Angiotensin II, mitochondria, cytoskeletal, and extracellular matrix connections: an integrating viewpoint

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Malfunctioning mitochondria strongly participate in the pathogenesis of cardiovascular damage associated with hypertension and other disease conditions. Eukaryotic cells move, assume their shape, resist mechanical stress, accommodate their internal constituents, and transmit signals by relying on the constant remodeling of cytoskeleton filaments. Mitochondrial ATP is needed to support cytoskeletal dynamics. Conversely, mitochondria need to interact with cytoskeletal elements to achieve normal motility, morphology, localization, and function. Extracellular matrix (ECM) quantity and quality influence cellular growth, differentiation, morphology, survival, and mobility. Mitochondria can sense ECM composition changes, and changes in mitochondrial functioning modify the ECM. Maladaptive ECM and cytoskeletal alterations occur in a number of cardiac conditions and in most types of glomerulosclerosis, leading to cardiovascular and renal fibrosis, respectively. Angiotensin II (ANG II), a vasoactive peptide and growth factor, stimulates cytosolic and mitochondrial oxidant production, eventually leading to mitochondrial dysfunction. Also, by inducing integrin/focal adhesion changes, ANG II regulates ECM and cytoskeletal composition and organization and, accordingly, contributes to the pathogenesis of cardiovascular remodeling. ANG II-initiated integrin signaling results in the release of transforming growth factor-β1 (TGF-β1), a cytokine that modifies ECM composition and structure, induces reorganization of the cytoskeleton, and modifies mitochondrial function. Therefore, it is possible to hypothesize that the depression of mitochondrial energy metabolism brought about by ANG II is preceded by ANG II-induced integrin signaling and the consequent derangement of the cytoskeletal filament network and/or ECM organization. ANG II-dependent TGF-β1 release is a potential link between ANG II, ECM, and cytoskeleton derangements and mitochondrial dysfunction. It is necessary to emphasize that the present hypothesis is among many other plausible explanations for ANG II-mediated mitochondrial dysfunction. A potential limitation of this proposal is that the results compiled here were obtained in different cells, tissues, and/or experimental models.

In addition to energy production, mitochondria also conduct other key cellular tasks, including the regulation of cytosolic calcium levels (29) and tissue oxygen gradients (104), H2O2 signaling (11), and the modulation of apoptosis (12). Importantly, mitochondria also receive, integrate, and transmit signals, thus playing a critical role in cellular responses to a variety of stimuli (38). In consequence, mitochondrial damage may lead to the impairment of various aspects of tissue functioning. Accumulating evidence supports the notion that malfunctioning mitochondria strongly participate in the pathogenesis of cardiovascular damage (43), associated with hypertension (26, 27, 107), cardiac failure, the metabolic syndrome and obesity (118), diabetes mellitus (23, 114), renal disease (44), atherosclerosis (96), as well as aging (25, 36, 46, 64).

Mitochondria and Angiotensin II

Angiotensin II (ANG II), a key component of the renin-angiotensin system (RAS), plays a pivotal role in the regulation of blood pressure, volume, and electrolyte balance. Derangements of the RAS contribute to hypertension and target-organ injury. ANG II can induce oxidant stress by enhancing the generation of both nitric oxide (NO) (88) and NAD(P)H oxidase-derived superoxide (92), thereby promoting peroxynitrite formation. ANG II can also induce endothelial NO synthase uncoupling, i.e., switching from NO to superoxide pro-
duction (74). In addition, ANG II was found to stimulate mitochondrial reactive oxygen species (mtROS) production. In rat endothelial cells, ANG II-activated mtROS generation activates redox-sensitive NF-κB, which is followed by a stimulation of vascular cell adhesion molecule-1 expression, a cytokine involved in atherosclerosis lesion formation (89). In mice, acute (24 h) and chronic (14 day) ANG II infusion led to a decreased cardiac expression of mitochondrial electron transport chain and Krebs cycle genes (65), supporting previous observations that indicated a role for ANG II in the depression of mitochondrial energy metabolism (16, 94, 99). Moreover, in rat vascular smooth muscle cells (VSMCs) and aorta in vivo, ANG II lowers mitochondrial membrane potential as a result of the stimulation of mtROS production (62). In cat myocardium, the response to stretch comprises the activation of NAD(P)H oxidase by ANG II, leading to mtROS release (13). Recently, it was shown that the molecular mechanisms involved in ANG II-mediated mitochondrial dysfunction (increased mitochondrial H$_2$O$_2$ production, and decreased mitochondrial glutathione, state 3 respiration, and membrane potential) include PKC activation, which in turn activates bovine aortic endothelial cell NAD(P)H oxidase and stimulates peroxynitrite formation (28). Notably, mitochondrial dysfunction was associated with endothelial dysfunction as indicated by the decrease of endothelial NO production (28).

Another link between ANG II and mitochondrial dysfunction is suggested by data showing that mitochondrial p66Shc plays a crucial role in ANG II-induced myocardial remodeling (42). p66Shc, a protein partially localized in the mitochondrial intermembrane space, was suggested to contribute to mitochondrial ROS production by subtracting electrons from cytochrome c and transferring them to oxygen to generate superoxide.

Other evidence indicates a direct interaction between ANG II and mitochondrial components. [125I]-labeled ANG II was detected in heart, brain, and smooth muscle cell mitochondria and nuclei (91, 98). In rat, adrenal zona glomerulosa, renin, angiotensigen, and angiotensin-converting enzyme (ACE) were detected within intramitochondrial dense bodies (85), and in rat cerebellar cortex mitochondria, ANG II immunoreactivity was also found (32). Therefore, it is apparent that the effects of ANG II on mitochondria may be either 1) dependent on NAD(P)H oxidase activation or 2) direct.

In this line, we have shown that in rodent models of hypertension, diabetes, and aging, ANG II blockade not only attenuates oxidant production but also improves mitochondrial function (23, 25–27). In support of the proposed direct actions of ANG II, recent data showed that in rat embryonic VSMCs, ANG II can exert effects from the cell interior, independently from extracellular actions, and seemingly through binding to intracellular receptors different from ANG II type 1 (AT$_1$) and type 2 (AT$_2$) receptors (35) or to AT$_1$ nuclear receptors (69). The internalization of ANG II can be mediated by the internalization of AT$_1$ receptors bound to ANG II, as was reported in rat aortic smooth muscle cells (1), hepatocytes (54), human VSMCs (8), and renal proximal tubule epithelial cells (103). In some cells, ANG II internalization is mediated by megalin, although this mechanism is thought to mark internalized ANG II for decomposition (39).

**Mitochondria and ANG II inhibitors.** The renal and cardiac benefits of ACE inhibitors and AT$_1$ receptor blockers in patients with hypertension, cardiovascular disease, and diabetes (10, 22, 86) seem to be at least partly independent from their blood pressure-lowering actions (10, 22, 82, 84). In this regard, RAS inhibitors were proposed to act as a magic bullet against oxidant stress (79).

To further investigate the cellular mechanisms responsible for the protective actions of RAS inhibitors, we set forth to study the effects of these drugs on mitochondria. First, we showed that ACE inhibition (enalapril) in aging mice prevented the lowering of mitochondrial number (34), attenuated age-related mitochondrial structure changes in myocardocytes and hepatocytes (unpublished observation), and significantly increased animal survival (34), suggesting that natural aging mechanisms had been altered in enalapril-treated animals. The latter action on mice survival is in agreement with recent data in rats (5). Later, we found that in aging rats, a long-term treatment with enalapril or AT$_1$ receptor blocker losartan improved kidney mitochondria functioning, when compared with mitochondria isolated from untreated old rats (25). In the same study, a general improvement in mitochondrial number and structure was observed, indicating that both RAS inhibitor strategies protect mitochondrial components and function from certain effects of aging. In another study, we showed that in spontaneously hypertensive rats, RAS inhibition, but not Ca$^{2+}$ channel blockade (amlodipine), protects kidney mitochondria from hypertension-related damage (26, 27). Furthermore, in streptozotocin-diabetic rats, a model of type 1 diabetes, losartan, but not amlodipine, protected kidney mitochondria function without normalizing plasma glucose (23). In accordance with our findings in the kidney, ANG II inhibition was shown to improve cardiac mitochondria energy production (75, 76, 80), and in captopril-treated diabetic rats, the expression of genes related to energy production was upregulated (19).

Concerning the potential factor(s) that may mediate the effects of ANG II inhibitors on mitochondrial function, a recent study suggests that mitochondrial NO may contribute to the mitochondrial effects of enalapril in the kidney (87). Other studies showed that AT$_1$ receptor blockers can modulate uncoupling protein (UCP) mRNA levels in mouse brown adipose tissue (3) and rat liver (21) or UCP protein content in rat kidney (25, 87). UCps, by uncoupling mitochondrial electron transport from ATP production, can affect mitochondrial energy output, as well as modulate mitochondrial oxidant production.

**Cytoskeleton, Extracellular Matrix, and Mitochondria**

The cytoskeleton influences mitochondrial morphology and function. The ability of eukaryotic cells to move, assume their shape, resist mechanical stress, and accommodate their internal constituents, including organelles, proteins, RNA, and other materials, depends on the proper functioning of the cytoskeleton. The three main types of cytoskeletal filaments, i.e., microtubules, microfilaments, and intermediate filaments, form a dynamic structure whose constant remodeling is necessary for normal cytoskeletal function. Thus cell survival depends on an equilibrium between the assembly and disassembly of tubulin, actin, and other types of proteins that compose microtubules, microfilaments, and intermediate filaments, respectively (53). The dynamic behavior of these filaments requires the input of energy that is provided by the hydrolysis of either ATP by tubulin or ATP by actin subunits.
Less is known on the mechanism of assembly and disassembly of intermediate filaments, but protein phosphorylation/dephosphorylation seems to be involved. The functional efficacy of cytoskeletal microtubules, microfilaments, and intermediate filaments is dependent on a number of accessory proteins that bind the filaments to each other and to other cell components. The accessory proteins include motor proteins that impel organelles or secretory vesicles along the filaments or move the filaments themselves by hydrolyzing ATP. Therefore, ATP-producing mitochondria are needed to support the energy requirements of cytoskeletal structure dynamics. Conversely, mitochondria need to interact with cytoskeletal elements to achieve normal organelle motility, morphology, localization, and function (9). Thus, in multicellular eukaryotes, mitochondria are transported along the microtubules, whereas in certain cells, such as neurons, actin filaments sustain short-length mitochondrial displacements (37). Also, actin filaments mediate the immobilization of mitochondria at intracellular locations of intense neuronal ATP consumption (9), and the association of mitochondria with actin filaments seems to facilitate the recruitment of proapoptotic cytosolic proteins to initiate apoptosis (101). It is apparent that an adequate distribution of mitochondria participates in sustaining organelle function and is necessary for cell survival (37).

The profoundly curved mitochondrial morphology is preserved, at least partly, by the attachment of mitochondria to the cytoskeletal tubulin and actin filaments (113), and the interaction between mitochondria and vimentin, an intermediate filament protein, also contributes to maintain mitochondrial morphology and organization (102).

Furthermore, the experimental depression of actin turnover stimulates the release of ROS from yeast mitochondria, thereby promoting apoptosis (40). This behavior indicates that the inability to reorganize the actin cytoskeleton in response to external or internal signals leads to the impairment of cell function. In mammalian cells, circumstantial evidence also points to the involvement of altered actin dynamics in prompting apoptosis, which seems to be mediated by increased mitochondrial ROS production (40).

Finally, because cytoskeletal filaments provide both a direct mechanical connection between most cell structures and an extensive negatively charged binding site for signaling molecules in response to activation of membrane receptors, the cytoskeleton participates in the transmission of signals from the plasma membrane to the cell interior (53).

In consideration of the above-mentioned functions, it is likely that the disturbance of the cytoskeleton interferes with normal mitochondrial and cellular function and may lead to pathological consequences. Interestingly, abundant evidence shows that ANG II induces cytoskeletal changes.

**ANG II and the Cytoskeleton.** In addition to its role as a vasoactive peptide, ANG II is a growth factor that variously regulates the genetic expression of extracellular matrix (ECM) proteins, other growth factors, cytokines, cell adhesion molecules, and vasoactive agents in vascular, cardiac, and kidney mesangial cells (59) and, in this way, can contribute to the pathogenesis of vascular remodeling in hypertension and atherosclerosis. Of note, ANG II (93) induces changes in cytoskeletal filament organization (filament polymerization, stress fiber formation, and assembly of focal adhesions; for focal adhesions, see *The cytoskeleton-ECM connection* (117), which also participate in pathological vascular alteration. Thus, in rat VSMCs, ANG II stimulates the formation of actin stress fibers and focal adhesions as a result of c-Src activation (51) and induces polymerization and contraction of actin filaments as a result of p38 MAPK activation by a pathway that involved ROS generation, but not the classic AT1 receptor signaling pathways (no activation of PKC or Tyr kinases) (72). In cultured rat VSMCs, ANG II stimulates tyrosine phosphorylation of paxillin and focal adhesion kinase (FAK), two components of actin-associated focal adhesions, via AT1 receptors (81), and these responses involve PKC-dependent and -independent pathways and are accompanied by cytoskeletal reorganization (formation of actin stress fiber sand focal adhesions) (108). Conversely, the disturbance of actin organization suppresses ANG II-mediated signaling (41).

In addition, ANG II prompts the phenotypic conversion of tubulointerstitial (115) and cardiac fibroblasts (14) into myofibroblasts, which express α-smooth muscle actin (α-SMA), which is not expressed in normal fibroblasts. ANG II also stimulates the expression of α-SMA in glomerular mesangial cells (55) and human VSMCs (2) and the intermediate filament protein desmin in glomerular epithelial cells (55). In agreement with the above data, chronic enalapril treatment prevented age-related and hyperoxaluria-related (106) α-SMA accumulation in rodent renal interstitium. Also, glomerular and cortical and medullary interstitial α-SMA staining was significantly lower in quinapril-treated than in untreated obese Zucker rats (90).

In summary, available evidence indicates that 1) mitochondrial function is altered in response to both cytoskeletal disturbance and ANG II and 2) ANG II changes cytoskeletal organization. Therefore, it is possible to hypothesize that the depression of mitochondrial energy metabolism brought about by ANG II is preceded by the ANG II-induced derangement of the cytoskeletal filament network.

**The ECM Influences Mitochondrial Morphology and Function.** The ECM plays a key role in the maintenance of tissue structure and function. ECM turnover is largely regulated by firmly controlled matrix metalloprotease (MMP) activities. An abnormal turnover of matrix components is associated with disease (71). Although ECM remodeling is critical for proper development and tissue repair, a faulty remodeling contributes to tissue fibrosis. Various high blood pressure conditions display cardiovascular and renal remodeling, which are manifested by tissue hypertrophy and inadequate changes in the quantity and quality of the ECM (17, 30) and which lead to the pathogenesis of fibrotic diseases.

Accumulating evidence shows that mitochondria may act as sensors for changes in ECM composition. Thus, in genetically modified mice, skeletal muscle collagen VI deficiency resulted in structural mitochondria alterations—abnormal cristae and modified matrix density—and mitochondrial dysfunction, leading to muscle cell apoptosis (50). Fibronectin, another ECM protein, conveys survival signals for many cell types, protecting them from apoptosis (56). Accordingly, a knockdown of fibronectin mRNA expression induced apoptosis in rat mesangial cells, and mitochondria-derived signaling was involved in the response (119). Also, under oxidative stress conditions, MMP-2 negatively regulates cardiac mitochondria function (122). Conversely, an alteration of mitochondrial functioning triggers ECM changes. Thus oxidative phosphorylation deficiency activates the transcription of ECM remodeling genes.
(including MMP) in cultured osteosarcoma cells (110). This evidence indicates that a delicate relationship seems to exist between the ECM and mitochondria. As described above for its ability to modify cytoskeletal organization, ANG II also alters the protein composition of the ECM.

**ANG II AND THE ECM.** As already mentioned in **ANG II AND THE CYTOSKELETON**, ANG II is involved in the regulation of ECM protein gene expression. In the heart, ECM proteins are mainly produced by fibroblasts; therefore, fibroblasts play a crucial role in cardiac fibrosis development. In cultured rat cardiac fibroblasts, ANG II increases the synthesis and secretion of collagen (21a), fibronectin and laminin (52), and osteopontin expression (4). When infused into rats, ANG II induces the increase of left ventricular fibronectin and collagens I and III mRNAs (61).

Rat VSMCs exposed to ANG II display increased levels of collagen protein (57), fibronectin (100), laminin and tenascin (97) mRNA, and protein. In vivo, the infusion of ANG II increases fibronectin mRNA and protein in rat aorta (60).

ANG II also stimulates the synthesis of ECM collagens, fibronectin, and laminin by renal mesangial cells, tubular cells, and interstitial fibroblasts. Seemingly, these effects are mediated by TGF-β and plasminogen activator inhibitor type 1, among other factors (73).

Pharmacological inhibition of the RAS attenuates ECM deposition in the kidney and in the vasculature (for review, see Ref. 59). In accordance with ANG II actions on ECM production, chronic enalapril treatment reduced age-associated renal (49) and myocardial (48) collagen deposition in female mice, hyperoxaluria-related TGF-β and collagen type III immunostaining in the tubulointerstitial area (106), cavernous tissue collagen type III immunolabeling in spontaneously hypertensive rats (105), and collagen accumulation in the heart, liver, and kidneys of streptozotocin-diabetic rats (24).

At this point, a new element can be added to the hypothesis proposed in **ANG II AND THE CYTOSKELETON**, as follows. A body of evidence indicates that 1) mitochondrial function is altered in response to cytoskeletal disturbance, ECM disturbance, and ANG II and 2) ANG II changes both cytoskeletal and ECM organization. Therefore, it is possible to hypothesize that the depression of mitochondrial energy metabolism brought about by ANG II is preceded by an ANG II-induced derangement of the cytoskeletal filament network and/or ECM organization.

**The cytoskeleton-ECM connection.** The cytoskeleton and the ECM are structurally and functionally connected through the integrins. Integrins are plasma membrane cell adhesion proteins that act as matrix receptors and bind ECM proteins, including collagen, fibronectin, vitronectin, and laminin, to the actin cytoskeleton. Importantly, the binding of integrins to their ligands activates intracellular signaling pathways that provide the cells with critical information concerning the nature of the surroundings (7), allowing them to respond to the environment. Integrins sense not only the biochemical composition but also the mechanical properties of the ECM. Integrin-mediated cell adhesion signaling conveys information that influences the growth, division, differentiation, morphology, survival, programmed death, and mobility properties of the cell (66). Integrin receptors cluster at clearly defined ranges of cell contact with the ECM, known as focal adhesions. Focal adhesions are large macromolecular assemblies involved in cell anchorage, which also function as biochemical signaling hubs by concentrating and directing signaling proteins. One of the earliest events in response to integrin stimulation is protein phosphorylation that results from the activation of protein-tyrosine kinases, such as members of the Src family and the cytoplasmic FAK (95). Other intracellular second message systems that are activated upon binding of integrins to their ligands include MAPKs, Rho family GTP-binding proteins,
calcium channels, phosphatidylinositol-4,5-bisphosphate, phospholipase C, the Na/H antiporter, tyrosine and serine/threonine kinases, phosphatases, and cyclin D1. Integrins also transduce messages from the inside out, whereby the intracellular milieu is able to regulate the adhesive activity of integrins.

Of note, integrin-mediated signaling was recently found to modify mitochondrial function, leading to changes in gene expression and without triggering apoptosis (116). In this context, ANG II was shown to exert intracellular effects by inducing integrin/focal adhesion changes.

ANG II AND INTEGRINS. Abundant evidence indicates that the effects of ANG II on ECM formation involve changes in integrin/focal adhesion signaling. In rat cardiac fibroblasts, ANG II, by acting on its AT_1 receptor, enhances α_5-, β_1-, β_3-, and β_5-integrins; osteopontin; and α-actinin mRNA and protein levels, and this effect is accompanied with an enhanced cell attachment to the ECM and a phosphorylation of FAK (58). In cultured rat VSMCs, ANG II stimulates paxillin and FAK phosphorylation via AT_1 receptors (81).

In adrenal glomerulosa cells, ANG II seems to inhibit the proliferation and initiation of steroidogenesis by interfering with extracellular matrix/integrin signaling at the level of paxillin binding to focal adhesions, which activates RhoA/B and Rac and disrupts actin stress fiber organization (83). In human umbilical vein endothelial cells, ANG II stimulates integrin-β_3 expression in part by activating NF-κB (67). Importantly, studies in vivo using genetically altered mice lacking α_1-integrin showed that

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**Table 1. Effects of ANG II on mitochondria, cytoskeleton, and extracellular matrix**

<table>
<thead>
<tr>
<th>Effect</th>
<th>Tissue/Cell Type</th>
<th>Experimental Model</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>Mitochondrial changes</td>
<td></td>
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<tr>
<td>↑ mitROS</td>
<td>Rat aortic ECs</td>
<td>Cell culture</td>
<td>89</td>
</tr>
<tr>
<td></td>
<td>Bovine aortic ECs</td>
<td>Cell culture and isolated mitochondria</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>Rat VSMCs</td>
<td>Cell culture</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>Rat aorta</td>
<td>In vivo ANG II infusion</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>Cat myocardium</td>
<td>Response to stretch</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Rodent heart</td>
<td>AngII in vivo infusion, mice</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mouse heart failure model</td>
<td>16, 94, 99</td>
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<tr>
<td></td>
<td></td>
<td>Rat heart failure model</td>
<td>16, 94, 99</td>
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<tr>
<td>↓ Mitochondrial respiratory function</td>
<td>Rat VSMCs</td>
<td>Cell culture</td>
<td>62</td>
</tr>
<tr>
<td>Cytoskeletal changes</td>
<td></td>
<td></td>
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<tr>
<td>Actin stress fiber and focal adhesion formation</td>
<td>Rat aortic VSMCs</td>
<td>Cell culture</td>
<td>51, 108</td>
</tr>
<tr>
<td>Polymerization and contraction of actin filaments</td>
<td>Rat aortic VSMCs</td>
<td>Cell culture</td>
<td>72</td>
</tr>
<tr>
<td>Formation of α-SMA</td>
<td>Tubulointerstitial fibroblasts</td>
<td>Cell culture</td>
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<td>Cardiac fibroblasts</td>
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<td></td>
<td>Glomerular mesangial cells</td>
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<td>Human VSMCs</td>
<td>Cell culture</td>
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<td></td>
<td>Glomerular epithelial cells</td>
<td>In vivo ANG II infusion</td>
<td>55</td>
</tr>
<tr>
<td>Extracellular matrix changes</td>
<td></td>
<td></td>
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<tr>
<td>↑ Synthesis and/or secretion of ECM proteins</td>
<td>Rat cardiac fibroblasts</td>
<td>Cell culture</td>
<td>4, 21a, 52</td>
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<td></td>
<td>Rat VSMCs</td>
<td>Cell culture</td>
<td>57, 97, 100</td>
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<td>Rat aorta</td>
<td>In vivo ANG II infusion</td>
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<td></td>
<td>Renal mesangial and tubular cells, and interstitial fibroblasts</td>
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<td>Changes in integrin expression or signaling</td>
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<tr>
<td>↑ Integrin expression</td>
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<td>58</td>
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<td>Human umbilical vein ECs</td>
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<td></td>
<td>Mouse aorta</td>
<td>ANG II administration to genetically altered mice</td>
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<td>Changes in integrin signaling</td>
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<td>Cell culture</td>
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<td>Adrenal glomerulosa cells</td>
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<tr>
<td></td>
<td>Mouse aorta</td>
<td>ANG II administration to genetically altered mice</td>
<td>70</td>
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</table>

mtROS, mitochondrial reactive oxygen species; α-SMA, α-smooth muscle actin; ECs, endothelial cells; VSMCs, vascular smooth muscle cells.

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**Table 2. Effects of ANG II on TGF-β_1 formation, and associated cytoskeletal and extracellular matrix changes**

<table>
<thead>
<tr>
<th>Effect</th>
<th>Tissue/Cell Type</th>
<th>Experimental Model</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>↑ TGF-β_1 production and signaling</td>
<td>Human hepatic stellate cells and rat cardiac fibroblasts</td>
<td>Cell culture</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>Human lung fibroblasts</td>
<td>Cell culture</td>
<td>109</td>
</tr>
<tr>
<td></td>
<td>Human kidney tubulo epithelial cells</td>
<td>Cell culture</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Human myocardium, cardiac fibroblasts</td>
<td>Biopsy incubation, cell culture</td>
<td>63</td>
</tr>
<tr>
<td>↑ TGF-β_1 depends on integrin signalling</td>
<td>Mouse VSMCs</td>
<td>Cell culture</td>
<td>6</td>
</tr>
<tr>
<td>↑ TGF-β_1 and fibronectin</td>
<td>Rat cardiac fibroblasts</td>
<td>Cell culture</td>
<td>77</td>
</tr>
<tr>
<td>↑ TGF-β_1 and collagen</td>
<td>Human myocardium</td>
<td>Biopsy incubation</td>
<td>63</td>
</tr>
</tbody>
</table>

TGF-β_1, transforming growth factor-β_1.
signaling through αβ3-integrin and focal adhesions is involved in ANG II-induced vascular hypertrophy (70).

ANG II, TGF-β, ECM, CYTOSKELETON, AND MITOCHONDRIA. In addition to the varied effects mentioned in ANG II AND THE CYTOSKELETON, ANG II plays a pivotal role in fibrosis, at least in part, by stimulating TGF-β1 production and enhancing TGF-β1 signaling in activated human hepatic stellate cells and rat cardiac fibroblasts (120), human lung fibroblasts (109), human kidney tubular epithelial cells (15), and human myocardium (63). TGF-β1 is a pleiotropic cytokine that plays a crucial role in the regulation of ECM assembly and remodeling, mainly by stimulating both the expression of collagens, fibronectin, and proteoglycans and the production of proteases that inhibit ECM breakdown. A persistent activation of TGF-β receptors leads to the anomalous connective tissue deposition associated with fibrotic disease (112). In mouse VSMCs, the induction of TGF-β1 and collagen type I release by ANG II is dependent on αβ3-integrin signaling (6). In rat cardiac fibroblasts, an ANG II-induced expression of fibronectin and TGF-β is mediated by a transactivation and downstream signaling of the epidermal growth factor receptor, and TGF-β contributes to the modulation of fibronectin mRNA stabilization (77). In the human myocardium, ANG II seems to increase collagen expression indirectly by first increasing TGF-β1 expression (63).

Apart from inducing ECM changes, the binding of TGF-β to plasma membrane receptors activates intracellular signaling pathways that are involved in the regulation of actin cytoskeleton reorganization (78, 111). These actin filament rearrangements contribute to the TGF-β effects on cell growth and differentiation (31).

Interestingly, TGF-β seems to interact with mitochondria. Recently, TGF-β1 was found to induce growth arrest and acquisition of senescent phenotypes in lung epithelial cells, and this effect was mediated by decreased mitochondrial complex IV activity and the subsequent increase of mitochondrial ROS production (121). The treatment of fetal hepatocytes with TGF-β1 was followed by an increased mitochondrial ROS generation, a lowering of mitochondrial membrane potential, and finally apoptosis (47). Also, TGF-β1 was found within the mitochondria in rat and mouse cardiac myocytes and rat hepatocytes (45) and in mouse T lymphocytes (20) where it seems to contribute to maintain mitochondrial structure and function.

These findings point to TGF-β1 as a potential link between ANG II, ECM, and cytoskeleton derangements and mitochondrial dysfunction. Interestingly, ANG II was recently shown to stimulate nuclear AT1a receptors directly to induce TGF-β1 transcription in rat renal cortical nuclei (68).

Summary and Hypothesis

Based on the above evidence, the hypothesis proposed in ANG II AND THE ECM can be completed as follows. A body of evidence indicates that 1) mitochondrial function is altered in response to cytoskeletal disturbance, ECM disturbance, and ANG II; 2) by acting on its AT1 receptors, ANG II changes both cytoskeletal and ECM organization; 3) ANG II exerts cytoskeletal and ECM effects by inducing integrin/focal adhesion changes; and 4) ANG II-initiated integrin signaling results in the release of TGF-β1, a cytokine that modifies ECM composition and structure, induces the reorganization of the cytoskeleton, and modifies mitochondrial function. Therefore, it is possible to hypothesize that the depression of mitochondrial energy metabolism brought about by ANG II is preceded by ANG II-induced integrin signaling and the consequent derangement of the cytoskeletal filament network and/or ECM organization. ANG II-dependent TGF-β1 release is a potential link between ANG II, ECM, and cytoskeleton derangements and mitochondrial dysfunction (Fig. 1). It is necessary to emphasize that the present hypothesis is among many other plausible explanations for ANG II-mediated mitochondrial dysfunction, since ANG II has complex, immediate effects. Thus the binding of ANG II to its AT1 receptor not only activates various G proteins and the JAK2/STAT pathway but also transactivates platelet-derived growth factor and epidermal growth factor receptors and, by homo- or heterodimerizing with AT1, AT2, bradykinin B2, β2-adrenergic, and dopamine D1 receptors, activates other signaling pathways (33). Also, as mentioned in Mitochondria and Angiotensin II, ANG II stimulates cellular ROS production, which can have a crucial effect on mitochondrial function (Fig. 1).

A potential limitation of this proposal is that the results compiled in the present report were obtained in different tissues or cellular systems, by using various experimental models (See Tables 1 and 2).

Further research is needed to confirm the veracity of the present proposal, which, if proven to be correct, would underscore the relevance of inhibiting the RAS to preserve mitochondrial structure and function in those conditions associated to the enhancement of ANG II signaling.

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