Review

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Angiotensins as therapeutic targets beyond heart disease

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The renin–angiotensin system (RAS) plays a pivotal role in cardiovascular and hydro-electrolyte homeostasis. Blockade of the RAS as a therapeutic strategy for treating hypertension and related cardiovascular diseases is well established. However, actions of the RAS go far beyond the targets initially described. In this regard, the recent identification of novel components of the RAS, including angiotensin-(1–7) [Ang-(1–7)], Ang-(1–9), and alamandine, have opened new possibilities for interfering with the development and manifestations of cardiovascular and non-cardiovascular diseases. In this article, we briefly review novel targets for angiotensins and its therapeutic implications in diverse areas, including cancer, inflammation, and glaucoma.

Introduction: the RAS

The RAS plays a crucial role in cardiovascular and hydro-electrolyte homeostasis. The formation of the biologically active end-products of this peptidic hormonal system is dependent on a limited proteolysis process starting with the cleavage of precursor, the glycoprotein angiotensinogen, by renin. This step occurs in the circulation but also in many organs and tissues [1]. The formation of the octapeptide angiotensin II (Ang II) from angiotensin I (Ang I), the product of angiotensinogen hydrolysis by renin, is mainly dependent of angiotensin converting enzyme (ACE), a dipeptidyl carboxyl-peptidase that is widely expressed in many tissues including in the endothelium, a strategic localization for the formation of circulating Ang II. The lung vascular territory plays a pivotal role in this process. In addition to Ang II, other biologically active end-products are formed – including Ang III, Ang IV, and Ang-(1–7) [1]. Furthermore, other two peptides, Ang A and alamandine, can be formed by replacement of asparagine by alanine, a process involving decarboxylation of the aspartate residue. Alamandine formation can also occur by hydrolysis of Ang A by ACE2 [2–4]. Ang III and IV formation is dependent of aminopeptidases, while the formation of Ang-(1–7) is dependent mainly on the hydrolysis of Ang II by ACE2 but is also generated by hydrolysis of Ang I by other peptidases including prolyl-endopeptidase, neutral-endopeptidase (NEP), and tymeth-oligopeptidase. Carboxypeptidases and prolyl-endopeptidases can also contribute to the formation of Ang-(1–7), acting on Ang II [1]. Figure 1 illustrates the current view of the renin–angiotensin cascade, showing most of the novel RAS biologically active components including Ang-(1–9), Ang A, and alamandine. Figure 2 shows the RAS receptors (and antagonists) involved in the known biological actions of angiotensins, including Mas (MAS1 proto-oncogene, G protein-coupled receptor) [5–9] and the novel putative receptor for alamandine, MrgD (MAS-related G protein-coupled receptor, member D) [2–4].

The therapeutic efficacy of the blockade of the RAS for treating hypertension and related cardiovascular diseases is well demonstrated. However, growing evidence indicates that the role of the RAS goes far beyond the targets initially identified. In fact, the list of biologically active end-products of the RAS is still growing, raising plenty of new possibilities to interfere with cardiovascular and non-cardiovascular diseases. This is particularly true for Ang-(1–7) and more recently, alamandine, which in most cases display activities opposed to those exerted by Ang II. In this brief review we select some of the novel targets for angiotensins to illustrate the growing list of possible therapeutic applications of interfering with the RAS, especially the new ACE2/Ang-(1–7)/Mas axis (Figure 3).

Cancer

Angiotensins have been reported to be involved in cancer pathogenesis (Figure 4). Inhibition of Ang II formation by ACE inhibitors (ACEI), or blockage of its receptor AT1, can have beneficial effects in cancer suppression. Egami and colleagues [10] described the reduction of tumor growth by ACEI through blockade of angiogenesis. However, losartan, an angiotensin receptor blocker (ARB), has been reported to increase tumor perfusion, thereby improving chemotherapy outcome by reduction of matrix production and fibroblast density, increasing drug and oxygen delivery [11]. ACEI and ARB also decrease the expression of vascular endothelial growth factor (VEGF) and tissue factors,

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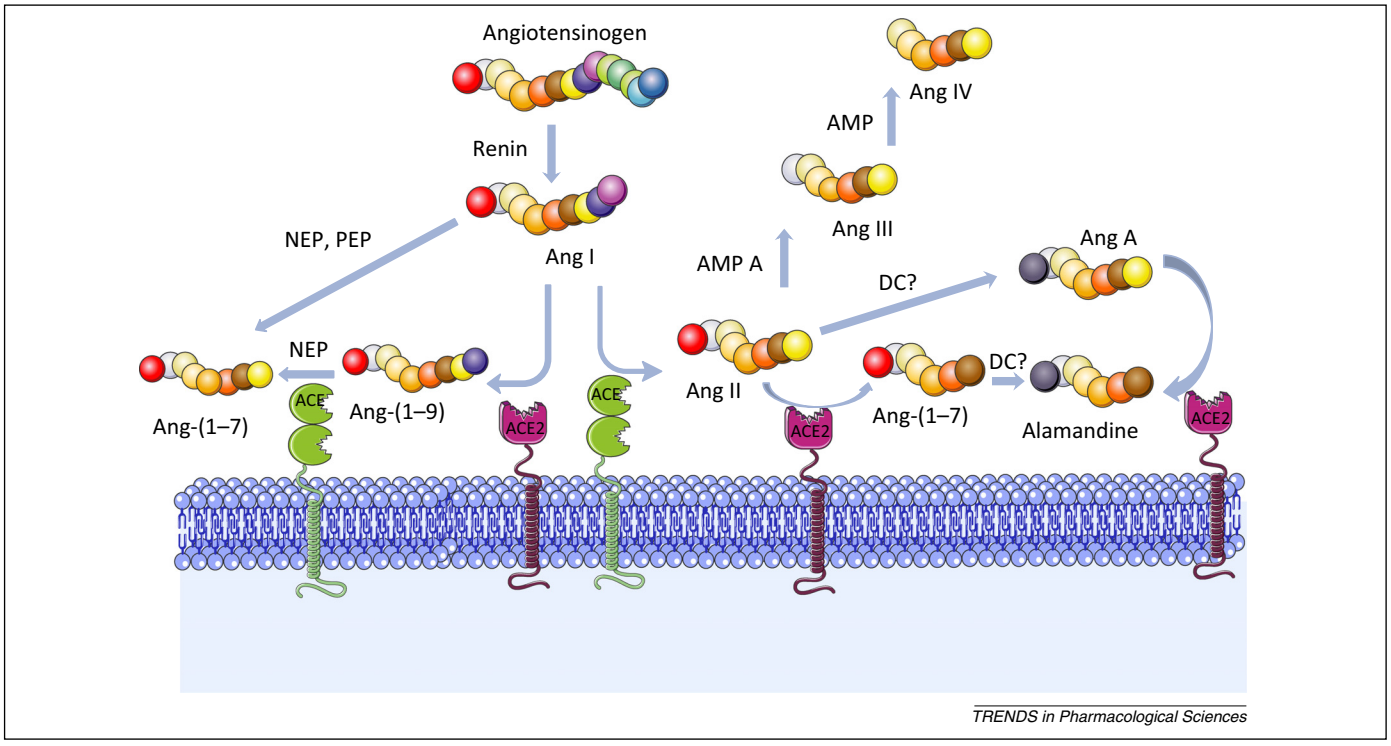

Figure 1. Simplified view of the renin–angiotensin system (RAS) cascade. Abbreviations: ACE, angiotensin converting enzyme; ACE2, angiotensin converting enzyme 2; Amp, aminopeptidase; Ang I, angiotensin I; Ang II, angiotensin II; Ang III, angiotensin III; Ang IV, angiotensin IV; Ang-(1–7), angiotensin (1–7); Ang-(1–9), angiotensin (1–9), Ang A, angiotensin A; DC, decarboxylase; NEP, neutral endopeptidase (neprilysin); PEP, prolylendopeptidase.

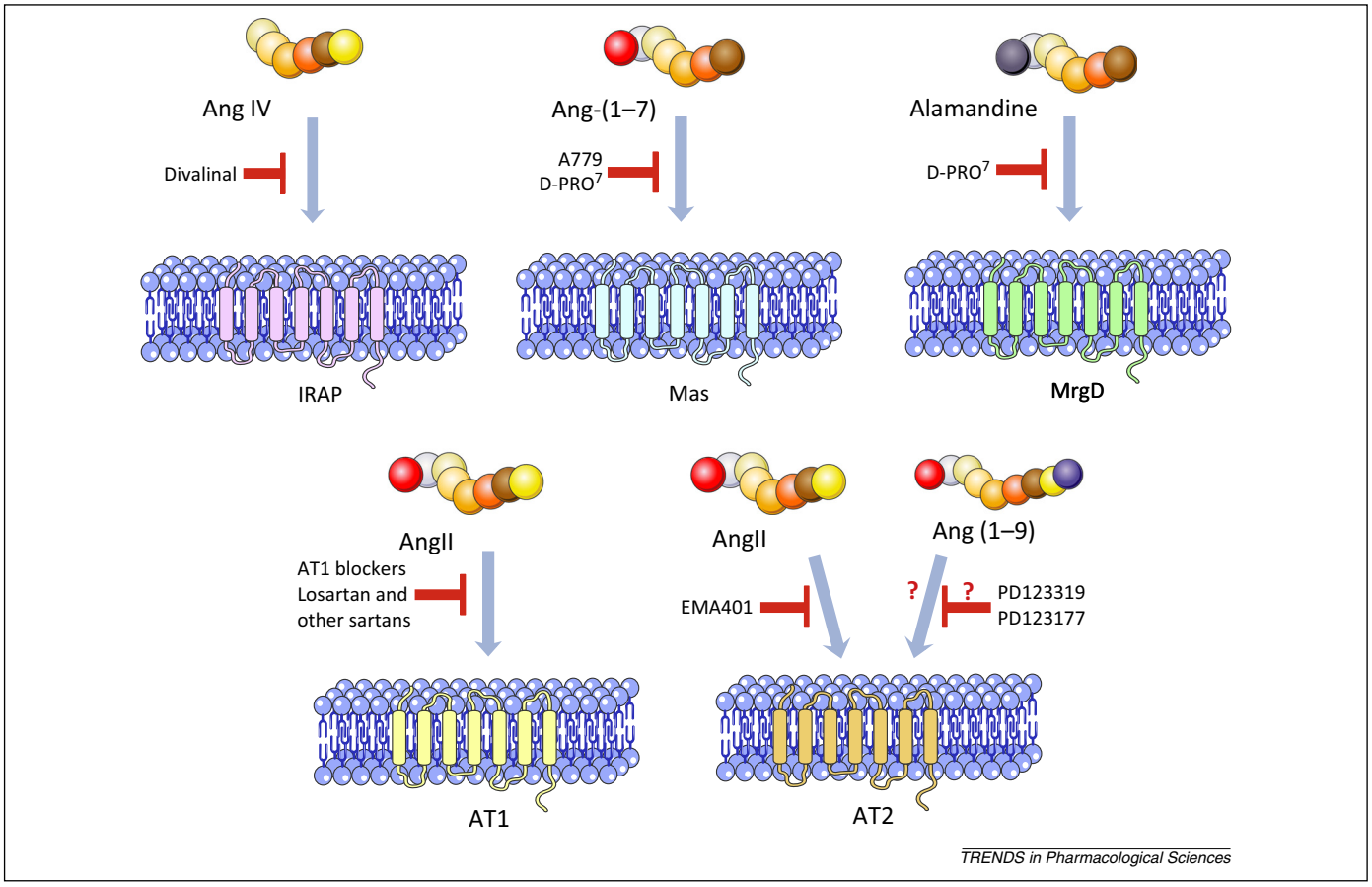

Figure 2. Agonists and antagonists of the known receptors of the renin–angiotensin system (RAS).

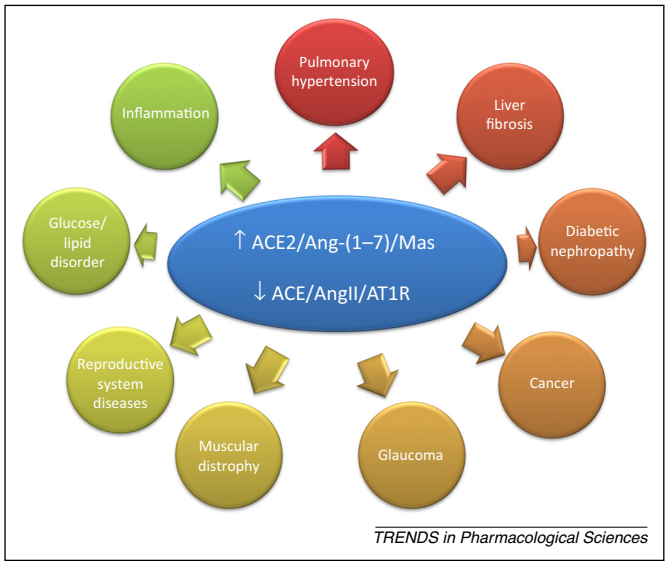

Figure 3. Blood pressure-independent targets for angiotensins.

which correlate with tumor progression [12]. Candesartan, an ARB, significantly reduced transforming growth factor β1 (TGF-β1) expression and suppressed tumor proliferation and stromal fibrosis [13], and it also significantly inhibited the growth of tumor xenografts in mice and tumor angiogenesis [10]. Ang II can also bind to the AT2 receptor, triggering actions that differ from AT1 receptor. AT2 overexpression in hepatocellular carcinoma (HCC) cell lines and orthotopic tumor grafts led to apoptosis mediated by activation of p38 MAPK, pJNK, caspase-8, and caspase-3, and by inactivation of pp42/44 MAPK (Erk1/2) [14]. A preclinical proof-of-concept of AT2 gene delivery through intratracheal administration to lung tumor showed successful tumoral apoptosis [15].

Combination of ACEI or ARB with other drugs has also been tested as a cancer treatment. Experiments in a mouse diabetes model showed that a hypoglycemic treatment (insulin or sulfonylurea) combined with anti-angiotensin such as renin inhibitor, aliskiren, or the ACEI, captopril, reduced liver metastasis [16]. Combined treatment with losartan, and anti-miR-155 showed synergistic antiproliferative action in endometrial cancer cells [17]. In addition, the combination of losartan and gemcitabine resulted in improved survival of rats with orthotopic pancreatic cancer due to diminished expression of VEGF and suppression of cancer cell proliferation [18]. The combination of losartan and AT2 agonist (CGP42112A) also synergistically decreased the survival of ovarian cancer cells and led to VEGF downregulation [19]. This combination of AT1 blocker and AT2 agonist was tested in prostate cancer, and led to decreased numbers of cancer cells [20]. A clinical trial

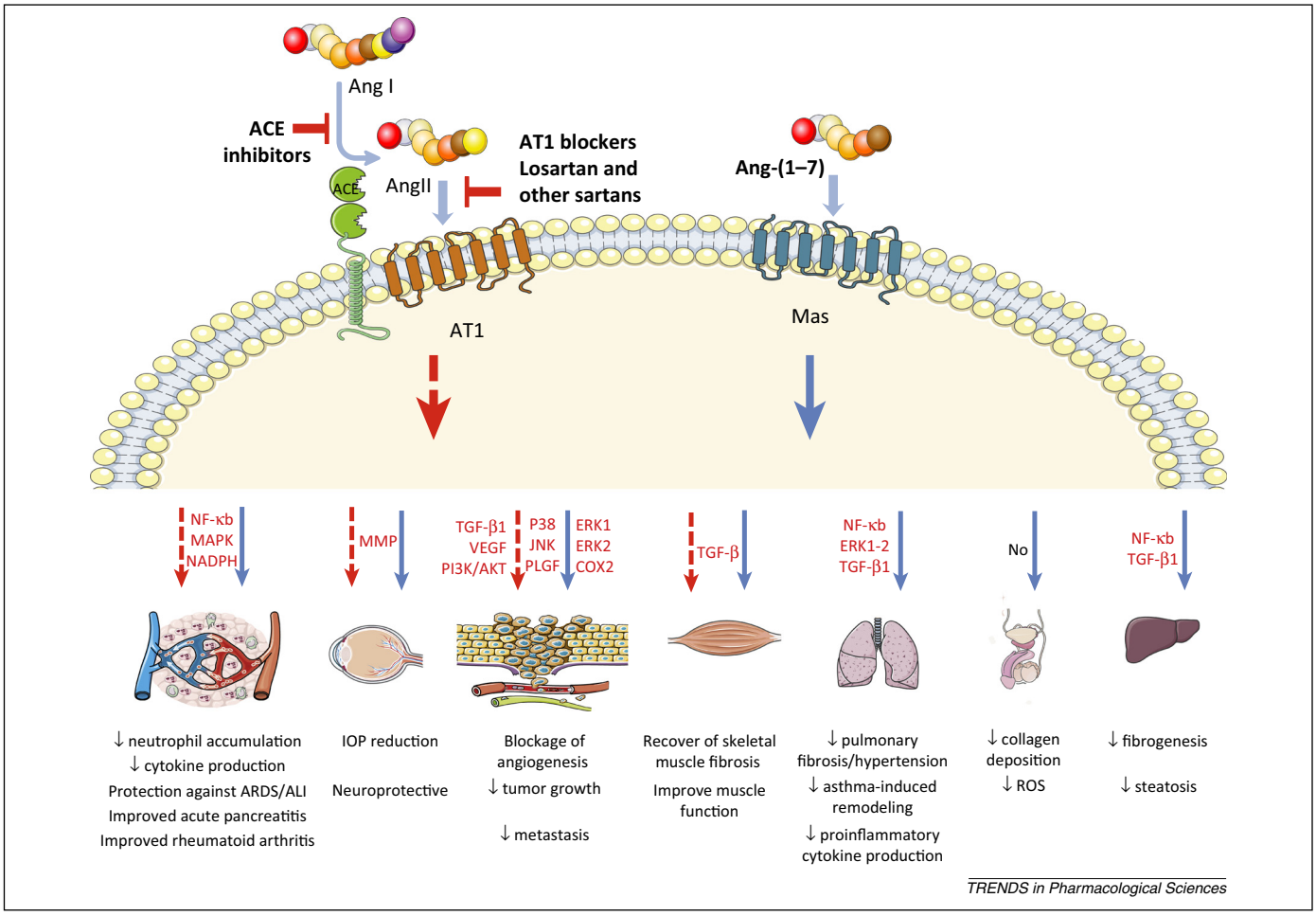

Figure 4. Molecular targets of the renin–angiotensin system (RAS), signaling molecules, and actions. Red dashed lines indicate blocked pathway; blue lines indicate activated pathways. Signaling molecules in red are molecules with inhibited expression, black denotes activated expression.

Phase I was also performed to choose the best dosage of another ARB, candesartan, and demonstrated its safety for use in combination with gemcitabine in patients with advanced pancreatic cancer [21].

Hypertensive patients under treatment with ACEI or ARB have recently been studied with several retrospective meta-analyses regarding the outcome in cancer treatment, with contradictory results. Several studies found no evidence of increased risks of cancer-specific mortality in cancer patients who used ACEI or ARBs, indicating the safety of these drugs, but others showed an increased susceptibility to develop cancer [22–28]. Other studies, however, found that ARBs and ACEI diminished the overall cancer risk of patients [22,29,30]. Independent studies with patients with esophageal squamous cell carcinoma receiving esophagectomy, upper-tract urothelial carcinoma, non-muscle-invasive bladder cancer, or advanced colon cancer showed that ACEI/ARB treatment was independently associated with superior overall survival [30–34]. An association between cancer and polymorphic variations in the coding region of the *ACE* gene, including single mutations (SNPs), deletions, and insertions, has been also discussed in the literature, with contradictory results. Some studies showed that *ACE* polymorphisms are not associated with different types of cancer, including lung, breast, gastric, and digestive system cancer [35–38]. By contrast, other studies showed an association between an *ACE* polymorphism (D/D genotype) and cancer, and found increased susceptibility to hepatocellular carcinoma [39], lymph node metastasis, oral cancer [40], and more advanced clinical stages of gastric cancer [41]. By contrast, some SNPs (G/G genotype) have been reported to be associated with some protection against breast cancer in the Brazilian population [42]. Further studies are obviously necessary to clarify whether these different outcomes are due to gender, race, environmental influences, or associated diseases.

As described for the cardiovascular system, Ang-(1–7) appears to have antiproliferative and antiangiogenic actions that oppose those of Ang II in cancer. Qian and colleagues [43] found that lung cancer has decreased expression of ACE2, and this correlates with poor clinical outcome. In further investigations, overexpression of ACE2 was tested in the A549 lung cancer cell line, and this resulted in decreased metastasis [43]. This modification upregulated the expression of E-cadherin, whose loss is linked to cancer cell invasion and metastasis, and diminished the effect of TGF-β, a key signaling pathway of this process [43].

Experiments *in vitro* using Ang-(1–7) treatment showed a marked decrease in the growth of cancer cells of different lineages, including lung cancer cells (A549, SK-LU-1, and SK-MES-1) [44] and prostate cancer cells (Du145 and LNCaP), as well as tumor xenografts [45] and orthotopic tumors [46]. The growth attenuation is a consequence of a decrease in the phosphorylation and activation of MAP kinases ERK1 and ERK2 in lung cancer cells [44] and in prostate tumor xenograft [46] after treatment with Ang-(1–7). Reduction in the levels of cyclooxygenase 2 mRNA and protein, which have been reported as procarcinogenic and contribute to new vessel formation, angiogenesis, and tumor growth, were also observed in lung cancer cells treated with Ang-(1–7) [47]. Other signaling pathways have also been demonstrated to be inactivated in tumoral cell lines treated with Ang-(1–7), such as PI3K/AKT, P38, and JNK, which reduce the cell migration and invasiveness of the cells through diminishing the expression of the metalloproteinases MMP-2 and MMP-9 [48]. These data could explain the anti-metastatic action of Ang-(1–7) in A549 human lung adenocarcinoma cells [48]. A recent phosphoproteome study performed by Verano-Braga and colleagues showed that Ang-(1–7) treatment led to (de)-phosphorylation in different signaling pathways involved in antiproliferative and antiangiogenic actions, such as MAPK, AKTS1 (a subunit of mTORC1), and HDAC1, among others. In this work they also showed dephosphorylation of the transcriptional factor FOXO1 after Ang-(1–7) treatment, and its consequent activation and translocation to the nucleus in the lung tumoral cell line A549 where it has antiproliferative action [49]

Another aspect modified by Ang-(1–7) in cancer is angiogenesis. The intratumoral vessel density of lung and prostate tumor xenografts was reduced in mice treated with Ang-(1–7), in agreement with the reduced CD34 immunoreactivity [50]. Expression analysis showed a reduction of VEGF-A protein and mRNA in lung and prostate cancer cells treated with this heptapeptide, and a reduction in placental growth factor (PlGF) in treated prostate cancer cells, showing that Ang-(1–7) diminishes neovascularization by reducing angiogenic factors. In addition, administration of Ang-(1–7) to prostate cancer cells increased the soluble fraction of VEGF receptor 1 (sFlt-1) which traps VEGF and PlGF, preventing activation of pro-angiogenic signaling, thereby potentiating the anti-angiogenic phenotype [45]. Following this line of reasoning, Abd-Alhaseeb and colleagues [51] also observed that the combination of AT1 blocker, olmesartan, with Ang-(1–7) diminished the intratumoral vessel density in Ehrlich's Carcinoma, together with reduction of insulin-like growth factor 1 (IGF-I) serum levels and the levels of its intratumoral receptor. Indeed, endothelial cell tubule formation and vessel formation in chick chorioallantoic membrane were reduced in the presence of Ang-(1–7) [50].

A clinical Phase I/II study was performed with Ang-(1–7) before and after chemotherapy in patients with breast cancer. This study also evaluated the maximum-tolerated dose of Ang-(1–7), and showed that Ang-(1–7) has no dose-limiting toxicity until 100 μg/kg [52]. The study also demonstrated that Ang-(1–7) could attenuate multilineage cytopenias following chemotherapy at a dosage of 100 μg/kg per day [52]. Another Phase I study with Ang-(1–7) administered subcutaneously was performed in patients with advanced solid tumors refractory to standard therapy [53]. Patients showed clinical improvement with stabilization of disease which was associated with decreased plasma levels of placental growth factor (PlGF), indicating an anti-angiogenic role of Ang-(1–7) [53]. For this study, a dose-limiting toxicity was found, 700 μg/kg, which was associated with stroke in one patient and with reversed neuropathy in other, thus 400 μg/kg was the recommended dose for a Phase II clinical trial [53]. Similarly, patients with sarcoma also treated with Ang-(1–7) displayed decreased PLGF

levels after treatment [54]. Other types of tumor have also been investigated for this molecular marker, but no statistical significance was found, probably due to the small size of the sample used [54].

Inflammation

The RAS can also play a role as an immunologic modulator, and can initiate not only innate but also acquired immunity [55–57]. Ang II is regarded as proinflammatory, and the activation of genes regulated by nuclear factor κB (NF-κB) has been seen in various studies with Ang II *in vitro* and *in vivo* [58–60]. Studies aimed at counteracting the actions of Ang II via Ang-(1–7), or by blockade of AngII/AT or AngII formation (ACEI), have shown promising results against some inflammatory pathologies (Figure 4).

Acute respiratory distress (ARDS) is the most severe form of acute lung injury (ALI), and has a mortality rate of at least 30–50% [61]. Losartan and irbesartan, AT1R blockers, showed protection against the strong inflammatory response in ARDS/ALI during sepsis [62,63]. A mouse model of cecal ligation and puncture was used in one of the studies which showed that losartan treatment inhibited the histological appearance of ALI/ARDS and prevented lung tissue activation of NF-κB and MAPK, leading to an improved survival after sepsis [62]. An endotoxin sepsis model showed that losartan prevented the reduction of ACE2 levels in lungs and diminished proinflammatory cytokines, improving lung injury and survival [64]. The administration of an ACEI also improved ALI/ARDS in a mouse model induced by oleic acid, and intercellular adhesion molecule-1 levels diminished in lung tissue [65]. Levels of ACE2 and Ang-(1–7) in ALI/ARDS were diminished in a mouse model induced by inhaled lipopolysaccharide (LPS), as shown by Wosten–van Asperen and colleagues [66]. Recently a cyclic form of Ang-(1–7) was developed [66]. It has been reported that this peptide derivative acts through Mas and is more stable than Ang-(1–7). Treatment with cyclized Ang-(1–7), and to lesser extent with losartan, improved lung function parameters and attenuated inflammatory mediators [66]. In another model using oleic acid, Ang-(1–7) infusion and its non-peptidic analog AVE09991 also protected mice and rats from acute lung injury, as evidenced by reduced lung edema, myeloperoxidase activity, histological lung injury score, and pulmonary vascular resistance [63]. These reductions are mediated by the Mas receptor because its antagonists, A779 and d-Pro7-angiotensin-(1–7), blocked these effects [63]. Ang-(1–7), ACEI, and ARB are therefore potential drugs to treat or prevent ALI/ARDS, and this is notable because no effective therapy is available. Studies with Ang-(1–7) also suggest its potential application in the treatment of asthma [67–69]. A model using chronic ovalbumin sensitization showed that Ang-(1–7) infusion diminished inflammatory cell infiltration and collagen deposition in the airways and lung parenchyma, and prevented bronchial hyper-responsiveness [69]. Another Mas agonist, AVE0991, was also tested and was found to prevent pulmonary remodeling, inflammation, and right ventricular hypertrophy in ovalbumin-sensitized mice [68].

The RAS is involved in another inflammatory pathology associated with high morbidity and mortality: acute pancreatitis (AP). The degree of severity of AP, induced by taurocholate, correlated with the tissue concentration of Ang II [70]. Blockade of the AT1 receptor with losartan improved AP, as shown by less histological damage, diminished myeloperoxidase activity, and reduced serum interleukin-6 levels. NADPH oxidase and the NF-κB signaling pathway are the main mediators of those actions because losartan treatment reduced depletion of IκBβ, elevated phospho-NF-κB p65 protein expression, and enhanced NFκB binding activity, in addition to reducing pancreatic glutathione and nitrotyrosine levels [71]. Furthermore, an ACE/ACE2 imbalance can correlate with the severity of AP, as shown in ACE2 knockout (KO) or transgenic mice: worsening of AP was seen in the ACE2 KO mice, whereas the opposite outcome was observed in mice with ACE2 overexpression [72]. Therefore, expression of ACE2 appears to confer resistance to this disease [72]. The Ang-(1–7) treatment *in vitro* had an anti-inflammatory role and activated endothelial nitric oxide synthase and nitric oxide signaling pathways, protecting the cell from developing AP [73].

Angiotensin-(1–7) is also a potential therapeutic target to treat rheumatoid arthritis, an inflammatory disease of the joints. Experiments were performed using two models of arthritis in which arthritis was induced in mice by antigen (methylated bovine serum albumin) and in rats by adjuvant (dried *Mycobacterium butyricum* in oil–water emulsion). These animals were then treated with Ang-(1–7) or the non-peptidic analog AVE099; treatment reduced inflammation as seen by histopathology. The treated animals also displayed decreased neutrophil accumulation and cytokine production [74]. Using the same models, losartan treatment was also tested. Independently of Mas activation, ARB treatment decreased inflammation and tissue injury. In addition, it reduced neutrophil recruitment, hypernociception, and cytokine production, as well as leukocyte rolling and adhesion [75]. Refaat and colleagues [76] also found that losartan alone reduced inflammation and improved arthritis, but the anti-inflammatory effects were more pronounced when combined with methotrexate.

The antinociceptive effect of RAS peptides was also evaluated in other studies. The receptor Mas was found to be expressed in dorsal root ganglia, and an antinociceptive effect of Ang-(1–7) treatment was demonstrated using the rat paw-pressure test together with prostaglandin E2 injection [77,78]. In addition, an oral active antagonist of the AT2 receptor, EMA401, has been reported to attenuate peripheral neuropathic pain in patients [79]. However, this antagonist was not fully characterized; it may interact with other RAS receptors, as has been shown for the AT2 antagonist PD123319 by Lautner *et al.* [80].

The RAS has also been implicated in inflammation of additional organs including liver [81], kidney [82], and brain [83–85], as well as in other processes such as asthma [67] and inflammatory pain [78,79] that will be not discussed in this review (reviewed in [86]). ARB and Ang-(1–7) have also been suggested as potential targets to treat these inflammatory disorders.

Glaucoma

Glaucoma, an eye disease that causes optic nerve lesion, can lead to irreversible blindness. This pathophysiology is mainly due to a deficiency of drainage of aqueous humor which can lead to elevated intraocular pressure (IOP), a major risk factor. The current anti-glaucoma therapy is focused on decreasing IOP. It currently includes prostaglandin analogs, sympathomimetics, β-blockers, and carbonic anhydrase inhibitors [87].

Components of the RAS including Ang-(1–7) are localized in ocular tissue in humans and in normotensive and hypertensive rats [88–90]. Recently, Mas was also localized in developing and adult mouse retina, with higher expression levels than the AT1 receptor [91]. An important finding regarding the role of the RAS in glaucoma was obtained with Ang-(1–7) treatment which lowered IOP when administered intravitreally in rabbits with normal IOP. The Mas receptor mediates this action because its antagonists abolish the effect [92] (Figure 4). A putative activator of ACE2, diminazene aceturate (DIZE), was also tested in glaucomatous rats. The administration of DIZE both systemically and topically (eye drops) was effective in lowering IOP, to the same proportion as one of the current market drugs, dorsolamide. DIZE was also protective against apoptosis of retinal cells [93]. In addition to the effects of DIZE and Ang-(1–7) on IOP reduction, adenovirus-mediated intraocular expression of ACE2 or of a fusion protein which produces Ang-(1–7) also conferred protection against diabetic retinopathy, as shown by diminished inflammation, retinal vascular leakage, acellular capillaries, and oxidative stress [94]. ACEI has long been described to be effective for treating glaucoma, diminishing IOP, and against diabetic retinopathy [87,95,96]. In addition, enaprilat, an ACEI, has shown efficacy to lower IOP in Sprague–Dawley rats to a greater degree than losartan. These results suggest that ACEI decrease IOP by another route in addition to reducing the availability of Ang II for interaction with AT1R. These mechanisms involve MMP and cytokine modulation as shown by experiments using MMP and cytokine inhibitors [97]. In addition to lowering IOP in normotensive and glaucoma human subjects, the use of ARB, Candesartan, in rats avoided retinal neuronal death by preventing ischemia [98].

Skeletal muscle disorders

Skeletal muscle disorders can arise from a wide range of causes including genetic predisposition to muscular weakness or the aging process in healthy individuals. Abnormal TGF-β signaling has been linked to several forms of muscular dystrophy including Becker, Emery–Dreifuss, and Duchene muscular dystrophy (DMD) [99]. Blockade of the Ang II receptor AT1 by losartan triggers the recovery of skeletal muscle fibrosis in *mdx* mice, an animal model for DMD, by reducing TGF-β-mediated canonical signaling in skeletal muscle fibers [100] (Figures 4 and 5).

The participation of ACE2/Ang-(1–7)/Mas receptor axis as an important regulator of skeletal muscle physiology has been demonstrated. *Mdx* mice, infused with a Mas antagonist (A-779), show highly deteriorated muscular architecture, as well as increases in fibrosis and TGF-β signaling, with diminished muscle strength (Figures 4 and 5). This was reinforced when skeletal muscles from *mdx*/Mas-KO mice were analyzed [101]. By contrast, infusion or oral administration of Ang-(1–7) into *mdx* mice normalizes skeletal muscle architecture, decreases local fibrosis, and improves muscle function *in vitro* and *in vivo* [101]. Similar beneficial effects of Ang-(1–7) were described in another mouse model of muscular dystrophy [102]. These results clearly indicate that under physiological conditions Ang-(1–7) has a beneficial role in diminishing the fibrotic response induced by chronic skeletal muscle damage, as in the *mdx* mice. The positive effects of Ang-(1–7) were mediated by the inhibition of TGF-β/Smad signaling which, in turn, led to reduction of the pro-fibrotic microRNA miR-21 [101]. At the cellular level, the numbers of skeletal muscle fibrotic fibroblasts (Tcf4-positive) [103] responsible for the fibrotic response [104–107] were increased in skeletal muscle of *mdx* mice compared to wild type, and infusion of Ang-(1–7) significantly decreased the number of fibroblasts [101].

ACE2, the enzyme responsible for Ang-(1–7) production, is found at the sarcolemma of skeletal muscle fibers in both wild type and *mdx* mice [108]. ACE2 overexpression in the tibialis anterior muscle of *mdx* mice using an recombinant adenovirus revealed that ACE2 localized in the sarcolemma, with a concomitant reduction in the fibrosis associated with tibialis anterior dystrophic muscles [108].

Skeletal muscle atrophy, which is characterized by loss of strength and muscle mass, is a pathological condition present after long periods of skeletal muscle inactivity. Under atrophic stimulus, Mas receptors increase [109] and infusion of Ang-(1–7) decreases skeletal muscle atrophy induced by Ang II [110]. Ang-(1–7) decreases the expression of TGF-β1 induced by Ang II, prevents Smad-2 phosphorylation and Smad-4 nuclear translocation, and decreases fibronectin levels, all of which are dependent on TGF-β1 induced by Ang II [111].

Thus, the evidence demonstrates that the ACE2/Ang-(1–7)/Mas axis is a pivotal regulator of the pathophysiology of several skeletal muscle diseases, and argues that the components of the axis are strong potential candidates for therapeutic use.

Erectile dysfunction

In the past few years several studies have reported the potential of Ang-(1–7)/ACE2 in the treatment of erectile dysfunction [112–115] (Figure 4). After the initial description of a pro-erectile effect of Ang-(1–7) [116,117], a beneficial effect of the heptapeptide was described in APOE KO mice fed with a western-type diet [112]. These mice presented increased collagen deposition, increased ROS production, and decreased erectile response as evaluated by the dose–response of carvenosal relaxation following acetylcholine administration. These alterations were attenuated/reversed by treatment with an oral formulation of Ang-(1–7). Similar beneficial effects were obtained with the putative ACE2 activator DIZE [112]. Likewise Benter and coworkers [113] showed a beneficial effect of Ang-(1–7) delivered by osmotic minipumps on erectile function in type 1 diabetic rats. Concerning blockers of the RAS, the results are not yet clear despite a clear deleterious effect of Ang II on erectile dysfunction [118,119]. These observations could be due to the direct effect of ACEI and ARBs on

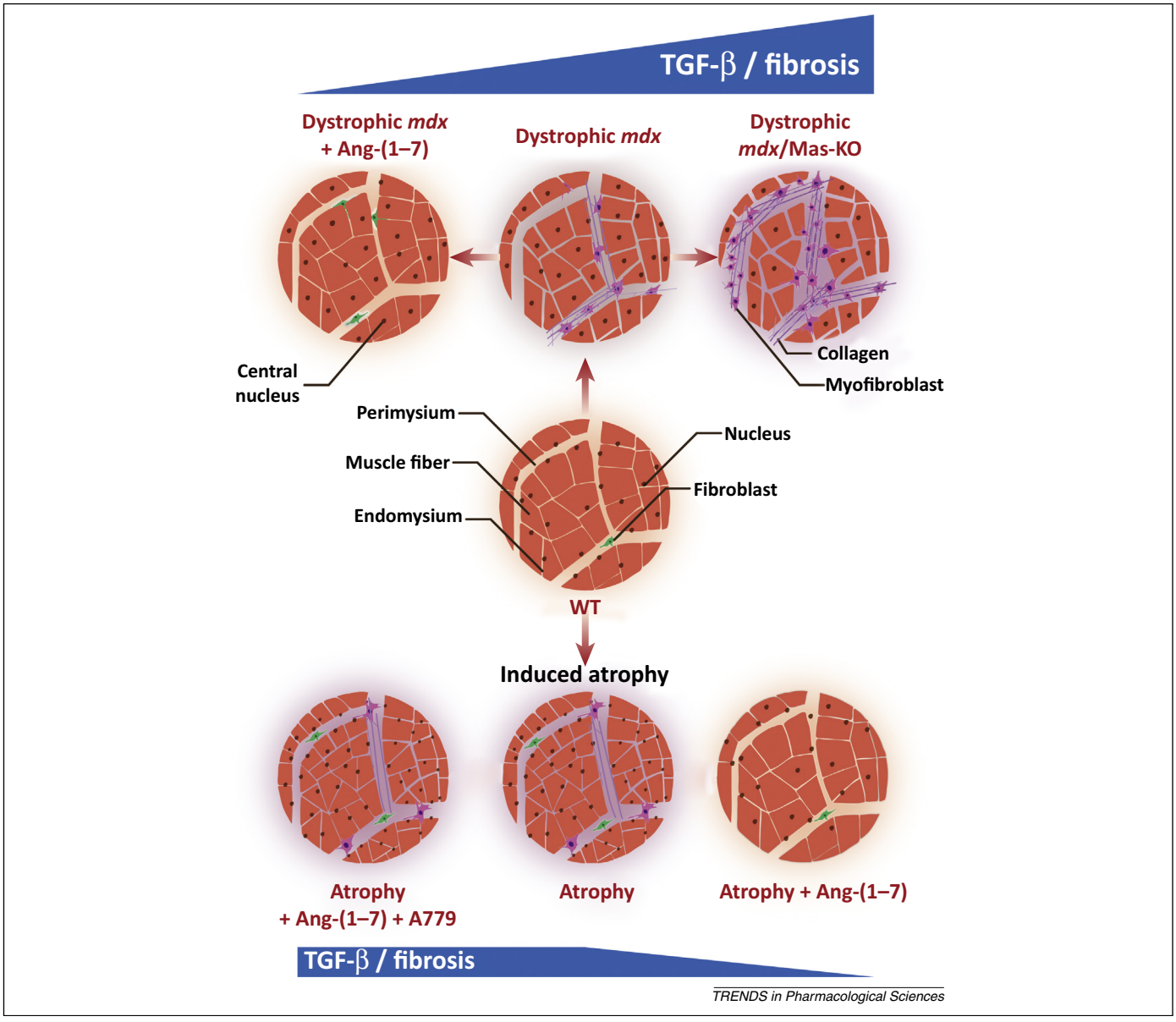

Figure 5. The figure shows (center) a diagram of a normal skeletal muscle cross-section indicating spaces containing extracellular matrix (perimysium and endomysium) and muscle fibers with peripherally located nuclei. Top (center): dystrophic skeletal muscle (*mdx*) showing the increase in fibrosis, centrally located nuclei and myofibroblasts. This increase in fibrotic components is exacerbated in *mdx*/Mas-knockout (KO) mice (right). By contrast, infusion of Ang-(1–7) diminishes fibrosis and the number of myofibroblasts (left) in the dystrophic muscle. Lower (center): skeletal muscle under atrophic conditions (Ang II-induced); this induced atrophy is avoided by Ang-(1–7) (right) which acts via the Mas receptor because A779 (antagonist of Mas) abolishes the anti-atrophic effect of Ang-(1–7) (left). Abbreviation: WT, wild type.

blood pressure, which may mask their direct effect on the corpus cavernosum.

Pulmonary fibrosis/hypertension

There is growing evidence that the ACE 2/Ang-(1–7)/Mas axis confers protection against lung fibrosis and pulmonary hypertension [120–124] (Figure 4). Ang-(1–7) has also been reported as a protective agent for another pulmonary disorder, asthma [67,68]. In the case of pulmonary fibrosis/hypertension, protection with Ang-(1–7) was achieved with endothraqueal gene transfer [125], osmotic minipump [121], or oral delivery [122]. Different models of pulmonary fibrosis were also used (monocrotaline, bleomycin, and neonatal hyperoxia). These observations suggest that pulmonary fibrosis is a target for Ang-(1–7). Similar results were obtained with ACE2 gene delivery, reinforcing the important role of the ACE2/Ang-(1–7)/Mas in pulmonary hypertension. Indeed, recombinant ACE2 administration in pigs diminished pulmonary arterial pressure and vascular resistance [126]. In addition, pharmacokinetic and pharmacodynamic studies with recombinant ACE2 have already been carried out successfully in patients [127]. In the model of neonatal hyperoxia-induced lung injury, in addition to a Mas agonist [cyclic-angiotensin-(1–7)], a protective role for a putative AT2 agonist [DKaAng-(1–7)] was reported, suggesting that AT2R stimulation could also be a pharmacological tool for attenuation of pulmonary hypertension. Another AT2 agonist, compound 21 (C21), was recently reported to also attenuate pathophysiology, and reversed the pulmonary fibrosis and prevented the right ventricular fibrosis associated with pulmonary hypertension induced by monocrotaline [128].

Liver fibrosis

The RAS appears to play a significant role in liver morphology and function. Increased activity of the ACE/Ang II/AT1 axis is related to fibrogenesis and steatosis, while an opposite role is exerted by ACE2/Ang-(1–7)/Mas ([129] for review) (Figure 4). A pronounced gene expression activation of the ACE2/Ang-(1–7)/Mas axis was reported following biliary duct occlusion, a maneuver associated with liver fibrosis [130–132]. Blockade of Ang-(1–7) in such conditions produced a worsening of liver fibrosis, indicating a protective role of the heptapeptide in the liver [131]. An anti-fibrotic effect was also observed upon AT1R blockade, suggesting that the balance between the two axes is important in maintaining fibrinogenesis homeostasis in the liver. These effects appear to be dependent on the influence of Ang II and Ang-(1–7) on stellate cells whose proliferation is stimulated by Ang II and inhibited by Ang-(1–7) [129].

The influence of the RAS is not restricted to fibrogenesis. Ang II facilitates steatosis while Ang-(1–7) has an anti-steatotic effect [129,133]. Furthermore, the liver is a target for important metabolic effects produced by the RAS, including neo-gluconeogenesis [134].

Concluding remarks

In the past few years there has been a remarkable expansion of our knowledge about the cardiovascular and non-cardiovascular role of the RAS. An important part of this advance was motivated by the physiological, pathophysiological, and therapeutic implications of the discovery of a novel dimension of the RAS, the ACE2/Ang-(1–7)/Mas axis. We have attempted in this review to address the pleiotropic role of the RAS and its two axes in the body. The therapeutic potential of Ang-(1–7)-like Mas agonists and maneuvers aimed to stimulate ACE2 activity are only beginning to be explored. Ongoing clinical studies/trials will be important to establish the possibility of expanding intervention in this system as a means to treat cardiovascular and non-cardiovascular diseases.

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