marewicz, E., Rakowski, T., Szastak, G., Wiecezorek-Surdacka, E., Chyrchel, B., Pryjma, J., Dudek, D. and Dubiel, J. S. (2011) Synergistic adverse prognostic effects of asymmetric dimethylarginine and endothelial progenitor-related cells deficiency after elective coronary angioplasty. *Int. J. Cardiol.* **152**, 400–403
- 70 Briguori, C., Testa, U., Riccioni, R., Colombo, A., Petrucci, E., Condorelli, G., Mariani, G., D'Andrea, D., De Micco, F., Rivera, N. V., Puca, A. A. et al. (2010) Correlations between progression of coronary artery disease and circulating endothelial progenitor cells. *FASEB J.* **24**, 1981–1988
- 71 Alev, C., Ii, M. and Asahara, T. (2011) Endothelial progenitor cells: a novel tool for the therapy of ischemic diseases. *Antioxid. Redox Signaling* **15**, 949–965
- 72 Salguero, G., Akin, E., Templin, C., Kotlarz, D., Doerries, C., Landmesser, U., Grote, K. and Schieffer, B. (2008) Renovascular hypertension by two-kidney one-clip enhances endothelial progenitor cell mobilization in a p47phox-dependent manner. *J. Hypertens.* **26**, 257–268
- 73 Imanishi, T., Hano, T. and Nishio, I. (2004) Angiotensin II potentiates vascular endothelial growth factor-induced proliferation and network formation of endothelial progenitor cells. *Hypertens. Res.* **27**, 101–108
- 74 Yin, T., Ma, X., Zhao, L., Cheng, K. and Wang, H. (2008) Angiotensin II promotes NO production, inhibits apoptosis and enhances adhesion potential of bone marrow-derived endothelial progenitor cells. *Cell Res.* **18**, 792–799
- 75 Roks, A. J. (2011) Angiotensin II deteriorates endothelial progenitor cells: good intentions with bad consequences. *Hypertension* **58**, 356–358
- 76 Endtmann, C., Ebrahimian, T., Czech, T., Arfa, O., Laufs, U., Fritz, M., Wassmann, K., Werner, N., Petoumenos, V., Nickenig, G. and Wassmann, S. (2011) Angiotensin II impairs endothelial progenitor cell number and function *in vitro* and *in vivo*: implications for vascular regeneration. *Hypertension* **58**, 394–403
- 77 Kobayashi, K., Imanishi, T. and Akasaka, T. (2006) Endothelial progenitor cell differentiation and senescence in an angiotensin II-infusion rat model. *Hypertens. Res.* **29**, 449–455
- 78 Imanishi, T., Hano, T. and Nishio, I. (2005) Angiotensin II accelerates endothelial progenitor cell senescence through induction of oxidative stress. *J. Hypertens.* **23**, 97–104

- 79 Min, T. Q., Zhu, C. J., Xiang, W. X., Hui, Z. J. and Peng, S. Y. (2004) Improvement in endothelial progenitor cells from peripheral blood by ramipril therapy in patients with stable coronary artery disease. *Cardiovasc. Drugs Ther.* **18**, 203–209
- 80 Sampaio, W. O., Souza dos Santos, R. A., Faria-Silva, R., da Mata Machado, L. T., Schiffrin, E. L. and Touyz, R. M. (2007) Angiotensin-(1–7) through receptor Mas mediates endothelial nitric oxide synthase activation via Akt-dependent pathways. *Hypertension* **49**, 185–192
- 81 Faria-Silva, R., Duarte, F. V. and Santos, R. A. (2005) Short-term angiotensin(1–7) receptor MAS stimulation improves endothelial function in normotensive rats. *Hypertension* **46**, 948–952
- 82 Heitsch, H., Brovkovych, S., Malinski, T. and Wiemer, G. (2001) Angiotensin-(1–7)-stimulated nitric oxide and superoxide release from endothelial cells. *Hypertension* **37**, 72–76
- 83 Costa, M. A., Lopez Verrilli, M. A., Gomez, K. A., Nakagawa, P., Pena, C., Arranz, C. and Gironacci, M. M. (2010) Angiotensin-(1–7) upregulates cardiac nitric oxide synthase in spontaneously hypertensive rats. *Am. J. Physiol. Heart Circ. Physiol.* **299**, H1205–H1211
- 84 Loot, A. E., Roks, A. J., Henning, R. H., Tio, R. A., Suurmeijer, A. J., Boomsma, F. and van Gilst, W. H. (2002) Angiotensin-(1–7) attenuates the development of heart failure after myocardial infarction in rats. *Circulation* **105**, 1548–1550
- 85 Benter, I. F., Yousif, M. H., Anim, J. T., Cojocel, C. and Diz, D. I. (2006) Angiotensin-(1–7) prevents development of severe hypertension and end-organ damage in spontaneously hypertensive rats treated with L-NAME. *Am. J. Physiol. Heart Circ. Physiol.* **290**, H684–H691
- 86 Rabelo, L. A., Xu, P., Todiras, M., Sampaio, W. O., Buttgerit, J., Bader, M., Santos, R. A. and Alenina, N. (2008) Ablation of angiotensin (1–7) receptor Mas in C57Bl/6 mice causes endothelial dysfunction. *J. Am. Soc. Hypertens.* **2**, 418–424
- 87 Tesanovic, S., Vinh, A., Gaspari, T. A., Casley, D. and Widdop, R. E. (2010) Vasoprotective and atheroprotective effects of angiotensin (1–7) in apolipoprotein E-deficient mice. *Arterioscler., Thromb., Vasc. Biol.* **30**, 1606–1613
- 88 Silva, D. M., Gomes-Filho, A., Olivon, V. C., Santos, T. M., Becker, L. K., Santos, R. A. and Lemos, V. S. (2011) Swimming training improves the vasodilator effect of angiotensin-(1–7) in the aorta of spontaneously hypertensive rat. *J. Appl. Physiol.* **111**, 1272–1277
- 89 Ren, Y., Garvin, J. and Carretero, O. A. (2002) Mechanism involved in bradykinin-induced efferent arteriole dilation. *Kidney Int.* **62**, 544–549
- 90 Porsti, I., Bara, A. T., Busse, R. and Hecker, M. (1994) Release of nitric oxide by angiotensin-(1–7) from porcine coronary endothelium: implications for a novel angiotensin receptor. *Br. J. Pharmacol.* **111**, 652–654
- 91 le Tran, Y. and Forster, C. (1997) Angiotensin-(1–7) and the rat aorta: modulation by the endothelium. *J. Cardiovasc. Pharmacol.* **30**, 676–682
- 92 Feterik, K., Smith, L. and Katusic, Z. S. (2000) Angiotensin-(1–7) causes endothelium-dependent relaxation in canine middle cerebral artery. *Brain Res.* **873**, 75–82
- 93 Stegbauer, J., Potthoff, S. A., Quack, I., Mergia, E., Clasen, T., Friedrich, S., Vonend, O., Woznowski, M., Konigshausen, E., Sellin, L. and Rump, L. C. (2011) Chronic treatment with angiotensin-(1–7) improves renal endothelial dysfunction in apolipoproteinE-deficient mice. *Br. J. Pharmacol.* **163**, 974–983
- 94 Benter, I. F., Yousif, M. H., Dhaunsi, G. S., Kaur, J., Chappell, M. C. and Diz, D. I. (2008) Angiotensin-(1–7) prevents activation of NADPH oxidase and renal vascular dysfunction in diabetic hypertensive rats. *Am. J. Nephrol.* **28**, 25–33
- 95 Sampaio, W. O., Henrique de Castro, C., Santos, R. A., Schiffrin, E. L. and Touyz, R. M. (2007) Angiotensin-(1–7) counterregulates angiotensin II signaling in human endothelial cells. *Hypertension* **50**, 1093–1098
- 96 Bodiga, S., Zhong, J. C., Wang, W., Basu, R., Lo, J., Liu, G. C., Guo, D., Holland, S. M., Scholey, J. W., Penninger, J. M. et al. (2011) Enhanced susceptibility to biomechanical stress in ACE2 null mice is prevented by loss of the p47^{phox} NADPH oxidase subunit. *Cardiovasc. Res.* **91**, 151–161
- 97 Machado, R. D., Santos, R. A. and Andrade, S. P. (2000) Opposing actions of angiotensins on angiogenesis. *Life Sci.* **66**, 67–76
- 98 Machado, R. D., Santos, R. A. and Andrade, S. P. (2001) Mechanisms of angiotensin-(1–7)-induced inhibition of angiogenesis. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **280**, R994–R1000
- 99 Petty, W. J., Miller, A. A., McCoy, T. P., Gallagher, P. E., Tallant, E. A. and Torti, F. M. (2009) Phase I and pharmacokinetic study of angiotensin-(1–7), an endogenous antiangiogenic hormone. *Clin. Cancer Res.* **15**, 7398–7404
- 100 Soto-Pantoja, D. R., Menon, J., Gallagher, P. E. and Tallant, E. A. (2009) Angiotensin-(1–7) inhibits tumor angiogenesis in human lung cancer xenografts with a reduction in vascular endothelial growth factor. *Mol. Cancer Ther.* **8**, 1676–1683
- 101 Anton, L., Merrill, D. C., Neves, L. A. and Brosnihan, K. B. (2007) Angiotensin-(1–7) inhibits *in vitro* endothelial cell tube formation in human umbilical vein endothelial cells through the AT(1–7) receptor. *Endocrine* **32**, 212–218
- 102 Menon, J., Soto-Pantoja, D. R., Callahan, M. F., Cline, J. M., Ferrario, C. M., Tallant, E. A. and Gallagher, P. E. (2007) Angiotensin-(1–7) inhibits growth of human lung adenocarcinoma xenografts in nude mice through a reduction in cyclooxygenase-2. *Cancer Res.* **67**, 2809–2815
- 103 Kostenis, E., Milligan, G., Christopoulos, A., Sanchez-Ferrer, C., Heringer-Walther, S., Sexton, P., Gemhardt, F., Kellett, E., Martini, L., Vanderheyden, P., Schultheiss, H. and Walther, T. (2005) G-protein-coupled receptor Mas is a physiological antagonist of the angiotensin II type 1 receptor. *Circulation* **111**, 1806–1813
- 104 Bussolati, B., Grange, C. and Camussi, G. (2011) Tumor exploits alternative strategies to achieve vascularization. *FASEB J.* **25**, 2874–2882
- 105 van Beusekom, H. M., Schoemaker, R., Roks, A. J., Zijlstra, F. and van der Giessen, W. J. (2007) Coronary stent healing, endothelialisation and the role of co-medication. *Neth. Heart J.* **15**, 395–396
- 106 Gironacci, M. M., Adamo, H. P., Corradi, G., Santos, R. A., Ortiz, P. and Carretero, O. A. (2011) Angiotensin (1–7) induces MAS receptor internalization. *Hypertension* **58**, 176–181
- 107 Silvestre, J. S., Tamarat, R., Senbonmatsu, T., Icchiki, T., Ebrahimian, T., Iglarz, M., Besnard, S., Duriez, M., Inagami, T. and Levy, B. I. (2002) Antiangiogenic effect of angiotensin II type 2 receptor in ischemia-induced angiogenesis in mice hindlimb. *Circ. Res.* **90**, 1072–1079
- 108 Yamada, K., Iyer, S. N., Chappell, M. C., Ganten, D. and Ferrario, C. M. (1998) Converting enzyme determines plasma clearance of angiotensin-(1–7). *Hypertension* **32**, 496–502
- 109 Yu, Y., Fukuda, N., Yao, E. H., Matsumoto, T., Kobayashi, N., Suzuki, R., Tahira, Y., Ueno, T. and Matsumoto, K. (2008) Effects of an ARB on endothelial progenitor cell function and cardiovascular oxidation in hypertension. *Am. J. Hypertens.* **21**, 72–77
- 110 Yoshida, Y., Fukuda, N., Maeshima, A., Yamamoto, C., Matsumoto, T., Ueno, T., Nojima, Y., Matsumoto, K. and Soma, M. (2011) Treatment with valsartan stimulates endothelial progenitor cells and renal label-retaining cells in hypertensive rats. *J. Hypertens.* **29**, 91–101
- 111 Yao, E. H., Fukuda, N., Matsumoto, T., Kobayashi, N., Katakawa, M., Yamamoto, C., Tsunemi, A., Suzuki, R., Ueno, T. and Matsumoto, K. (2007) Losartan improves the impaired function of endothelial progenitor cells in hypertension via an antioxidant effect. *Hypertens. Res.* **30**, 1119–1128
- 112 Yu, Y., Wang, Y., Zhou, L. N. and Zheng, F. (2011) ARB treatment prevents the decrease in endothelial progenitor cells and the loss of renal microvasculature in remnant kidney. *Am. J. Nephrol.* **33**, 550–557

- 113 Thum, T., Fraccarollo, D., Galuppo, P., Tsikas, D., Frantz, S., Ertl, G. and Bauersachs, J. (2006) Bone marrow molecular alterations after myocardial infarction: impact on endothelial progenitor cells. *Cardiovasc. Res.* **70**, 50–60
- 114 Cheng, X. W., Song, H., Sasaki, T., Hu, L., Inoue, A., Bando, Y. K., Shi, G. P., Kuzuya, M., Okumura, K. and Murohara, T. (2011) Angiotensin type 1 receptor blocker reduces intimal neovascularization and plaque growth in apolipoprotein E-deficient mice. *Hypertension* **57**, 981–989
- 115 You, D., Cochain, C., Loinard, C., Vilar, J., Mees, B., Duriez, M., Levy, B. I. and Silvestre, J. S. (2008) Hypertension impairs postnatal vasculogenesis: role of antihypertensive agents. *Hypertension* **51**, 1537–1544
- 116 Wang, C. H., Verma, S., Hsieh, I. C., Chen, Y. J., Kuo, L. T., Yang, N. I., Wang, S. Y., Wu, M. Y., Hsu, C. M., Cheng, C. W. and Cherng, W. J. (2006) Enalapril increases ischemia-induced endothelial progenitor cell mobilization through manipulation of the CD26 system. *J. Mol. Cell. Cardiol.* **41**, 34–43
- 117 Bahlmann, F. H., de Groot, K., Mueller, O., Hertel, B., Haller, H. and Fliser, D. (2005) Stimulation of endothelial progenitor cells: a new putative therapeutic effect of angiotensin II receptor antagonists. *Hypertension* **45**, 526–529
- 118 Reinhard, H., Jacobsen, P. K., Lajer, M., Pedersen, N., Billestrup, N., Mandrup-Poulsen, T., Parving, H. H. and Rossing, P. (2010) Multifactorial treatment increases endothelial progenitor cells in patients with type 2 diabetes. *Diabetologia* **53**, 2129–2133
- 119 Pelliccia, F., Pasceri, V., Cianfrocca, C., Vitale, C., Speciale, G., Gaudio, C., Rosano, G. M. and Mercuro, G. (2010) Angiotensin II receptor antagonism with telmisartan increases number of endothelial progenitor cells in normotensive patients with coronary artery disease: a randomized, double-blind, placebo-controlled study. *Atherosclerosis* **210**, 510–515
- 120 Porto, I., Di Vito, L., De Maria, G. L., Dato, I., Tritarelli, A., Leone, A. M., Niccoli, G., Capogrossi, M. C., Biasucci, L. M. and Crea, F. (2009) Comparison of the effects of ramipril versus telmisartan on high-sensitivity C-reactive protein and endothelial progenitor cells after acute coronary syndrome. *Am. J. Cardiol.* **103**, 1500–1505
- 121 Townamchai, N., Praditpornsilpa, K. and Eiam-Ong, S. (2010) Endothelial progenitor cells in Asian kidney transplant patients. *Transplant Proc.* **42**, 1690–1694
- 122 Honda, A., Matsuura, K., Fukushima, N., Tsurumi, Y., Kasanuki, H. and Hagiwara, N. (2009) Telmisartan induces proliferation of human endothelial progenitor cells via PPAR γ -dependent PI3K/Akt pathway. *Atherosclerosis* **205**, 376–384
- 123 Steinmetz, M., Brouwers, C., Nickenig, G. and Wassmann, S. (2010) Synergistic effects of telmisartan and simvastatin on endothelial progenitor cells. *J. Cell. Mol. Med.* **14**, 1645–1656
- 124 Yusuf, S., Sleight, P., Pogue, J., Bosch, J., Davies, R. and Dagenais, G. (2000) Effects of an angiotensin-converting-enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients. *N. Engl. J. Med.* **342**, 145–153
- 125 Hansson, L., Lindholm, L. H., Niskanen, L., Lanke, J., Hedner, T., Niklason, A., Luomanmaki, K., Dahlöf, B., de Faire, U., Morlin, C. et al. (1999) Effect of angiotensin-converting-enzyme inhibition compared with conventional therapy on cardiovascular morbidity and mortality in hypertension: the Captopril Prevention Project (CAPPP) randomised trial. *Lancet* **353**, 611–616
- 126 Hansson, L., Lindholm, L. H., Ekblom, T., Dahlöf, B., Lanke, J., Schersten, B., Wester, P. O., Hedner, T. and de Faire, U. (1999) Randomised trial of old and new antihypertensive drugs in elderly patients: cardiovascular mortality and morbidity in the Swedish Trial in Old Patients with Hypertension-2 study. *Lancet* **354**, 1751–1756
- 127 The Acute Infarction Ramipril Efficacy (AIRE) Study Investigators (1993) Effect of ramipril on mortality and morbidity of survivors of acute myocardial infarction with clinical evidence of heart failure. *Lancet* **342**, 821–828
- 128 Schartl, M., Bocksch, W. G., Dreyse, S., Beckmann, S., Franke, O. and Hunten, U. (1994) Remodeling of myocardium and arteries by chronic angiotensin converting enzyme inhibition in hypertensive patients. *J. Hypertens. Suppl.* **12**, S37–S42
- 129 Mancini, G. B., Henry, G. C., Macaya, C., O'Neill, B. J., Pucillo, A. L., Carere, R. G., Wargovich, T. J., Mudra, H., Luscher, T. F., Klibaner, M. I. et al. (1996) Angiotensin-converting enzyme inhibition with quinapril improves endothelial vasomotor dysfunction in patients with coronary artery disease. The TREND (Trial on Reversing ENdothelial Dysfunction) Study. *Circulation* **94**, 258–265
- 130 Cacciatore, F., Bruzzese, G., Vitale, D. F., Liguori, A., de Nigris, F., Fiorito, C., Infante, T., Donatelli, F., Minucci, P. B., Ignarro, L. J. and Napoli, C. (2011) Effects of ACE inhibition on circulating endothelial progenitor cells, vascular damage, and oxidative stress in hypertensive patients. *Eur. J. Clin. Pharmacol.* **67**, 877–883
- 131 Cangiano, E., Marchesini, J., Campo, G., Francolini, G., Fortini, C., Carra, G., Miccoli, M., Ceconi, C., Tavazzi, L. and Ferrari, R. (2011) ACE inhibition modulates endothelial apoptosis and renewal via endothelial progenitor cells in patients with acute coronary syndromes. *Am. J. Cardiovasc. Drugs.* **11**, 189–198
- 132 Muller, P., Kazakov, A., Jagoda, P., Semenov, A., Bohm, M. and Laufs, U. (2009) ACE inhibition promotes upregulation of endothelial progenitor cells and neoangiogenesis in cardiac pressure overload. *Cardiovasc. Res.* **83**, 106–114
- 133 You, D., Cochain, C., Loinard, C., Vilar, J., Mees, B., Duriez, M., Levy, B. I. and Silvestre, J. S. (2008) Combination of the angiotensin-converting enzyme inhibitor perindopril and the diuretic indapamide activate postnatal vasculogenesis in spontaneously hypertensive rats. *J. Pharmacol. Exp. Ther.* **325**, 766–773
- 134 Sakai, N., Wada, T., Matsushima, K., Bucala, R., Iwai, M., Horiuchi, M. and Kaneko, S. (2008) The renin-angiotensin system contributes to renal fibrosis through regulation of fibrocytes. *J. Hypertens.* **26**, 780–790
- 135 Chen, G., Lin, S. C., Chen, J., He, L., Dong, F., Xu, J., Han, S., Du, J., Entman, M. L. and Wang, Y. (2011) CXCL16 recruits bone marrow-derived fibroblast precursors in renal fibrosis. *J. Am. Soc. Nephrol.* **22**, 1876–1886
- 136 Xu, J., Lin, S. C., Chen, J., Miao, Y., Taffet, G. E., Entman, M. L. and Wang, Y. (2011) CCR2 mediates the uptake of bone marrow-derived fibroblast precursors in angiotensin II-induced cardiac fibrosis. *Am. J. Physiol. Heart Circ. Physiol.* **301**, H538–H547
- 137 Sopol, M. J., Rosin, N. L., Lee, T. D. and Legare, J. F. (2011) Myocardial fibrosis in response to angiotensin II is preceded by the recruitment of mesenchymal progenitor cells. *Lab. Invest.* **91**, 565–578
- 138 Makino, S., Fukuda, K., Miyoshi, S., Konishi, F., Kodama, H., Pan, J., Sano, M., Takahashi, T., Hori, S., Abe, H., Hata, J., Umezawa, A. and Ogawa, S. (1999) Cardiomyocytes can be generated from marrow stromal cells *in vitro*. *J. Clin. Invest.* **103**, 697–705
- 139 Abdel-Latif, A., Bolli, R., Tleyjeh, I. M., Montori, V. M., Perin, E. C., Hornung, C. A., Zuba-Surma, E. K., Al-Mallah, M. and Dawn, B. (2007) Adult bone marrow-derived cells for cardiac repair: a systematic review and meta-analysis. *Arch. Intern. Med.* **167**, 989–997
- 140 Dawn, B., Abdel-Latif, A., Sanganalmath, S. K., Flaherty, M. P. and Zuba-Surma, E. K. (2009) Cardiac repair with adult bone marrow-derived cells: the clinical evidence. *Antioxid. Redox Signaling* **11**, 1865–1882
- 141 Xing, Y., Lv, A., Wang, L. and Yan, X. (2012) The combination of angiotensin II and 5-azacytidine promotes cardiomyocyte differentiation of rat bone marrow mesenchymal stem cells. *Mol. Cell. Biochem.* **360**, 279–287
- 142 Numasawa, Y., Kimura, T., Miyoshi, S., Nishiyama, N., Hida, N., Tsuji, H., Tsuruta, H., Segawa, K., Ogawa, S. and Umezawa, A. (2011) Treatment of human mesenchymal stem cells with angiotensin receptor blocker improved efficiency of cardiomyogenic transdifferentiation and improved cardiac function via angiogenesis. *Stem Cells* **29**, 1405–1414

- 143 Noiseux, N., Gneccchi, M., Lopez-Illasaca, M., Zhang, L., Solomon, S. D., Deb, A., Dzau, V. J. and Pratt, R. E. (2006) Mesenchymal stem cells overexpressing Akt dramatically repair infarcted myocardium and improve cardiac function despite infrequent cellular fusion or differentiation. *Mol. Ther.* **14**, 840–850
- 144 Gneccchi, M., He, H., Noiseux, N., Liang, O. D., Zhang, L., Morello, F., Mu, H., Melo, L. G., Pratt, R. E., Ingwall, J. S. and Dzau, V. J. (2006) Evidence supporting paracrine hypothesis for Akt-modified mesenchymal stem cell-mediated cardiac protection and functional improvement. *FASEB J.* **20**, 661–669
- 145 Iwanami, J., Mogi, M., Li, J. M., Tsukuda, K., Min, L. J., Sakata, A., Fujita, T., Iwai, M. and Horiuchi, M. (2008) Deletion of angiotensin II type 2 receptor attenuates protective effects of bone marrow stromal cell treatment on ischemia-reperfusion brain injury in mice. *Stroke* **39**, 2554–2559
- 146 Reinecke, K., Lucius, R., Reinecke, A., Rickert, U., Herdegen, T. and Unger, T. (2003) Angiotensin II accelerates functional recovery in the rat sciatic nerve *in vivo*: role of the AT2 receptor and the transcription factor NF- κ B. *FASEB J.* **17**, 2094–2096
- 147 Mogi, M., Li, J. M., Iwanami, J., Min, L. J., Tsukuda, K., Iwai, M. and Horiuchi, M. (2006) Angiotensin II type-2 receptor stimulation prevents neural damage by transcriptional activation of methyl methanesulfonate sensitive 2. *Hypertension* **48**, 141–148
- 148 Borlongan, C. V., Glover, L. E., Tajiri, N., Kaneko, Y. and Freeman, T. B. (2011) The great migration of bone marrow-derived stem cells toward the ischemic brain: therapeutic implications for stroke and other neurological disorders. *Prog. Neurobiol.* **95**, 213–228
- 149 Bang, O. Y., Lee, J. S., Lee, P. H. and Lee, G. (2005) Autologous mesenchymal stem cell transplantation in stroke patients. *Ann. Neurol.* **57**, 874–882
- 150 Iwai, M., Liu, H. W., Chen, R., Ide, A., Okamoto, S., Hata, R., Sakanaka, M., Shiuchi, T. and Horiuchi, M. (2004) Possible inhibition of focal cerebral ischemia by angiotensin II type 2 receptor stimulation. *Circulation* **110**, 843–848
- 151 Tsukuda, K., Mogi, M., Iwanami, J., Min, L. J., Jing, F., Oshima, K. and Horiuchi, M. (2011) Irbesartan attenuates ischemic brain damage by inhibition of MCP-1/CCR2 signaling pathway beyond AT receptor blockade. *Biochem. Biophys. Res. Commun.* **409**, 275–279
- 152 Dahlof, B., Devereux, R. B., Kjeldsen, S. E., Julius, S., Beevers, G., de Faire, U., Fyhrquist, F., Ibsen, H., Kristiansson, K., Lederballe-Pedersen, O. et al. (2002) Cardiovascular morbidity and mortality in the Losartan Intervention For Endpoint reduction in hypertension study (LIFE): a randomised trial against atenolol. *Lancet* **359**, 995–1003
- 153 Fagan, S. C., Kozak, A., Hill, W. D., Pollock, D. M., Xu, L., Johnson, M. H., Ergul, A. and Hess, D. C. (2006) Hypertension after experimental cerebral ischemia: candesartan provides neurovascular protection. *J. Hypertens.* **24**, 535–539
- 154 Kozak, A., Ergul, A., El-Remessy, A. B., Johnson, M. H., Machado, L. S., Elewa, H. F., Abdelsaid, M., Wiley, D. C. and Fagan, S. C. (2009) Candesartan augments ischemia-induced proangiogenic state and results in sustained improvement after stroke. *Stroke* **40**, 1870–1876
- 155 Kozak, W., Kozak, A., Johnson, M. H., Elewa, H. F. and Fagan, S. C. (2008) Vascular protection with candesartan after experimental acute stroke in hypertensive rats: a dose-response study. *J. Pharmacol. Exp. Ther.* **326**, 773–782
- 156 Sandset, E. C., Bath, P. M., Boysen, G., Jatuzis, D., Korv, J., Luders, S., Murray, G. D., Richter, P. S., Roine, R. O., Terent, A. et al. (2011) The angiotensin-receptor blocker candesartan for treatment of acute stroke (SCAST): a randomised, placebo-controlled, double-blind trial. *Lancet* **377**, 741–750
- 157 Cassis, L. A., Police, S. B., Yiannikouris, F. and Thatcher, S. E. (2008) Local adipose tissue renin-angiotensin system. *Curr. Hypertens. Rep.* **10**, 93–98
- 158 Thatcher, S., Yiannikouris, F., Gupte, M. and Cassis, L. (2009) The adipose renin-angiotensin system: role in cardiovascular disease. *Mol. Cell. Endocrinol.* **302**, 111–117
- 159 Okuno, A., Tamemoto, H., Tobe, K., Ueki, K., Mori, Y., Iwamoto, K., Umesono, K., Akanuma, Y., Fujiwara, T., Horikoshi, H. et al. (1998) Troglitazone increases the number of small adipocytes without the change of white adipose tissue mass in obese Zucker rats. *J. Clin. Invest.* **101**, 1354–1361
- 160 Matsushita, K., Wu, Y., 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
- 161 Schling, P. and Löffler, G. (2001) Effects of angiotensin II on adipose conversion and expression of genes of the renin-angiotensin system in human preadipocytes. *Horm. Metab. Res.* **33**, 189–195
- 162 Sharma, A. M., Janke, J., Gorzelnik, K., Engeli, S. and Luft, F. C. (2002) Angiotensin blockade prevents type 2 diabetes by formation of fat cells. *Hypertension* **40**, 609–611
- 163 Bujak-Gizycka, B., Madej, J., Wolkow, P. P., Olszanecki, R., Drabik, L., Rutowski, J. and Korbut, R. (2007) Measurement of angiotensin metabolites in organ bath and cell culture experiments by liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS). *J. Physiol. Pharmacol.* **58**, 529–540
- 164 Gupte, M., Boustany-Kari, C. M., Bharadwaj, K., Police, S., Thatcher, S., Gong, M. C., English, V. L. and Cassis, L. A. (2008) ACE2 is expressed in mouse adipocytes and regulated by a high-fat diet. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **295**, R781–R788
- 165 Santos, S. H., Fernandes, L. R., Mario, E. G., Ferreira, A. V., Porto, L. C., Alvarez-Leite, J. I., Botion, L. M., Bader, M., Alenina, N. and Santos, R. A. (2008) Mas deficiency in FVB/N mice produces marked changes in lipid and glycemic metabolism. *Diabetes* **57**, 340–347
- 166 Santos, S. H., Braga, J. F., Mario, E. G., Porto, L. C., Rodrigues-Machado Mda, G., Murari, A., Botion, L. M., Alenina, N., Bader, M. and Santos, R. A. (2010) Improved lipid and glucose metabolism in transgenic rats with increased circulating angiotensin-(1–7). *Arterioscler., Thromb., Vasc. Biol.* **30**, 953–961
- 167 Lee, R. M., Bader, M., Alenina, N., Santos, R. A., Gao, Y. J. and Lu, C. (2011) Mas receptors in modulating relaxation induced by perivascular adipose tissue. *Life Sci.* **89**, 467–472
- 168 Yamada, T., Kondo, T., Numaguchi, Y., Tsuzuki, M., Matsubara, T., Manabe, I., Sata, M., Nagai, R. and Murohara, T. (2007) Angiotensin II receptor blocker inhibits neointimal hyperplasia through regulation of smooth muscle-like progenitor cells. *Arterioscler., Thromb., Vasc. Biol.* **27**, 2363–2369
- 169 Kim, Y. M., Jeon, E. S., Kim, M. R., Jho, S. K., Ryu, S. W. and Kim, J. H. (2008) Angiotensin II-induced differentiation of adipose tissue-derived mesenchymal stem cells to smooth muscle-like cells. *Int. J. Biochem. Cell Biol.* **40**, 2482–2491
- 170 Ohtani, K., Egashira, K., Ihara, Y., Nakano, K., Funakoshi, K., Zhao, G., Sata, M. and Sunagawa, K. (2006) Angiotensin II type 1 receptor blockade attenuates in-stent restenosis by inhibiting inflammation and progenitor cells. *Hypertension* **48**, 664–670
- 171 Sata, M., Saiura, A., Kunisato, A., Tojo, A., Okada, S., Tokuhisa, T., Hirai, H., Makuuchi, M., Hirata, Y. and Nagai, R. (2002) Hematopoietic stem cells differentiate into vascular cells that participate in the pathogenesis of atherosclerosis. *Nat. Med.* **8**, 403–409
- 172 Zerneck, A., Schober, A., Bot, I., von Hundelshausen, P., Liehn, E. A., Mopps, B., Mericskay, M., Gierschik, P., Biessen, E. A. and Weber, C. (2005) SDF-1 α /CXCR4 axis is instrumental in neointimal hyperplasia and recruitment of smooth muscle progenitor cells. *Circ. Res.* **96**, 784–791

- 173 Yokoi, H., Yamada, H., Tsubakimoto, Y., Takata, H., Kawahito, H., Kishida, S., Kato, T., Matsui, A., Hirai, H., Ashihara, E. et al. (2010) Bone marrow AT1 augments neointima formation by promoting mobilization of smooth muscle progenitors via platelet-derived SDF-1 α . *Arterioscler., Thromb., Vasc. Biol.* **30**, 60–67
- 174 Yoon, Y. S., Wecker, A., Heyd, L., Park, J. S., Tkebuchava, T., Kusano, K., Hanley, A., Scadova, H., Qin, G., Cha, D. H. et al. (2005) Clonally expanded novel multipotent stem cells from human bone marrow regenerate myocardium after myocardial infarction. *J. Clin. Invest.* **115**, 326–338
- 175 Mangi, A. A., Noiseux, N., Kong, D., He, H., Rezvani, M., Ingwall, J. S. and Dzau, V. J. (2003) Mesenchymal stem cells modified with Akt prevent remodeling and restore performance of infarcted hearts. *Nat. Med.* **9**, 1195–1201
- 176 Galmiche, M. C., Koteliensky, V. E., Briere, J., Herve, P. and Charbord, P. (1993) Stromal cells from human long-term marrow cultures are mesenchymal cells that differentiate following a vascular smooth muscle differentiation pathway. *Blood* **82**, 66–76
- 177 Iwata, H., Manabe, I., Fujiu, K., Yamamoto, T., Takeda, N., Eguchi, K., Furuya, A., Kuro-o, M., Sata, M. and Nagai, R. (2010) Bone marrow-derived cells contribute to vascular inflammation but do not differentiate into smooth muscle cell lineages. *Circulation* **122**, 2048–2057
- 178 Groenewegen, H. C., Onuta, G., Goris, M., Zandvoort, A., Zijlstra, F., van Gilst, W. H., Rozing, J., de Smet, B. J., Roks, A. J. and Hillebrands, J. L. (2008) Non-bone marrow origin of neointimal smooth muscle cells in experimental in-stent restenosis in rats. *J. Vasc. Res.* **45**, 493–502
- 179 Zerneck, A., Bot, I., Djalali-Talab, Y., Shagdarsuren, E., Bidzhekov, K., Meiler, S., Krohn, R., Schober, A., Sperandio, M., Soehnlein, O. et al. (2008) Protective role of CXC receptor 4/CXC ligand 12 unveils the importance of neutrophils in atherosclerosis. *Circ. Res.* **102**, 209–217
- 180 Kinner, B., Zaleskas, J. M. and Spector, M. (2002) Regulation of smooth muscle actin expression and contraction in adult human mesenchymal stem cells. *Exp. Cell Res.* **278**, 72–83
- 181 Imasawa, T., Utsunomiya, Y., Kawamura, T., Zhong, Y., Nagasawa, R., Okabe, M., Maruyama, N., Hosoya, T. and Ohno, T. (2001) The potential of bone marrow-derived cells to differentiate to glomerular mesangial cells. *J. Am. Soc. Nephrol.* **12**, 1401–1409
- 182 Matsushita, K., Morello, F., Wu, Y., Zhang, L., Iwanaga, S., Pratt, R. E. and Dzau, V. J. (2010) Mesenchymal stem cells differentiate into renin-producing juxtaglomerular (JG)-like cells under the control of liver X receptor- α . *J. Biol. Chem.* **285**, 11974–11982
- 183 Marques, F. D., Ferreira, A. J., Sinisterra, R. D., Jacoby, B. A., Sousa, F. B., Caliani, M. V., Silva, G. A., Melo, M. B., Nadu, A. P., Souza, L. E., Irigoyen, M. C., Almeida, A. P. and Santos, R. A. (2011) An oral formulation of angiotensin-(1–7) produces cardioprotective effects in infarcted and isoproterenol-treated rats. *Hypertension* **57**, 477–483
- 184 Ferreira, A. J., Shenoy, V., Yamazato, Y., Sriramula, S., Francis, J., Yuan, L., Castellano, R. K., Ostrov, D. A., Oh, S. P., Katovich, M. J. and Raizada, M. K. (2009) Evidence for angiotensin-converting enzyme 2 as a therapeutic target for the prevention of pulmonary hypertension. *Am. J. Respir. Crit. Care Med.* **179**, 1048–1054
- 185 Savergnini, S. Q., Beiman, M., Lautner, R. Q., de Paula-Carvalho, V., Allahdadi, K., Pessoa, D. C., Costa-Fraga, F. P., Fraga-Silva, R. A., Cojocar, G., Cohen, Y. et al. (2010) Vascular relaxation, antihypertensive effect, and cardioprotection of a novel peptide agonist of the MAS receptor. *Hypertension* **56**, 112–120
- 186 Kluskens, L. D., Nelemans, S. A., Rink, R., de Vries, L., Meter-Arkema, A., Wang, Y., Walther, T., Kuipers, A., Moll, G. N. and Haas, M. (2009) Angiotensin-(1–7) with thioether bridge: an angiotensin-converting enzyme-resistant, potent angiotensin-(1–7) analog. *J. Pharmacol. Exp. Ther.* **328**, 849–854
- 187 de Vries, L., Reitzema-Klein, C. E., Meter-Arkema, A., van Dam, A., Rink, R., Moll, G. N. and Akanbi, M. H. (2010) Oral and pulmonary delivery of thioether-bridged angiotensin-(1–7). *Peptides* **31**, 893–898
- 188 Wosten-van Asperen, R. M., Lutter, R., Specht, P. A., Moll, G. N., van Woensel, J. B., van der Loos, C. M., van Goor, H., Kamlic, J., Florquin, S. and Bos, A. P. (2011) Acute respiratory distress syndrome leads to reduced ratio of ACE/ACE2 activities and is prevented by angiotensin-(1–7) or an angiotensin II receptor antagonist. *J. Pathol.* **225**, 618–627
- 189 Durik, M., van Veghel, R., Kuipers, A., Rink, R., Haas Jimoh Akanbi, M., Moll, G., Danser, A.H.J. and Roks, A.J.M. (2012) The effect of the thioether-bridged, stabilized angiotensin-(1–7) analogue cyclic Ang-(1–7) on cardiac remodeling and endothelial function in rats with myocardial infarction. *Int. J. Hypertens.* **2012**, 536426
- 190 Jones, E. S., Del Borgo, M. P., Kirsch, J. F., Clayton, D., Bosnyak, S., Welungoda, I., Hausler, N., Unabia, S., Perlmutter, P., Thomas, W. G. et al. (2011) A single β -amino acid substitution to angiotensin II confers AT2 receptor selectivity and vascular function. *Hypertension* **57**, 570–576
- 191 Kaschina, E., Grzesiak, A., Li, J., Foryst-Ludwig, A., Timm, M., Rompe, F., Sommerfeld, M., Kemnitz, U. R., Curato, C., Namsolleck, P. et al. (2008) Angiotensin II type 2 receptor stimulation: a novel option of therapeutic interference with the renin-angiotensin system in myocardial infarction? *Circulation* **118**, 2523–2532
- 192 Rehman, A., Leibowitz, A., Yamamoto, N., Rautureau, Y., Paradis, P. and Schiffrin, E. L. (2012) Angiotensin type 2 receptor agonist compound 21 reduces vascular injury and myocardial fibrosis in stroke-prone spontaneously hypertensive rats. *Hypertension* **59**, 291–299
- 193 Kobayashi, M. and Spector, M. (2009) *In vitro* response of the bone marrow-derived mesenchymal stem cells seeded in a type-I collagen-glycosaminoglycan scaffold for skin wound repair under the mechanical loading condition. *Mol. Cell. Biomech.* **6**, 217–227