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Review

Molecular and cellular mechanisms of the inhibitory effects of ACE-2/ANG1-7/Mas axis on lung injury

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ABSTRACT

An established body of recent literature has demonstrated potent inhibitory effects of the angiotensin converting enzyme-2 (ACE-2)/ANG1-7/ Mas axis on acute lung injury and lung fibrogenesis. One of the mechanisms of this inhibition is the enzymatic action of ACE-2 to degrade its main substrate angiotensin (ANG) II, thereby reducing the injurious and profibrotic activities of this octapeptide. Another, potentially more important mechanism is the production by ACE-2 of the heptapeptide ANG1-7, which inhibits the actions of ANGII through its own receptor Mas, the product of the oncogene of the same name. Very recent efforts to define the molecular and cellular mechanisms of ANG1-7/Mas action have revealed a number of similar, but mechanistically distinct, pathways by which ANG1-7 and Mas act on various lung cell types to inhibit lung injury and fibrosis. In this review we summarize the beneficial actions of the ANG1-7/Mas pathway, specifically on lung cells in non-neoplastic lung injury. We also review the currently known down-stream signaling mechanisms of the ANG1-7/Mas pathway in various lung cell types known to be key in acute injury and fibrogenesis.

KEYWORDS: ANG1-7, Mas, apoptosis, signaling, lung injury

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INTRODUCTION

In recent years, many publications support the concept that excessive apoptosis of alveolar epithelial cells (AECs) is associated with progressive lung diseases such as idiopathic pulmonary fibrosis (IPF), acute lung injury (ALI) and chronic obstructive pulmonary disease (COPD) [1, 2]. Numerous experimental studies with both animal models and human tissues *ex vivo* now support the theory that activation of a local angiotensin (ANG) system plays a major role in lung injury. A subset of these studies demonstrated the existence of an intrinsic (i.e., all components expressed by the target cell itself) angiotensin system in lung alveolar epithelial cells. For example, in response to apoptosis inducers such as bleomycin, Fas ligand or tumor necrosis factor-alpha (TNF-α), angiotensinogen (AGT) mRNA and protein are produced *de novo* by AECs [3, 4, 5]. Once synthesized, AGT is cleaved by AEC proteases to generate the effector peptide angiotensin II (ANGII), which induces apoptosis in AECs through binding to AT1 receptor [3].

The heptapeptide Angiotensin 1-7 (ANG1-7) is produced by cleavage of the octapeptide ANGII by angiotensin converting enzyme-2 (ACE-2), which is also expressed constitutively by AECs. Recently, many experimental studies have demonstrated that ANG1-7 plays anti-apoptotic, anti-proliferative and anti-fibrotic roles in various lung cell types both *in vitro* and *in vivo* [6, 7, 8]. Further, radioligand-binding studies have demonstrated

that ANG1-7 acts through its receptor Mas, which belongs to the G-protein coupled receptor (GPCR) family [9] and is the product of the Mas oncogene. A considerable body of literature has shown that ANGII/AT1 receptor-mediated lung injury is counteracted by the ANG1-7/Mas axis [10, 11, 12, 13]. In this review we summarize the beneficial/protective actions of the ANG1-7/Mas axis on lung cells in non-neoplastic lung injury. Exactly how the ANG1-7/Mas axis affects injurious signaling pathways is currently a topic of intense focus. Hence we also discuss the downstream signaling mechanisms of the ANG1-7/ Mas pathway recently shown to be active in various lung cells.

Inhibitory actions of the ANG1-7/Mas pathway on pulmonary injury

The local ANG system is activated after tissue injury in a variety of organs to promote repair, but abnormalities in the process promote fibrosis. Many experimental studies have elucidated the contribution of AEC apoptosis to the pathogenesis of lung fibrosis [14, 15, 16]. Many years ago and more recently, seminal research works reported data to support the concept that the death of AECs, by itself, could create a profibrotic microenvironment without the involvement of an inflammatory response [17]. Consistent with this concept, blockade of apoptosis of AECs during lung injury by angiotensin receptor blockers (ARBs), ACE inhibitors (ACEi) or by a broad-spectrum caspase inhibitor decreased the fibrotic response in animal models [18]. However, issues that might limit the applicability of this approach to human subjects have been discussed, such as potential side effects, gender differences and in the case of ARBs, the potential for systemic hypotension in some patients [19]. Although ACE inhibitors have shown to reduce lung fibrogenesis in some animal models, clinical trials of ACE inhibitors in humans have failed to detect beneficial effects on lung fibrosis. This might be explained by the presence of other enzymes independent of ACE that could generate ANGII. Thus, it is crucial to understand the underlying mechanisms of the counter-regulatory axis ACE-2/ANG1-7/Mas, which may hold potential for future therapeutics for lung diseases.

In AECs, constitutively expressed ACE-2 converts the pro-apoptotic octapeptide ANGII to the anti-apoptotic heptapeptide ANG1-7, and thereby limits the accumulation of ANGII to promote cell survival [8]. Evidence for a beneficial role of ACE-2/ANG1-7 is strengthened by *in vivo* studies of experimental animals that used genetic manipulation of ACE-2 or specific inhibitors of ACE-2 to establish a protective role of the enzyme [20]. Previous work in this laboratory showed that ACE-2 is protective against experimental fibrosis, but is down-regulated in both human lung fibrosis and experimental lung fibrosis in animal models [21]. Uhal *et al.* demonstrated that ACE-2 mRNA, protein and enzymatic activity were severely decreased in lung biopsy specimens isolated from IPF patients and also in the lung tissue of experimental animals made fibrotic by administration of bleomycin [21]. In these studies, intratracheal administration of either ACE-2-specific siRNAs or DX600, a competitive inhibitor of ACE-2, enhanced bleomycin-induced lung collagen accumulation. Moreover, in the lungs of animals in which ACE-2 was manipulated in these ways, ANGII levels were increased and the resulting increase in lung collagen was blocked by an ANG receptor blocker. Together, these studies showed that ACE-2 is protective against lung fibrogenesis by controlling local ANGII generation.

In the lungs of patients with IPF, many alveolar epithelial cells are proliferating in the so-called “hyperplastic epithelium” described by pathologists, whereas AECs in the normal lung are primarily quiescent [2]. On this basis, it was hypothesized that cell cycle regulation plays an important role in ACE-2 expression by AECs. This was verified in a recent study that showed significant differences in ACE-2 mRNA, protein and enzymatic activity in sub-confluent (proliferating) vs. post-confluent (quiescent) human lung cells in culture, and within normal or fibrotic human lung specimens [22]. The data clearly showed a down-regulation of ACE-2 mRNA, protein and enzymatic activity in proliferating cells and an up-regulation in quiescent cells. Additionally, the up-regulation of ACE-2 that occurs in cells approaching density-dependent quiescence *in vitro* is blocked by the transcription blocker actinomycin D or by

an inhibitor of JNK phosphorylation. Taken together, these results illustrated the cell cycle-dependent and JNK-mediated regulation of ACE-2 expression in AECs.

Recent work in our laboratory showed that both ANGII generation and c-Jun N terminal kinase (JNK) phosphorylation are required events in AEC apoptosis and subsequent lung fibrosis [23]. It was speculated that ACE-2, as well as its product ANG1-7, might regulate AEC apoptosis. This theory was confirmed by the findings that ANG1-7 could block JNK phosphorylation, caspase activation and nuclear fragmentation in a cultured mouse lung epithelial cell line (MLE-12 cells) or in primary cultures of rat lung alveolar type II epithelial cells [23]. Furthermore, pretreatment with A779 (D-ala^7-Ang1-7), a specific antagonist of the Mas receptor, prevented the inhibitory actions of ANG1-7 and thus implicated the involvement of Mas receptor [23]. A subsequent study of the human type II epithelial cell-derived cell line A549 and primary cultures of human lung AECs evaluated apoptosis of these cells, induced by either MG132 (a proteasome inhibitor and inducer of ER stress) or by the surfactant protein C (SPC) BRICHOS domain mutation G100S (an inducer of the Unfolded Protein Response and ER stress), one of several recently discovered SPC mutations that cause interstitial lung disease. In response to either of these inducers, the apoptosis was completely abrogated by ANG1-7 [24]. Specifically, ANG1-7 prevented the induction of caspases, loss of mitochondrial membrane potential, cytochrome c release, JNK phosphorylation and nuclear fragmentation in the cultured human AECs. Further, the Mas antagonist A779 blocked the inhibition of apoptosis by ANG1-7 and demonstrated the involvement of Mas. This study also demonstrated a reduction of ACE-2 expression when the cultured AECs were challenged with the proteasome inhibitor MG132 or the SPC mutant G100S. This reduction was prevented by an inhibitor of the ACE-2 ectodomain shedding enzyme ADAM17/TACE (TAPI-2). Together, these data demonstrate that AEC apoptosis is mediated by the autocrine ANGII/ANG1-7 system expressed by these cells, and suggest that the hepta-peptide ANG1-7 may hold therapeutic potential for lung diseases in which the UPR and/or ER stress play a role in pathogenesis. The exact mechanisms of the activation of the ANG system in response to ER stress are currently unclear but are under investigation.

Consistent with these observations, Shenoy *et al.* demonstrated that intratracheal administration of a lentiviral-packaged ANG1-7 expression construct or ACE-2 cDNA into Sprague Dawley (SD) rats significantly inhibited bleomycin-induced collagen deposition, expression of transforming growth factor-β (TGF-β) mRNA and AT1 receptor protein levels in the rat lungs [25]. Additionally, protective effects against lung fibrosis were also obtained by overexpression of ACE-2. This study is consistent with a previously published study by Li *et al.*, which demonstrated that exposure of cultured rat or human AECs to bleomycin *in vitro* caused a robust expression of angiotensinogen (AGT) mRNA and the processed peptide ANGII, both of which are required for the apoptotic response of these cells [26]. Attempts to determine which profibrotic genes might be activated in response to ANGII have shown the induction of TGF-β and α-collagen-1 mRNA levels *in vitro* in human fetal lung (HFL-1) cells exposed to the octapeptide [27]. Pre- or co-incubation with ANG1-7 prior to the application of ANGII inhibited the induction of the profibrotic genes. However in this study, pre-incubation with A779 did not prevent the inhibitory actions of ANG1-7. The cause for this incongruity is not clear, but may depend on the cell type specificity (fibroblast vs. epithelial cell) or the experimental conditions that were used [18].

Acute respiratory distress syndrome (ARDS) is one of the most devastating forms of acute lung injury (ALI). Each year in the United States around 200,000 patients suffer from ARDS [10]. Apoptosis of AECs has been discovered in the lungs of ARDS patients and was associated with increased Fas/FasL expression [28]. It is currently believed that anomalies of the ANG system contribute to the pathogenesis of ARDS. About 60% of ARDS patients are shown to develop pulmonary fibrosis with increased mortality rates [29]. A considerable number of *in vivo* studies demonstrates the beneficial actions of the ACE-2/ANG1-7 axis in acute lung injury in several animal models (see Figure 1). For example,

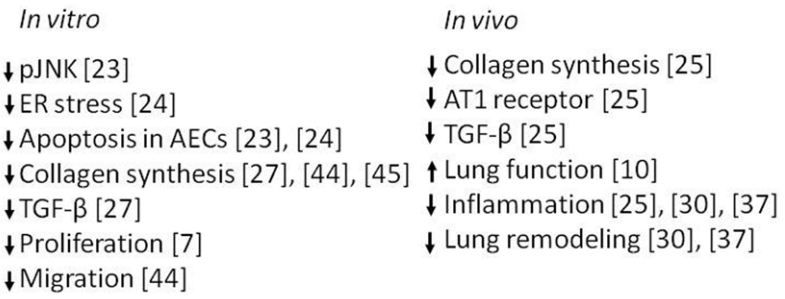

Figure 1. Known actions of ANG1-7 in lung injury. *In vitro* studies of lung fibroblasts have demonstrated the down-regulation of profibrotic genes. Further, angiotensin (ANG) II-induced apoptosis of alveolar epithelial cells (AECs) and JNK phosphorylation were reversed by treating the epithelial cells with ANG1-7. *In vivo* studies have shown the reduction of collagen levels, TGF-β1 and lung inflammation in response to ANG1-7. Moreover, ANG1-7 enhanced lung function in experimental mice after lung injury. Parentheses indicate key references.

intratracheal administration of lipopolysaccharide (LPS) induced acute lung injury in C57BL/6 mice, which resulted in substantial induction of collagen accumulation, pulmonary edema and inflammation [30]. However, subcutaneous infusion of ANG1-7 significantly reduced hydroxyproline levels (a marker of total collagen) as well as TGF-β1 and Smad2/3 protein levels. Treatment with A779 prevented the protective effect of ANG1-7 on collagen deposition and lung remodeling, observations that provide *in vivo* evidence that Mas mediates the protective role of ANG1-7 on lung injury and fibrogenesis.

Similarly, overexpression of a recombinant form of ACE-2 prevented ALI induced by acid aspiration or sepsis in mice [31]. The same authors also demonstrated that mice deficient in ACE had markedly decreased ALI. On the other hand, the importance of ACE-2 is strengthened by the use of ACE-2 knockout mice [32]. Experimental ARDS induced in mice by acid aspiration was more severe in ACE-2 knockouts compared to wildtype controls that express functional ACE-2; the loss of ACE-2 in the knockout mice increased neutrophil accumulation and worsened pulmonary edema. These studies demonstrated the protective role of ACE-2 *in vivo* in models of ALI and showed that part of this defensive role is due to limitation of the accumulation of ANGII. Recently, many studies have accumulated to support the view that an imbalance between the enzymatic activity of ACE and ACE-2 determines the local tissue levels of ANGII and ANG1-7. For example, a study by Wosten-van Asperen *et al.* conducted to determine pulmonary ACE and ACE-2 activity in patients with ARDS, demonstrated increased ACE activity and decreased ACE-2 activity compared to the control group [12]. In an animal model of ARDS, the reduction of ACE-2 activity was also present, but could be reestablished by *in vivo* treatment with ANG1-7. These findings are promising but since the clinical study was limited to fourteen ARDS patients, larger clinical studies are needed for confirmation. Along the same line of thinking, Imai *et al.* demonstrated that pharmacological inhibition of AT1 receptor or ACE-knockout mice showed improved ALI symptoms in the absence of functional AT1 receptor or ACE [33]. On the basis of this work and that summarized in preceding paragraphs, it has been theorized that ACE/ANGII/AT1 can promote ALI, but the counter-regulatory axis ACE-2/ANG1-7 is protective against ALI.

Related experimental studies showed that ALI in mice following hindlimb ischemia-reperfusion (LIR) is also due to the dysregulation of the ANG system [34]. Changes in the ACE/ACE-2 mRNA level and protein levels were measured after 2 hour of hind limb ischemia in mice. In addition, ANGII and ANG1-7 levels in the blood serum and in lung tissues were measured by enzyme-linked inmunosorbent assay. In the beginning of the reperfusion period, the authors found higher levels of ANG1-7 than ANGII, but in later stages of reperfusion ANGII levels were higher than ANG1-7 levels. This change agreed with varying levels of ACE/ACE-2 expression [34]. Consistent with other works mentioned above, genetic deletion

of ACE-2 showed increased disease progression in this model. Collectively, the above studies all demonstrate the protective role of ACE-2 in lung injury.

On the other hand, the efficacy of ANG1-7 administration *in vivo* has been less well documented to date, in part due to the many proteases that degrade the heptapeptide very rapidly. This problem was bypassed by the addition of a thioether ring to the peptide to form cyclic ANG1-7 (cANG1-7), which has been shown to increase resistance to proteolytic degradation *in vivo* in Sprague Dawley rats [35]. Experimental studies have found enhanced stability of the cANG1-7 and also evidence that it binds to the ANG1-7 receptor Mas with high affinity. Efficacy of the cyclic analog of ANG1-7 was confirmed by the abrogation of LPS-induced acute lung injury by treatment with cANG1-7 *in vivo* [10]. These authors further found that cANG1-7 acted very quickly (< 4 hrs) to improve lung function and increase oxygenation. Thus, administration of the modified heptapeptide has a protective role against LPS-induced experimental ARDS. Nevertheless, the ability of cANG1-7 to inhibit acid aspiration-induced and sepsis-induced ARDS also needs to be validated in animal models.

It was also demonstrated that agonists of the Mas receptor or angiotensin type II receptor may hold therapeutic benefits against chronic lung disease (CLD) by counterbalancing ANGII induced pulmonary inflammation. Wagenaar *et al.* demonstrated cardiopulmonary effects by examination of lung and heart histopathology in neonatal rats challenged with constant exposure to 100% oxygen for 10 days in the presence of cANG1-7 or an AT2 agonist [36]. Additionally, mRNA levels of crucial genes that are involved in the ANG system and alveolar development were evaluated. Treatment with the agonists reduced the influx of macrophages and neutrophils into the lungs. However, treatment with the agonists did not affect alveolar development in neonatal rats with CLD.

A non-peptide compound AVE 0991 (AVE) has also shown to mimic the beneficial effects of ANG1-7 in a murine model of ovalbumin (OVA)-induced chronic allergic lung inflammation. Mice were challenged with OVA in the presence or absence of AVE [37]. While OVA increased airway and pulmonary vascular wall thickness, OVA + AVE-treated mice displayed reduced airway wall and pulmonary vasculature thickness. Further, cytokine levels and airway contractile response were also reduced in mice treated with AVE compound. Together, these studies suggested the potential of analogues of ANG1-7 in the treatment of chronic pulmonary remodeling associated with asthma.

The protective effects of ACE-2 and ANG1-7 have also been demonstrated in models of pulmonary hypertension (PH). In animal models of PH, ANGII contributes to pulmonary remodeling and binding of ANGII to AT1 receptor is increased in rats with experimental pulmonary hypertension [38]. Moreover, ACE expression *in vivo* is also increased in these animals [39]. However, preliminary clinical trials did not have major success in demonstrating beneficial effects of ACE inhibitors or ARBs on COPD-related PH [40]. Monocrotaline-induced animal models of PH have revealed that experimental overexpression of ACE-2 can inhibit and reverse the induction in right ventricular pressure, suggesting ACE-2 as a potential therapy [41, 42]. The development of a plant-based oral delivery system, consisting of purified ACE-2 and ANG1-7 bioencapsulated in plant cells, has displayed protection against experimental PH [43]. The bioencapsulation defends against gastric enzymatic degradation and improved systemic absorption from the intestine. Sprague-Dawley rats with monocrotaline-induced experimental PH were treated with bioencapsulated ACE-2 and ANG1-7, which significantly halted the disease progression in these animals. This novel approach of delivery system using transplastomic technology may be beneficial for future treatments of other types of lung injuries, but needs to be investigated further. As discussed, ultimately these experimental studies may provide ideas to develop novel therapeutic strategies to control lung diseases and conceivably other diseases that involve the ANG system.

Signaling mechanisms underlying ACE-2/ANG1-7/Mas action in lung cells

The recent advances discussed above have enhanced our understanding of the tissue specific

renin-angiotensin system (RAS), and mainly the counter-regulatory role of the ACE-2/ANG1-7/Mas axis which opposes the many deleterious actions of the ACE/ANGII/AT1 axis. However, the exact intracellular signaling mechanisms of the ANG1-7/Mas pathway are currently unclear in lung cells. A number of cell signaling mechanisms are thought to be involved downstream of ANG1-7 binding to Mas, but only a few studies have explored this subject in lung injury. In this section we summarize the experimental studies that demonstrate the downstream signaling pathways that are involved in ANG1-7/Mas signaling in lung cells.

A recent study from our lab demonstrated that ANGII-induced JNK phosphorylation and apoptosis in AECs were potently blocked by ANG1-7 [23]. At baseline (without added inducers and in serum-free media), ANG1-7 levels in the medium of cultured AECs are ~10-fold higher than ANGII levels, and thereby function to maintain cell survival [23]. The same study demonstrated that ANG1-7 could potently reduce the JNK phosphorylation induced by ANGII, and JNK phosphorylation is required for AEC apoptosis. In accord with these observations, it was hypothesized that ANG1-7 binding to Mas activates a JNK-selective phosphatase which reduces the accumulation of phospho-JNK as a cell survival mechanism. This idea is currently being investigated through the use of gene knockdown strategies. In an earlier study of primary cultures of AECs, it was observed that ANGII binding to AT1 activates protein kinase C (PKC) and that PKC activation, as well as JNK phosphorylation, is required for apoptosis [8]. Other recent studies showed that blockade of protein kinase A (PKA) by a specific inhibitor led to a rapid increase in phospho-JNK, which suggests a possible role for PKA in AEC apoptosis (unpublished data). Therefore, it will be of high interest to investigate the possible involvement of the cAMP/PKA pathway in the inhibitory actions of ANG1-7 on lung cells.

Other signaling pathways of ANG1-7/Mas have been reported by various research groups studying lung injury and fibrosis. Meng and colleagues showed that the ACE-2/ANG1-7/Mas axis protects against pulmonary fibrosis by inhibiting the MAPK/NF-κB pathway in homogenates of whole lung tissue, thereby reducing markers of fibrosis such as α-collagen-I and synthesis of TGF-β [44]. The authors found, in studies of rat lung and in human fetal lung (HFL)-1 cells, that the resistance of fibroblasts to bleomycin- or ANGII-induced apoptosis was prevented by ANG1-7 through inhibition of the MAPK/NF-κB pathway. Moreover, they found the activation of caspase-dependent mitochondrial apoptotic pathway and BAX protein in response to ANG1-7 in lung fibroblasts. The inhibitory effects of ANG1-7 could be blocked by A779, the Mas receptor blocker, thus showing the involvement of Mas. However, these authors also demonstrated that administration of ANG1-7 alone activated ERK1/2 and moreover, blunted JNK phosphorylation in the HFL-1 cells. The dual effects of ANG1-7 that were shown in this study were explained by the state of activation of the ACE/ANGII/AT1 axis and a possible mechanism of ANG1-7 acting through AT1 receptor. However, no data were provided to support the proposed mechanism of ANG1-7 action through AT1 rather than Mas, and thus this concept should be evaluated carefully, since cell type specificity and the ratio of AT1 vs. Mas receptors are likely to play roles in the activation of this pathway. A later study from the same research group revealed that the ANG1-7/Mas axis protects against fibroblast migration by inhibiting the NADPH oxidase-4 (NOX-4)-derived ROS-mediated RhoA/Rho kinase pathway [45].

Recent reports have established the contribution of oxidative stress to the pathogenesis of pulmonary fibrosis [46, 47]. NOX-4 is an important source for the generation of reactive oxygen species (ROS) believed to be involved in initiating lung fibrosis. Consistent with this notion, the induction of α-collagen-I synthesis and fibroblast migration by ANGII was abrogated by an inhibitor of RhoA/Rock pathway (Y-27632) or by siRNA-mediated silencing of NOX-4. A direct inhibitory effect of ANG1-7 was investigated by the ability of ANG1-7 to block ANGII-induced RhoA and Rock-2 mRNA induction. Additionally, the authors showed that lentiviral-mediated expression of ACE-2 suppressed ANGII-induced fibroblast migration and collagen synthesis by blockade of the RhoA/Rho kinase pathway. In agreement with this study, another group showed that ANGII-induced human airway smooth muscle cell

(HASMC) contraction was reversed by ANG1-7 through the RhoA/Rho kinase signaling pathway [48]. In this study, HASMCs that were isolated from main bronchus biopsies obtained from lung resection donors were incubated with a RhoA/Rho kinase inhibitor Y-27632, which blocked ANGII induced HASMC contraction. These studies clearly demonstrate a down-regulation of RhoA/Rho kinase signaling pathways by ANG1-7 acting through its receptor Mas. However, further studies are required in other cell types in the lung and to identify the factor(s) that may influence this pathway.

Hashim *et al.* studied bronchoalveolar lavage fluid (BALF) in allergen-challenged mice and demonstrated that ovalbumin increased total cell numbers of neutrophils, eosinophils and lymphocytes, but this was attenuated by ANG1-7 through suppression of ERK1/ERK2 [49]. Although the exact mechanism of the suppression is unknown, ANG1-7 attenuated the ovalbumin-induced phosphorylation of ERK1/ERK2 and IκB-α, all of which was prevented by pre-treatment with the Mas receptor antagonist A779 [49]. Transcriptional regulation of the ANG1-7/Mas pathway is poorly understood in any organ system. To date, only one study has reported studies of the transcriptional regulation (or at least partial regulation) of the ANG1-7/Mas pathway in A549 cells [50]. Forkhead box protein O 1 (FOXO-1) is a transcription factor that regulates cell growth and apoptosis; when treated with ANG1-7, FOXO-1 transcriptional factor in A549 cells was phosphorylated and translocated to the nucleus upon stimulation [50]. However, further studies are required to understand the regulation of this system *in vivo*. Several groups have shown the involvement of vascular endothelial growth factor (VEGF), cyclooxygenase (COX-2) and PI3K/AKT pathway in A549s and

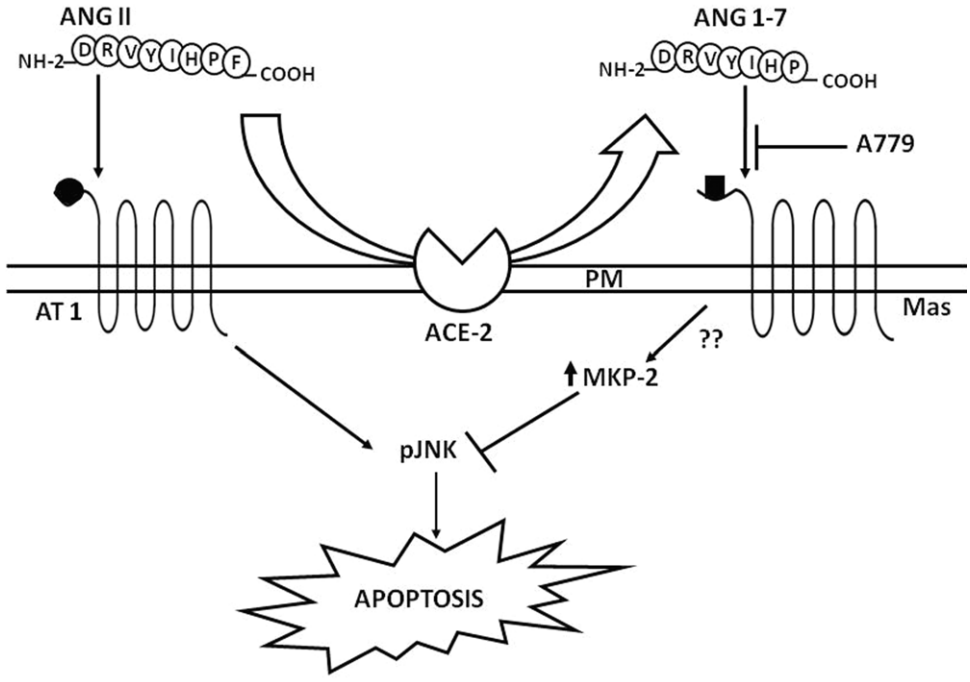

Figure 2. Known signaling mechanisms in alveolar epithelial cells (AECs). The octapeptide angiotensin II (ANGII) induces JNK phosphorylation and apoptosis through the AT1 receptor [23]. Angiotensin converting enzyme-2 (ACE-2) degrades the pro-apoptotic ANGII to the anti-apoptotic ANG1-7, which inhibits both JNK phosphorylation and apoptosis through the Mas receptor. These inhibitory effects of ANG1-7 are blocked by A779, a specific antagonist of Mas. Current studies suggest that ANG1-7/Mas activation prevents JNK phosphorylation by constitutively activating the JNK-selective map kinase phosphatase-2 (MKP-2) and further, demonstrate the involvement of the Mas receptor in MKP-2 activation. The mechanism(s) by which Mas activation induces MKP-2 (??) are currently unclear. PM - plasma membrane.

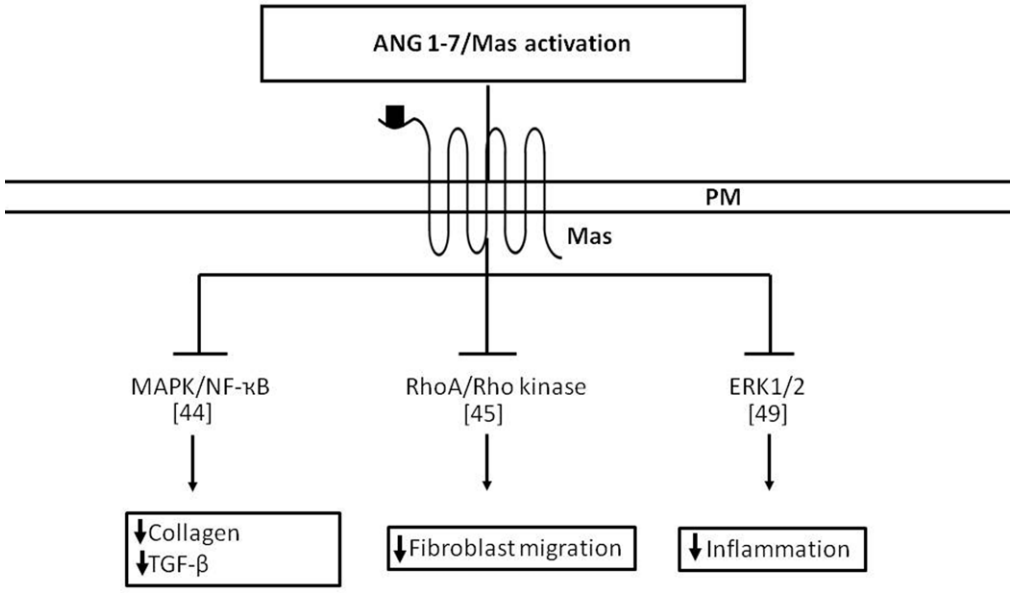

Figure 3. Downstream signaling pathways of the ANG1-7/Mas pathway in non-epithelial lung cell types. In lung fibroblasts (Left), ANG1-7/Mas protects against bleomycin-induced lung fibrosis by inhibiting the mitogen-activated protein kinase (MAPK)/NF-κB pathway. Fibroblast migration (middle) was prevented by inhibition of the RhoA/Rho kinase pathway. Ovalbumin-induced infiltration of lung tissues by eosinophils, lymphocytes and neutrophils (Right) were prevented by ANG1-7 through suppression of ERK1/2. Parentheses indicate key references. PM - plasma membrane.

demonstrated the ability of ANG1-7 to prevent tumor angiogenesis [7, 51, 52]. Nevertheless, further study is needed to determine the role of these pathways in non-neoplastic lung injury.

SUMMARY AND PERSPECTIVE

Evidence to date clearly demonstrates potent protective actions of angiotensin 1-7, acting through its receptor Mas, on lung injury and fibrogenesis through a variety of mechanisms including, but not limited to, inhibition of inflammation, chronic tissue remodeling and/or collagen deposition, as well as reduction of apoptosis in epithelial cells vs. reduction of cell proliferation and migration in stromal cells (see Figure 1). Clearly, opposing actions such as the latter listed above point to different and potentially opposite actions of ANG1-7/Mas on different cell types within the same organ. This diversity of cell type-dependent signaling pathways downstream of Mas activation (see Figures 2 and 3) emphasizes the importance of elucidating ANG1-7/Mas signaling in individual cell types of the lung, each of which is known to be key in the various lung diseases for which current therapies are limited and/or lacking. We suggest that future studies of this nature be conducted with well-differentiated primary cell cultures obtained from human lung, to help avoid potentially conflicting results due to species differences or the state of cell differentiation in culture.

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CONFLICT OF INTEREST STATEMENT

No conflict of interest.

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