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Angiotensin receptors as determinants of life span

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Abstract Angiotensin II (Ang II), the central product of renin-angiotensin system, has a role in the etiology of hypertension and in pathophysiology of cardiac and renal diseases in humans. Other functions of Ang II include effects on immune response, inflammation, cell growth and proliferation, which are largely mediated by Ang II type 1 receptor (AT_1). Several experimental studies have demonstrated that Ang II acts through AT_1 as a mediator of normal aging processes by increasing oxidant damage to mitochondria and in consequences by affecting mitochondrial function. Recently, our group has demonstrated that the inhibition of Ang II activity by targeted disruption of the *Agtr1a* gene encoding Ang II type 1A receptor (AT_{1A}) in mice translates into marked prolongation of life span. The absence of AT_{1A} protected multiple organs from oxidative damage and the alleviation of aging-like phenotype was associated with increased number of mitochondria and upregulation of the prosurvival gene sirtuin 3. AT_1 receptor antagonists have been proven safe and well-tolerated for chronic use and are used as a key component of the modern therapy for hypertension and cardiac failure, therefore Ang II/AT_1 pathway represents a feasible therapeutic strategy to prolong life span in humans.

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Abbreviation

RAS	renin-angiotensin system
Ang II	angiotensin II
ACE	angiotensin converting enzyme
AT_1	Ang II type 1 receptor
AT_2	Ang II type 2 receptor
NO	nitric oxide
eNOS	endothelial nitric oxide synthase
ROS	reactive oxygen species
ACEi	angiotensin-converting enzyme inhibitors
ARBs	angiotensin II receptor blockers
SIRT	sirtuin
Nampt	nicotinamide phosphoribosyltransferase
IGF-1	insulin growth factor-1

Introduction

The mean life span has been increasing steadily over the course of human evolution, and in the last century, the human life expectancy in developed countries has nearly doubled as indicated by an increase from around 50 to 75–80 years [12]. Before 1950, most of the gain in life expectancy was due to marked decrease in death rates at younger ages. In the second half of the 20th century, the observed reduction in mortality at ages above 65 years could be ascribed to the delay in the onset of several age-related disorders and to the increased capacity to prevent organ damage, as a consequence of improved biomedical and nutritional conditions [47]. Therefore, ensuring disease-free survival and not merely survival per se represents an

attractive and desirable goal for society as a whole. Healthy aging and longevity depend on the dynamic interaction between biological and environmental factors, including medical care, healthy diet, and lifestyle. However, emerging evidence from model organisms has indicated that several genetic factors might play a role in longevity, putting the attention on several molecular candidates involved in pathways contributing to protect organs from degeneration and diseases.

In the last 20 years, one of the main goals of our research was to find out therapeutic strategies to protect the kidney from progressive renal injury with the final aim to reduce the need of dialysis in patients. To this aim, our efforts have been devoted to identify factors implicated in the progression of chronic kidney disease, whose incidence is increasing worldwide at an alarming rate. Experimental and clinical evidence is available that blockade of the renin angiotensin system (RAS) by angiotensin converting enzyme inhibitors (ACEi) and angiotensin II receptor blockers (ARB) is effective in slowing the progression of kidney disease due to the drugs' ability to reduce proteinuria [53]. When ACEi and ARB were given in combination to rats genetically predisposed to progressive nephropathy, reduction of glomerular sclerosis was even more evident, particularly in those glomeruli that had less severe lesion to begin with. This shows that remodeling of glomerular architecture is possible, which would imply some form of regeneration of the capillary network [53]. Recent clinical trials suggested that inhibition of RAS might actually prevent nephropathy in patients with chronic renal failure of nondiabetic origin (the Ramipril Efficacy in Nephropathy study) [57]. The effectiveness of ACEi in protecting the kidney against the development of microalbuminuria, which is a major risk factor for cardiovascular events and death, has been also documented in patients with type 2 diabetes (the Bergamo Nephrologic Diabetes Complications Trial study) [56].

Strategies able to reduce renal disease progression could translate into a decreased incidence of cardiovascular events. A tremendous body of research, both experimental and clinical, has unequivocally shown that pharmacologic blockade of RAS, beyond the renal protection, reduces cardiovascular risk more effectively than other antihypertensive treatments [54]. Inhibition of RAS prevents end-organ damage associated with aging [14], in line with evidence that angiotensin II (Ang II) promotes the onset and the progression of vascular senescence, associated with vascular, functional, and structural changes contributing to age-related vascular disease [43].

In the present review, we focus on the recent emerging data suggesting a role of Ang II in aging. In addition we highlight the mechanisms by which Ang II via AT_1 could affect life span in mammals.

The renin-angiotensin system

Renin-angiotensin system is considered to be the major regulator of blood pressure and fluid homeostasis. The main effector molecule of the RAS, Ang II, is produced from the substrate angiotensinogen through sequential enzymatic cleavages by renin and angiotensin converting enzyme (ACE). In particular renin cleaves angiotensinogen, forming Ang I that in turn is converted to Ang II by ACE (Fig. 1). ACE is a circulating enzyme found in the endothelial cells of the lung, vascular endothelium, and cell membranes of the kidney, heart, and brain. ACE also degrades bradykinin to inactive fragments, reducing the serum levels of endogenous vasodilators [8].

Ang II causes increases in systemic and local blood pressure via its vasoconstrictive effect, influences renal tubuli to retain sodium and water, and stimulates aldosterone release from adrenal gland [69]. Besides being a potent vasoconstrictor, Ang II exerts several prominent nonhemodynamic effects including proliferative, proinflammatory, and profibrotic activities [58].

At the cellular level, responsiveness to Ang II is conferred by the expression of two classes of pharmacologically distinct rhodopsin-like G protein-associated receptors, the type 1 and the type 2 receptors (AT_1 and AT_2) [61, 69]. AT_1 has been cloned in a number of species and two subtypes [59], named AT_{1A} and AT_{1B}, have been identified in rat and mouse. AT_{1A} is the predominantly expressed receptor in different body districts including kidney, liver, heart, blood vessels, adrenal glands, and cardiovascular control centers in the brain [11], and is considered the closest murine homolog to the single human AT_1. AT_{1A} confers most of the classical actions of Ang II such as blood pressure increase [36], aldosterone release from the adrenal zona glomerulosa [1], salt retention in renal proximal tubuli [42], and stimulation of the sympathetic nervous system via receptors in the brain [17]. The expression of the AT_{1B} appears to be more prominent in the anterior pituitary gland and the adrenal zona glomerulosa. AT_{1B} regulates blood pressure when AT_{1A} is absent [48].

The expression of the AT_2 is high in the fetus, but low in adult tissues. AT_2 is expressed in the adrenal medulla, uterus, ovary, vascular endothelium, and distinct brain areas [65]. AT_2 interacts with and modulates actions perpetuated by the AT_1, possibly antagonizing many of its effects. The binding of Ang II to the AT_2 activates vasorelaxation of conduit and resistant arteries and improves resistance artery remodeling, promotes cardiovascular protection against ischemia-reperfusion injury and acute myocardial infarction, inhibits cardiac fibrosis, and protects the kidney from ischemic injury [60]. In a mouse model of renal ablation, the lack of AT_2 aggravates renal injury and reduces survival [6].

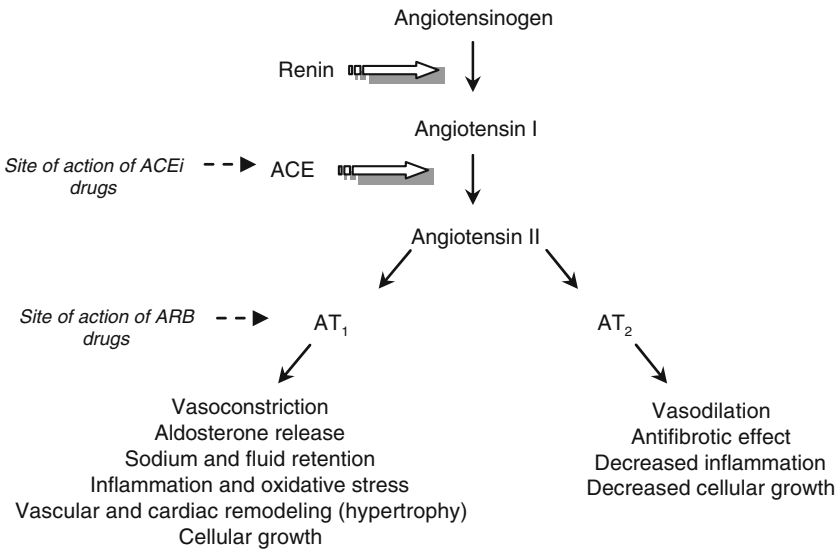

Fig. 1 Overview of RAS. *ACE* angiotensin converting enzyme; *AT_1* angiotensin type 1 receptor; *AT_2* angiotensin type 2 receptor; *ARB* angiotensin II receptor blockers

Link between angiotensin II and oxidative stress

Angiotensin II is known to contribute to oxidative stress damage by stimulating the generation of both nitric oxide (NO) and NAD(P)H oxidase-derived superoxide in the cytosol of different cell types including endothelial, vascular smooth muscle, fibroblast and tubular epithelial cells [51, 55]. The interaction between NO and superoxide generates peroxynitrite, a cytotoxic anion that inhibits mitochondrial electron transport, destroys DNA and cellular proteins, leading to oxidative stress damage [52]. Furthermore, Ang II can induce endothelial nitric oxide synthase (eNOS) uncoupling, switching from NO to superoxide production [46]. Ang II stimulates both cytosolic and mitochondrial reactive oxygen species (ROS) generation [64] (Fig. 2). The direct interaction between Ang II and mitochondrial components has been suggested by the presence of Ang II in mitochondria of brain, heart, and smooth muscle cell in rodent [22]; moreover, renin, angiotensinogen, and ACE were also detected within intramitochondrial dense bodies [50].

One of the most prominent theories to explain aging is the "free-radical theory" of aging which was initially proposed by Harman in 1950 s [30]. It postulates that the loss of cell functional capacity associated to senescence results from the accumulation of ROS-inflicted oxidative stress damage to different molecules, leading to lipid peroxidation, protein oxidation and oxidative modifications in nuclear and mitochondrial DNA [21].

Reactive oxygen species are generated in multiple compartments and by multiple enzymes within the cells, including NAD(P)H oxidases on plasma membranes, lipid metabolism within the peroxisomes, and various cytosolic enzymes such as cyclooxygenase. The majority of intracellular ROS production derives from mitochondrial matrix and the space between the inner and outer mitochondrial membrane. Mitochondria utilize more than 90% of cellular oxygen to produce energy. While most oxygen is transformed into water, 1–2% of it forms superoxide [7]. Reactive oxygen species compromise mitochondrial integrity and function, leading to a decreased mitochondrial ATP generation, with a subsequent increased release of ROS by the mitochondria themselves, initiating a vicious cycle of

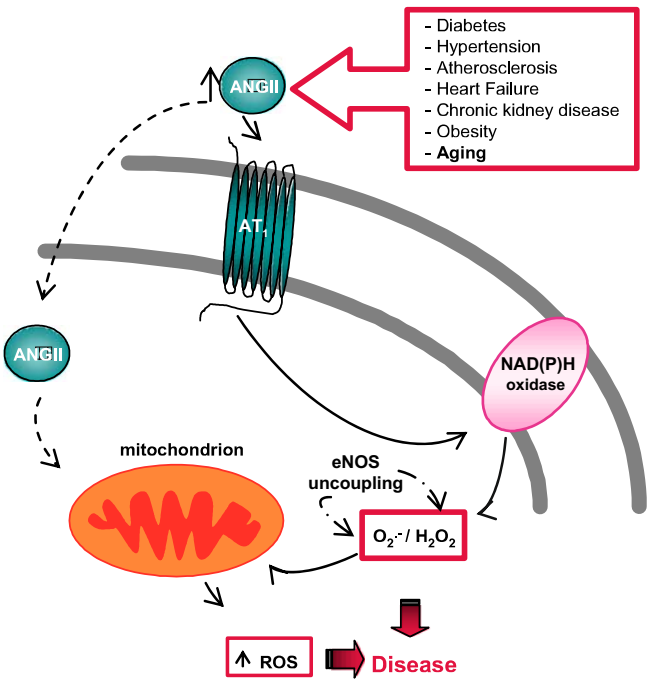

Fig. 2 Schematic representation of Ang II-induced oxidative stress damage. Ang II via AT_1 promotes oxidative stress by activating NAD(P)H oxidase-derived superoxide generation and by inducing eNOS uncoupling switching from NO to superoxide production. Ang II may also have a direct action on mitochondrial ROS production, independent of AT_1 signaling

progressively increasing oxidative stress [23]. The aging process is frequently associated to a reduction in mitochondrial number and several changes in mitochondrial structure, such as swelling, shortening of the cristae, and matrix vacuolization [10, 31, 67].

Under normal physiological conditions, the capacity of Ang II to promote oxidative stress is tightly regulated [20, 27]. By contrast, in conditions associated with RAS overactivation, such as aging [3, 68, 70], the dysregulation of Ang II-dependent ROS generation may become a significant contributor to cell oxidation and tissue damage. RAS overactivation exerts deleterious effects on renal and cardiac functions documented by the increase of Ang II peptide in urines [25] and increased generation of Ang II in the heart [28] of old animals. A recent in vitro study has demonstrated that the production of ROS induced by Ang II via AT_1 led to DNA damage, and consequently to accelerated aging of human vascular smooth muscle cells [32]. Cell senescence following ROS production has been proposed to be mediated by two different mechanisms of DNA damage: a telomere-independent pathway via the induction of stress induced premature senescence (SIPS) and a telomere-dependent mechanism via accelerated attrition of telomeres. This hypothesis has been confirmed by data showing that the critical DNA damage induced by AT_1-mediated ROS production both increased SIPS expressions that promoted cell cycle arrest and markedly accelerated the rate of telomere loss that is associated with reduced cellular proliferation and premature cell senescence [32].

All these findings demonstrate the crucial role of oxidative stress on the aging process and strongly support the involvement of Ang II in tissue senescence by virtue of its ability to mediate the release of oxidant species.

Protective effect of inhibiting angiotensin II on aging

Angiotensin-converting enzyme inhibitors and ARBs are two widely used classes of anti-hypertensive drugs that inhibit RAS at different levels. ACEi inhibit Ang II formation by binding to the active site of the enzyme that converts Ang I into Ang II, and ARBs prevent Ang II from binding to its receptors.

In aging animals, the cardiovascular protective effects occurred after RAS inhibition was associated with an increased NOS activity [26]. Moreover, in old animals, both enalapril and losartan treatments significantly increased NO production in heart homogenate, while reduced hydrogen peroxide formation [15]. In spontaneously hypertensive rats, the inhibition of RAS was able to reverse the naturally age-related and advanced myocardial hypertrophy and fibrosis by attenuating Ang II-mediated oxidative stress, as documented by reduced expression of NAD(P)H oxidative components p22phox, p47phox, and gp91phox in old hearts [37]. Furthermore, oxygen radicals mediated the accelerated cerebral endothelial dysfunction that occurs with age, and more importantly, old mice lacking AT_1 did not develop these age-related cerebral circulation damages [45].

Other studies performed in normal adult rats have clearly shown that chronic treatment with ACEi or ARB reduced kidney damage associated with age. Old animals treated with enalapril and losartan presented lower glomerular and tubulointerstitial fibrosis, reduced monocyte or macrophage infiltrates, and decreased tubular atrophy than untreated aged animals [24].

The beneficial effect of RAS inhibition involves the preservation of renal mitochondria from aging in rats. Enalapril and losartan treatments prevented the age-associated decline in the renal mitochondrial capacity for energy production and attenuated the age-associated increase in mitochondrial oxidant production [19].

A similar protective effect of RAS inhibition was also observed in the liver from old rats. In these animals the maintenance of an adequate mitochondrial function during aging was due to the enhanced transcription levels of the genes nuclear respiratory factor 1 and peroxisome proliferators activator receptor gamma coactivator-1α that are involved in mitochondrial respiration and biogenesis, respectively. These positive effects on mitochondria maintained the integrity of the hepatocyte system, and prevented liver fibrosis and the infiltration of inflammatory cells during aging [18].

The development of gene-targeting technology in mice has provided new insight into the role of RAS genes in regulating blood pressure, body fluid homeostasis, and fetal development. Mice that are unable to generate Ang II because of targeted mutation in the angiotensinogen ($Agt^{-/-}$) or angiotensin-converting enzyme ($Ace^{-/-}$) genes had a severe phenotype characterized by reduced survival, low blood pressure, and abnormal kidney morphology. A similar phenotype was observed in mice lacking both Ang II type 1 receptors ($AT_{1A}^{-/-}$ $AT_{1B}^{-/-}$) [49].

The disruption of the gene encoding AT_{1A} (*Agtr1a*)—the major mouse AT_1 receptor isoform—did not cause severe postnatal mortality or the structural abnormalities seen in the kidneys of the knockout models described above. Taking advantage from this mouse model, we have recently investigated the role of the AT_1 in end-stage organ damage. A prospective observational study was performed in homozygous mice deficient for the AT_{1A} and wild-type controls. $AT_{1A}^{-/-}$ mice substantially outlived their wild-type littermates by 26% (Fig. 3) and had normal body weight and physical activity as reflected by their ability to perform on a rotating system that evaluates motor coordination and vitality. Reduction in food intake of 20% and 40% in

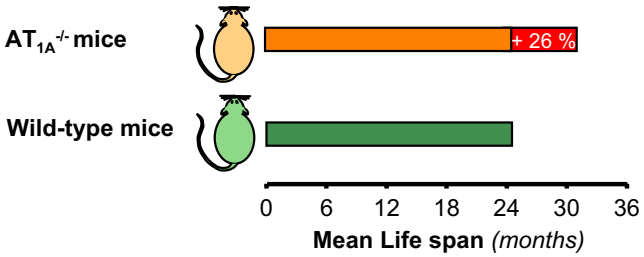

Fig. 3 Mean life span of AT_{1A}-deficient mice (31.20±2.31 mo) and their wild-type littermates (24.81±3.10 mo)

laboratory animals extends their life span by up to 50% [29]. Reduced caloric intake did not contribute to prolonged survival in $AT_{1A}^{-/-}$ mice, since daily food intake was virtually identical between $AT_{1A}^{-/-}$ and wild-type mice. $AT_{1A}^{-/-}$ grew up normally, and the body weights increased comparably to wild-type littermates ruling out the possibility that small body size could be responsible of extended life span as previously described [9].

Aging $AT_{1A}^{-/-}$ mice developed fewer aortic atherosclerotic lesions and less cardiac injury, as reflected by the reduction of myocyte size and fibrosis, with lower deposition of interstitial collagen with respect to wild-type mice [5]. These data point to a direct effect of Ang II via AT_{1A} on atherosclerotic lesion generation and on extracellular matrix deposition by cardiac fibroblasts.

Furthermore, aging mice lacking the AT_{1A} showed reduced production of peroxinitrite in hearts and aortas, as compared to wild-type animals, indicating a possible role of Ang II via AT_{1A} into the production of ROS [5]. Given the crucial role of mitochondria in producing peroxynitrite during aging processes [39], ultrastructural analysis of mitochondria was performed in proximal tubular cells of the kidney of $AT_{1A}^{-/-}$ mice that possess a large number of mitochondria and are highly dependent on mitochondrial energy production for proper function [33]. The lack of AT_{1A} protected the cells from the loss of mitochondria during aging [5], demonstrating that Ang II negatively influences mitochondrial number and function by promoting oxidative stress, and that the absence of AT_{1A} strongly attenuated the functional and structural changes that occur in kidney mitochondria following oxidative stress increase upon age [5].

Role of sirtuins in longevity associated with $AT_{1A}^{-/-}$ mice

Recent evidences have suggested that mitochondrial activity could be regulated by the expression of enzymes belonging to the Sirtuin family [16]. Sirtuins are nicotinamide adenine dinucleotide (NAD)-dependent deacetylases proteins highly conserved from *Escherichia Coli* to humans and associated with longevity, mitochondrial and cell cycle regulation, apoptosis, and DNA damage repair [29]. In humans and mice, there are seven different sirtuins (SIRT1-7), and three are located in the mitochondria (SIRT3, 4, and 5). Among

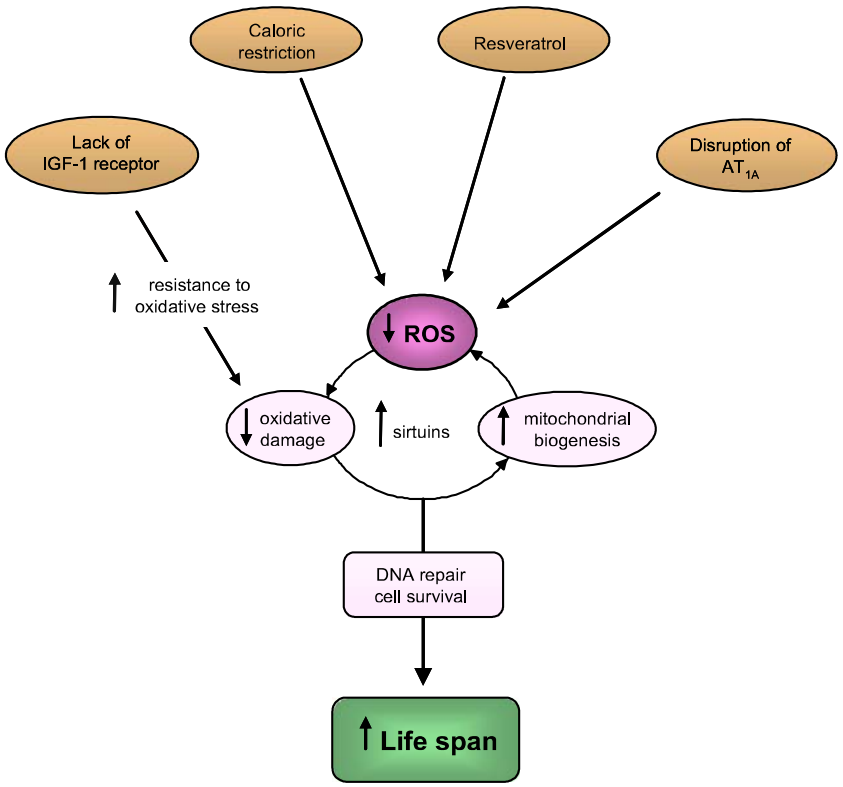

Fig. 4 Different pathways involved in life span regulation preserve mitochondrial and cellular wellness and promote longevity by modulating ROS production and sirtuin expression

them, SIRT3 has an apparent direct link to extended life span in humans, in fact mutations in an enhancer region of the *Sirt3* gene that potentially upregulate its expression were found at a high frequency in long-lived individuals [4]. Under oxidative stress, SIRT3 overexpression protects the cardiomyocytes against Bax-mediated apoptosis by deacetylating the substrate Ku70, promoting the binding of Ku70 to Bax, and hence blocking the Bax activation [66]. Of note, SIRT3 regulates adaptive thermogenesis and decreases mitochondrial membrane potential and reactive oxygen species production, while increasing cellular respiration [62]. For these reasons SIRT3 acts as sensor of small reactive oxygen species that could lead to mitochondrial damage and activates specific cellular signaling pathway to counteract oxidative stress such as the expression of MnSOD antioxidant protein [38]. The recent identification of the two substrates such as acetyl coenzyme A synthetase and glutamate dehydrogenase as targets of SIRT3 revealed that this molecule controls a regulatory network involved in energy metabolism and in mechanisms of caloric restriction and life span determination [40, 41].

SIRT3 could exert its action only in the presence of the cosubstrate NAD^+, and the concentration of NAD^+ determines cell survival. In the context of nutrient restriction, mitochondria dictate cell survival through the upregulation of nicotinamide phosphoribosyltransferase (Nampt) that boosts mitochondrial NAD^+ concentration [71]. Altogether these findings prompted us to study *Sirt3* and *Nampt* survival genes in $AT_{1A}^{-/-}$ mice. Transcript levels of both *Nampt* and *Sirt3* were increased in kidneys from $AT_{1A}^{-/-}$ mice with respect to wild-type animals. The finding that candesartan, an AT_1 receptor antagonist, prevented Ang II-induced *Nampt* and *Sirt3* mRNA reduction in cultured tubular epithelial cells suggested a possible biochemical link between Ang II and survival genes, which conceivably operates via the AT_{1A}. Furthermore, experiments showing that *Nampt* gene silencing by siRNA limited the reduction of *Sirt3* mRNA induced by Ang II would indicate a causative role of Nampt in modulating *Sirt3* gene transcription in response to Ang II [5].

Caloric restriction prolongs the life span through an increase of sirtuins [29, 63]. In rodents and humans the levels of Sir2 ortholog SIRT1, that targets numerous regulatory factors affecting stress management and metabolism, increase in response to caloric restriction and this increase causes favorable changes in metabolism and stress tolerance [13].

The sirtuins are also involved in prolonged survival induced by resveratrol [2], a small molecule found in red wine which activates SIRT1 and mimics the anti-aging effect of caloric restriction. The effect of resveratrol on life span is associated with increased mitochondrial number and is dependent on the upregulation of Sir2 [35]. Moreover, resveratrol downregulates AT_1 through SIRT1 activation in cultured vascular smooth muscle cells and mouse aorta implying that inhibition of the AT_1 contributes to resveratrol-induced longevity [44].

In the kidney from $AT_{1A}^{-/-}$ mice the levels of SIRT1 were comparable to wild-type mice, suggesting that the increased longevity of this mouse strain is independent from the SIRT1 pathway.

All these findings support a role of SIRT3 in the prolongation of life span, and the manipulation of the RAS system provides a remarkable beneficial effect on longevity by reducing oxidative stress and upregulating survival genes (Fig. 4).

Conclusions

Chronic activation of RAS plays an important role in the promotion of end-stage organ damage associated with aging by increasing tissue and mitochondrial oxidative stress. Therapies targeting RAS (ACEi and ARBs) reduce age-associated cardiovascular and renal damage and preserve the number and the function of mitochondria. A stronger protective effect, demonstrated by a significant prolongation of life span, was observed in genetically modified mice, which lack the AT_{1A} gene. In these mice, the longevity is the consequence of reduced mitochondrial damage due to the attenuation of oxidative stress and the upregulation of *Nampt* and *Sirt3* survival genes.

The extension of the life span observed in $AT_{1A}^{-/-}$ mice is comparable to that of mice lacking the insulin growth factor-1 (IGF-1) receptor [34]. However the manipulation of the latter pathway in humans is not imminently feasible. In contrast, Ang II type 1 receptor antagonists have been proven safe, well-tolerated for chronic use and represent a key component of the modern therapy for hypertension and cardiac failure. Thus, the inhibition of AT_1 could represent a possible therapeutic strategy for diseases of aging and possibly for extending the life span. Further studies are necessary to deepen the role of AT_1 in humans and to understand whether the receptor function is similar to that found in animals.

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