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REVIEW ARTICLE

Is the anti-aging effect of ACE2 due to its role in the renin-angiotensin system?—Findings from a comparison of the aging phenotypes of ACE2-deficient, Tsukuba hypertensive, and Mas-deficient mice—

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Abstract

Angiotensin converting enzyme 2 (ACE2) functions as an enzyme that produces angiotensin 1-7 (A1-7) from angiotensin II (AII) in the renin-angiotensin system (RAS). We evaluated aging phenotypes, especially skeletal muscle aging, in ACE2 systemically deficient (ACE2 KO) mice and found that ACE2 has an antiaging function. The characteristic aging phenotype observed in ACE2 KO mice was not reproduced in mice deficient in the A1-7 receptor Mas or in Tsukuba hypertensive mice, a model of chronic AII overproduction, suggesting that ACE2 has a RAS-independent antiaging function. In this review, the results we have obtained and related studies on the aging regulatory mechanism mediated by RAS components will be presented and summarized.

Keywords Renin-angiotensin system · ACE2 · Aging · Sarcopenia · Skeletal muscle

Introduction

The renin-angiotensin system (RAS), a circulatory regulatory system, consists of two axes: the ACE-angiotensin II (AII)-AT1 axis and the ACE2-angiotensin 1-7 (A1-7)-Mas axis, which plays an antagonistic role to the ACE-AII-AT1 axis. Of these axes, many clinical and basic studies have already shown that excessive activation of the ACE-AII-AT1 axis promotes aging [1, 2]. Therefore, the ACE2-A1-7-Mas axis was thought to have an antiaging function, but there were no sufficient findings to support this hypothesis. Within the ACE2-A1-7-Mas axis, ACE2 is known as a multifunctional molecule that controls amino acid absorption in the intestinal tract in addition to its function in the RAS and has recently attracted attention as a receptor for SARS-CoV-2. Detailed studies are therefore needed to determine whether ACE2, if it has a regulatory role in aging, is RAS dependent. To address this question, we examined whether ACE2 has an antiaging function and whether its mechanism is RAS-dependent using ACE2-deficient (ACE2 KO) mice and Mas-deficient (Mas KO) mice, which are receptors for A1-7, as well as Tsukuba hypertensive mice, a model of chronic overproduction of AII. In our series of investigations, we have uncovered new findings that deepen our understanding of the regulatory mechanisms of aging by RAS components other than ACE2 and will present them in this review along with the background findings.

RAS and the regulation of aging and skeletal muscle function

Although the ACE-AII-AT1 axis is essential for maintaining blood pressure, development, growth, and structural maintenance of the kidney [3], excessive activation of this axis has been shown to lead to multiorgan dysfunction [4–8] and accelerated aging [1, 2].

Initially, administration of ACE inhibitors [9] or AT1 inhibitors [10] to hypertensive rats prolonged their lifespan,

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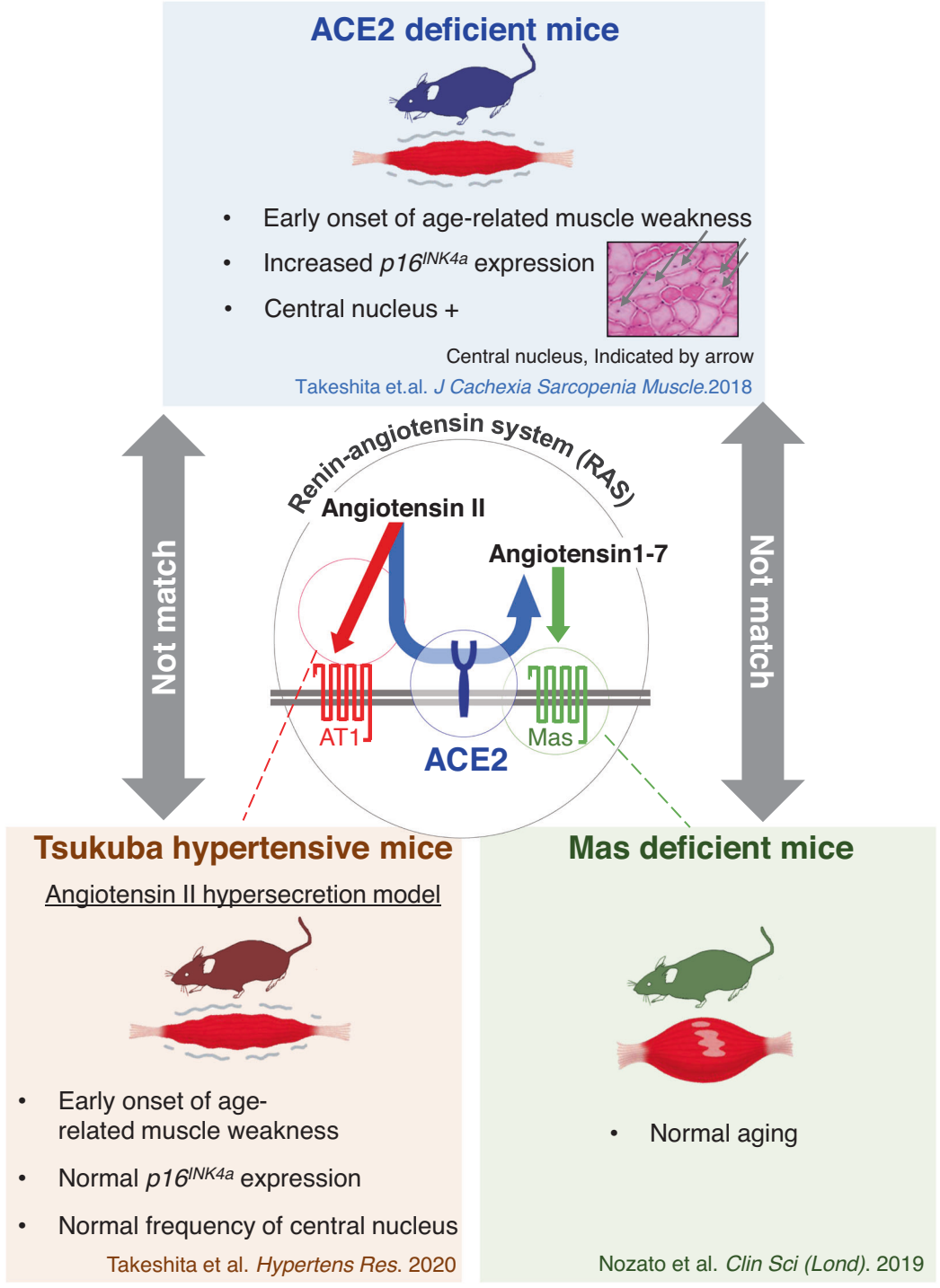
Graphical Abstract

We evaluated the aging phenotype of ACE2 systemically deficient (ACE2 KO) mice, particularly skeletal muscle aging, and found that ACE2 has an antiaging function. The characteristic aging phenotype observed in ACE2 KO mice was not reproduced in Mas KO mice, angiotensin 1-7 receptor-deficient mice or in Tsukuba hypertensive mice, a model of chronic angiotensin II overproduction, suggesting that the antiaging functions of ACE2 are independent of the renin-angiotensin system (RAS).



which was thought to be due in part to their antihypertensive effects. However, since this lifespan-prolonging effect of ACE inhibitors has also been confirmed in normotensive rats [11], the effects of RAS inhibitors are not necessarily mediated by antihypertensive effects. Additionally, in AT1a, a main homolog of the AT1 receptor in rodents, deficient mice (AT1a KO), compared to wild-type (WT) mice, a higher mitochondrial number and less oxidative stress in multiple organs, higher expression of nicotinamide phosphoribosyl transferase (Nampt) and Sirtuin 3 in the kidney, and a longer lifespan have been reported [12, 13].

Excessive age-related loss of skeletal muscle is associated with mortality and disability in elderly individuals and is considered an aging-related disease called sarcopenia [14, 15]. Currently, no established pharmacological treatment for sarcopenia exists, but observational studies have reported that ACE inhibitors suppress skeletal muscle functional decline in elderly women [16], and it is expected that inhibition of ACE-AII-AT1 activation will have a deterrent effect on the pathological progression of sarcopenia. Overactivation of the ACE-AII-AT1 pathway in skeletal muscle inhibits the IGF-1-AKT-mTOR pathway, which promotes muscle protein synthesis [17–20], promotes muscle protein degradation via induction of the ubiquitin ligases Atrogin-1 and MuRF-1, which are specifically expressed in skeletal muscle [21, 22], and promotes NF-κB-dependent inflammation and mitochondrial damage [23, 24] via enhanced signaling downstream of TGF-β [25] and ROS generation [26]. Furthermore, since treatment with losartan, an AT1 blocker, improves muscle regeneration and skeletal muscle fibrosis via inhibition of the TGF-β and MAPK signaling pathways [27, 28] in a mouse model of muscular dystrophy, physiological concentrations of AII stimulation may contribute to exacerbation of muscle atrophy. It has also been reported that AT1a KO mice exhibit reduced age-related muscle weakness via inhibition of the C1q-Wnt/β-catenin signaling pathway, which is known to promote impairment of skeletal muscle regeneration [13].

On the other hand, the ACE2-A1-7-Mas axis, which is considered a counterregulatory system of the ACE-AII-AT1 axis, is believed to have vasodilatory, antiproliferative, anti-inflammatory, and antifibrotic effects through the synthesis of nitric oxide, inhibition of MAP kinase signaling (ERK1/2, p38, JNK), inhibition of ROS formation, and inhibition of TGF-β-SMAD signaling [29–31]. It is also known to have a positive effect on metabolism, improving insulin resistance and dyslipidemia by activating the A1-7-Mas pathway [30].

In addition, the A1-7-Mas pathway was shown to have skeletal muscle protective effects via phosphorylation of AKT, suppression of the increases in Atrogin-1 and MuRF-1, and suppression of TGF-β signaling [32, 33] in various mouse models of induced skeletal muscle damage, with AII [33–35], endotoxin injection [36], disuse atrophy [37], and gene induction of muscular dystrophy [32]. Administration of A1-7 also inhibited muscle weakness and reduced muscle fiber quality in mice with denervation-induced muscle atrophy and muscle damage [38] and in a mouse model of chronic liver disease [39]. Furthermore, administration of A1-7 inhibited the increase in skeletal muscle autophagy observed in mice treated with lipopolysaccharide (LPS) [40]. Administration of the Mas agonist AVE0991 reduced adverse tumor effects, such as weight loss, and improved activity and muscle wasting in cancer cachexia mice by increasing appetite [41]. Regarding the ACE2-A1-7-Mas axis and aging, it has been shown that the expression level of Mas decreases with age in mouse arteries [42] and that the vasodilatory response to exogenous A1-7 administration decreases with age in isolated aortic vessels from female mice [43], but there are only limited findings about the ACE2-A1-7-Mas axis.

To elucidate the roles of the ACE2-A1-7-Mas axis, we assessed skeletal muscle functions by measuring grip strength in ACE2 KO and Mas KO mice with or without A1-7 (Fig. 1) and Tsukuba hypertensive (TH) mice (Fig. 2). We briefly review the RAS regulation of aging and skeletal muscle function according to our findings.

The anti-aging function of ACE2

ACE2 is a molecule identified as a homolog of angiotensin converting enzyme (ACE) from a cDNA library of left ventricular samples from patients with idiopathic dilated cardiomyopathy [44, 45]. In addition to A1-7, ACE2 produces angiotensin 1-9 from angiotensin I in the RAS, but the catalytic efficiency of ACE2 is approximately 400 times higher for AII than for AI [46]. From the physiological function of suppression of ACE-AII-AT1, we hypothesized that the ACE2-A1-7-Mas axis has antiaging effects and evaluated the aging phenotype, mainly skeletal muscle function, in ACE2 KO mic [47]. WT and ACE2 KO mice were kept until 25 months of age, which represents the senescence stage, and changes in muscle strength over time were monitored by measuring grip strength (Fig. 1). A newly developed method with higher accuracy than the conventional method is used to measure grip strength [48]. At 3 months of age, the grip strength of ACE2 KO mice was equivalent to that of WT mice and not significantly different, but from 6 months of age, the grip strength of ACE2 KO mice was lower than that of WT mice (Figs. 1, 3) [47]. Histological analysis of tibialis anterior (TA) muscle sampled at 25 months of age revealed that the central nucleus frequency, which increases in skeletal muscle with aging [49], was significantly more frequent in ACE2 KO mice than in WT mice (Figs. 1, 3) [47]. Analysis of aging-related gene expression in skeletal muscle revealed that the expression of $p16^{INK4a}$ was markedly increased in the TA,

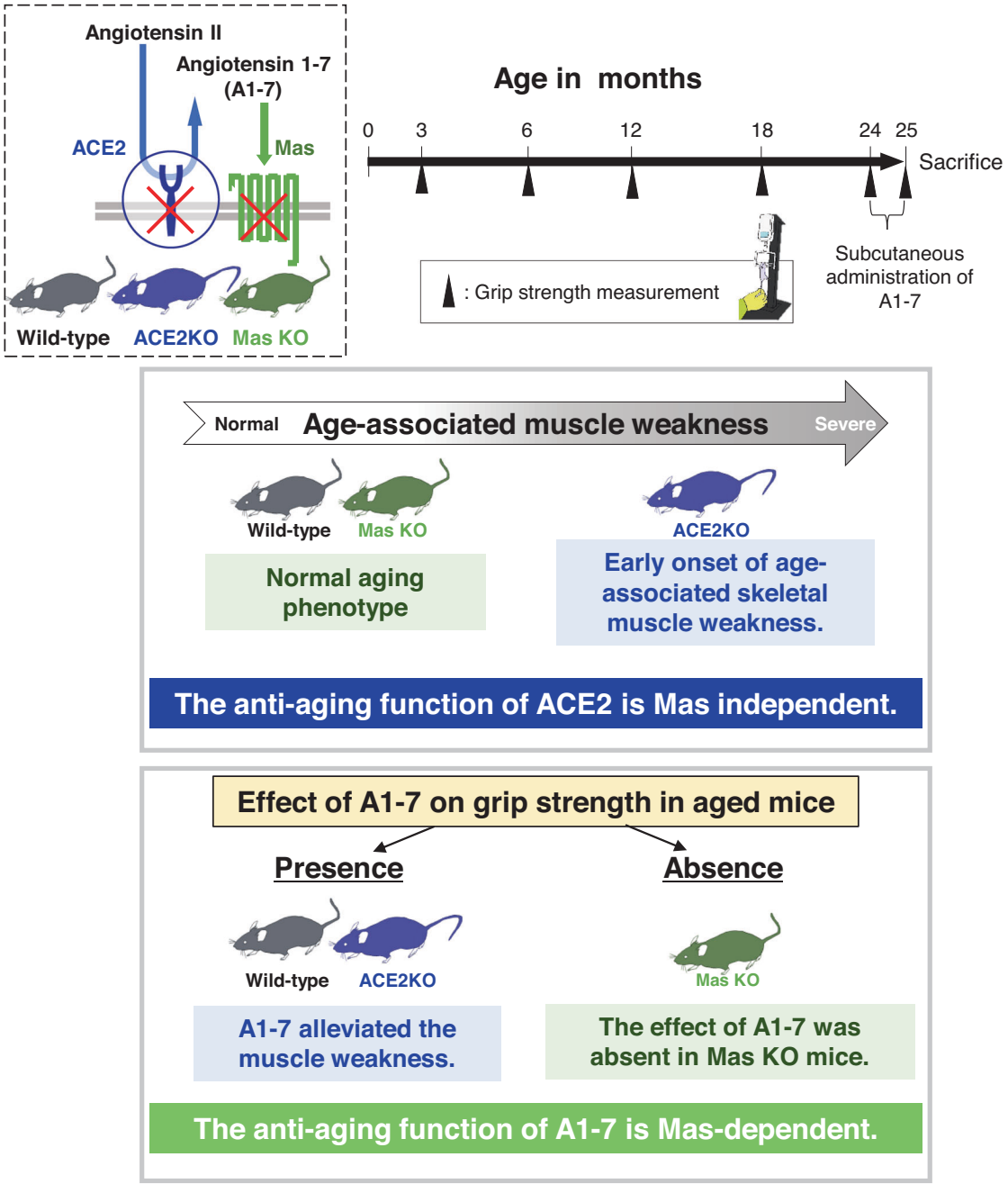


Fig. 1 Mas-deficient (Mas KO) mice did not exhibit the premature aging traits seen in ACE2-deficient (ACE2 KO) mice, suggesting a Mas-independent antiaging effect of ACE2. In contrast, Mas KO mice showed no effects of angiotensin 1-7 (A1-7) on muscle strength, suggesting that A1-7 exerts its antiaging effects in a Mas-dependent manner

extensor digitorum longus (EDL) and quadriceps (QM) of 25-month-old ACE2 KO mice compared to WT mice (Figs. 1, 3) [50]. Similar to $p16^{INK4a}$ and *p19*, *p21*, a known aging-related gene [51], was significantly downregulated in the skeletal muscle of ACE2 KO mice compared to WT mice, and *p53* expression was not significantly different between ACE2 KO and WT mice [47]. In mouse skeletal muscle, the expression patterns of the *p21* and *p53* genes have been reported to change such that their expression is enhanced at the age of 10 or 15 months, which is middle-aged for mice, and then returns and increases again with aging, but the enhanced expression of $p16^{INK4a}$ is observed only at very old ages, suggesting that the enhanced expression of $p16^{INK4a}$ in ACE2 KO mouse skeletal muscle indicates accelerated aging [52].

In addition, no significant difference was observed between WT and ACE2 KO mice in the cross-sectional area (CSA) of muscle fibers in TA muscle, but in the EDL muscle,

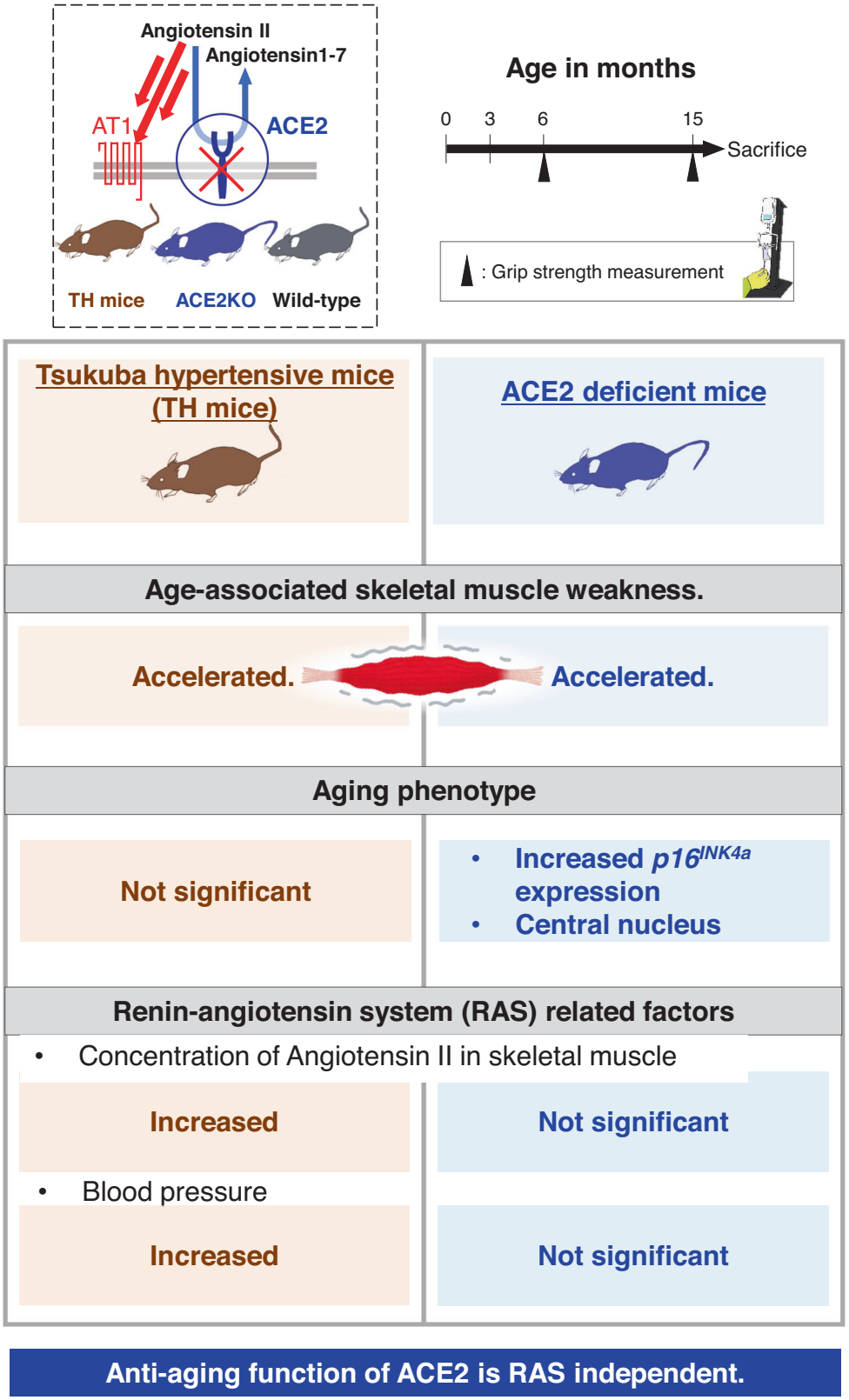


Fig. 2 Tsukuba hypertensive (TH) mice showed exacerbated skeletal muscle dysfunction with enhanced renin-angiotensin system (RAS)-related factors, while ACE2 KO mice showed proaging traits that were not mediated by effects on RAS-related factors, including blood pressure. In addition, the aging traits observed in the skeletal muscle of ACE2 KO mice were not reproduced in TH mice

ACE2 KO mice had a smaller CSA than WT mice, indicating that the effect of ACE2 deficiency varies depending on the type of skeletal muscle [50].

No difference in glucose tolerance, activity, or food intake was observed between WT and ACE2 KO mice, suggesting that the mechanism of exacerbated muscle weakness in

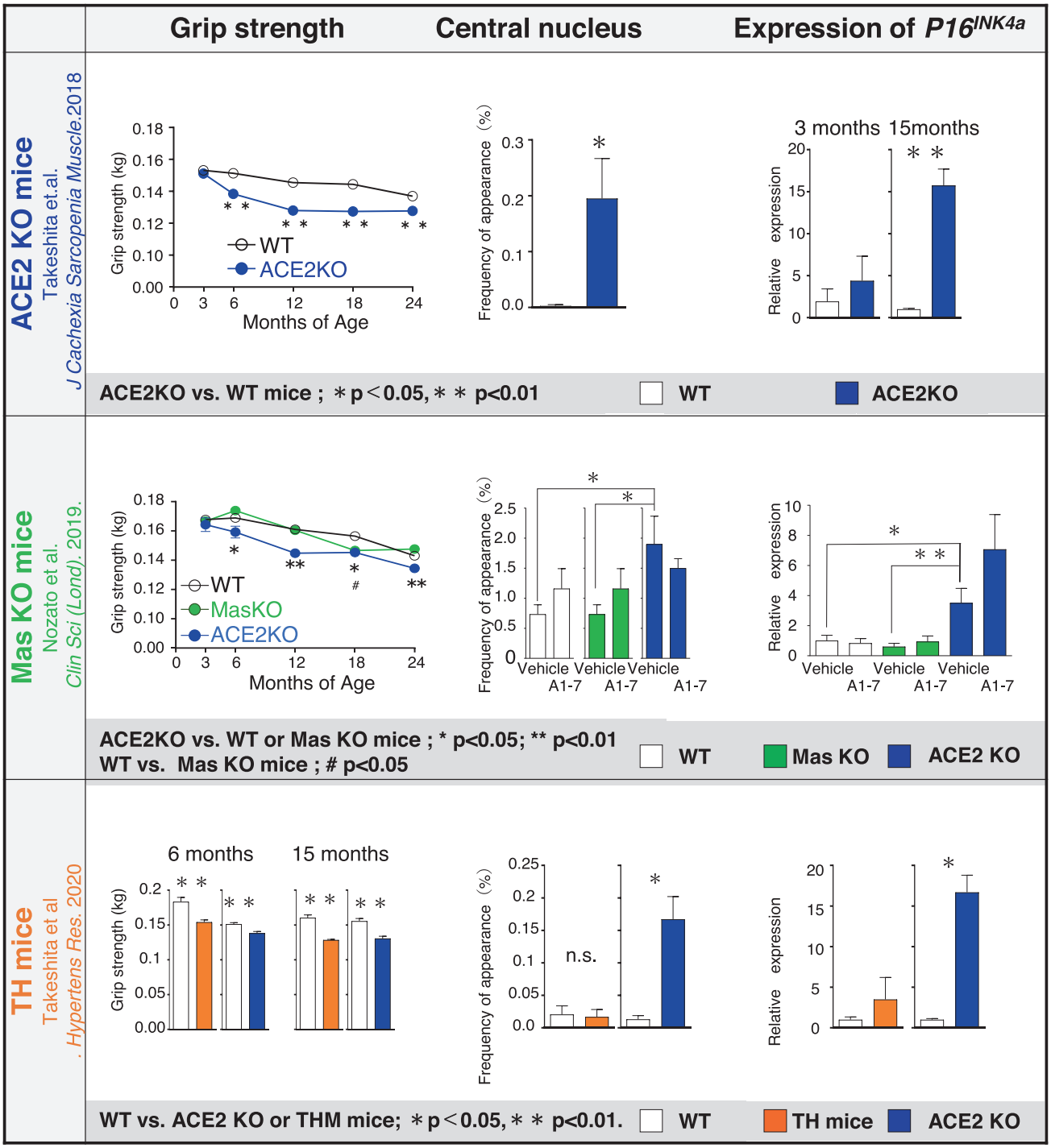


Fig. 3 List of the main results of our series of studies presented in this review

ACE2 KO mice involves a different factor than metabolism. Furthermore, at 12 and 18 months of age, ACE2 KO mice weighed significantly less than WT mice. Comparison of multiple organ masses showed that this difference in body weight was dependent on reduced adipose tissue, and increased expression of the $p16^{INK4a}$ gene was also observed in adipose tissue [50]. Moreover, ACE2 KO mice had thinner skin than WT mice [50].

In addition to these studies, vehicle (saline) or A1-7 was subcutaneously administered to both WT and ACE2 KO

mice at 24 months of age for 4 weeks via an osmotic pump to evaluate skeletal muscle function, such as grip strength and aging phenotype [47, 50] (Fig. 1). Grip strength was increased by administration of A1-7 in both WT and ACE2 KO mice [47] (Figs. 1, 3).

Regarding the CSA of muscle fibers, A1-7 treatment did not change the CSA in the TA but increased the CSA in the EDL [47, 50]. Microarray analysis of gene expression in skeletal muscle revealed that administration of A1-7 upregulates Pax3, a paralog of Pax7 present in muscle satellite cells [53, 54], in the EDL [50]. However, there was no effect of A1-7 treatment on the frequency of central nucleus or $p16^{INK4a}$ expression in both TA and EDL (Figs. 1, 3) [47, 50].

To clarify the mechanism by which ACE2 exerts its antiaging function, since ACE2 plays a major role in AII degradation and A1-7 in the RAS, we next assessed the aging phenotype in mice with systemic deficiency of the Mas receptor (Mas KO), which is the receptor for A1-7 (Figs. 1, 3) [50]. ACE2 KO mice had lower grip strength than WT mice beginning at 6 months of age, while Mas KO mice had lower muscle strength than WT mice only at 18 months of age and had almost the same grip strength as WT mice at other ages, including 24 months. In addition, Mas KO mice did not show enhanced expression of $p16^{INK4a}$ or increased central nucleus frequency in skeletal muscle, as observed in ACE2 KO mice [50]. In a similar experiment, Mas KO mice were treated with A1-7 starting at 24 months of age and did not show the enhanced grip strength or bone density seen in WT and ACE2 KO mice [50]. Taken together, our studies demonstrate that the effects of ACE2 on physiological aging are independent of the endogenous production of A1-7 by ACE2, but A1-7 administration enhances muscle strength and bone density in old mice in a Mas-dependent manner (Figs. 1, 3).

Next, considering the possibility that degradation of AII, another function of ACE2 in the RAS, is an antiaging function of ACE2, we examined whether a mouse model of chronic AII hypersecretion exhibited the same proaging traits as ACE2 KO mice (Fig. 2) [55].

Differential aging phenotypes in chronic AII hypersecretion model mice and ACE2 KO mice

To elucidate the mechanism of the antiaging effects of ACE2, instead of injecting AII into young mice, which has been used in previous basic research, we used Tsukuba hypertensive (TH) mice, which carry the human renin and angiotensinogen genes, as a model of chronic AII hypersecretion [56, 57]. To verify whether the exacerbation of muscle weakness after 6 months of age seen in ACE2 KO mice is reproduced in TH mice, muscle strength was assessed at two time points, 6 and 15 months of age [55] (Fig. 2). The results showed that TH mice had significantly lower grip strength at 6 and 15 months of age than WT mice as well as ACE2 KO mice [50] (Figs. 2, 3). We further evaluated the gastrocnemius muscle (GM) for aging-related genes, including $p16^{INK4a}$, *p19*, *p21*, and *p53*. Similar to the TA results, the expression of $p16^{INK4a}$ and the frequency of central nuclei were significantly higher in the GM of ACE2 KO mice than in the GM of WT mice, whereas the GM of TH mice was not significantly different from that of WT mice [55] (Figs. 2, 3).

Regarding the phenotypic differences between TH and ACE2 KO mice in the RAS, it has been reported that blood pressure is significantly higher in TH mice than in WT mice [56, 57], while the tissue and plasma phenotypes of blood pressure and AII in ACE2 KO mice differ depending on the type of mice used [58, 59]. We have confirmed that blood pressure and tissue, plasma, and skeletal muscle levels of AII in our mice are not significantly different from those in wild-type mice [50, 60]. While the concentration of AII in skeletal muscle (TA) was significantly higher in TH mice than in WT mice, there was no significant difference in ACE2 KO mice compared to WT mice [55] (Figs. 2, 3). Gene expression of RAS components in skeletal muscle was not significantly different between ACE2 KO and WT mice but was significantly higher in TH mice than in WT mice, including the Mas receptor gene. This enhancement of Mas expression in the skeletal muscle of TH mice is considered to be a response to AII-induced inflammation, since it has been previously reported that muscle atrophy induced by AII or LPS administration and disuse atrophy induced by cast immobilization enhance Mas expression in skeletal muscle [61], supporting the previously reported protective effect of the Mas receptor in skeletal muscle. Unlike ACE2 KO mice, TH mice are thought to have increased production of A1-7 in conjunction with increased production of AII, which, together with the increased expression of Mas in TH mice, is consistent with previous reports that binding of A1-7 to Mas acts in a myoprotective manner in injured skeletal muscle conditions.

On the other hand, RAS-related genes in the skeletal muscle of ACE2 KO mice were not significantly different from those in WT mice. Thus, the muscle weakness observed in ACE2 KO mice may be unrelated to changes in blood pressure or RAS activation.

Mechanism of the antiaging effect of ACE2

Our series of studies has demonstrated that ACE2 has RAS-independent antiaging effects, and that A1-7 contributes to the recovery of age-related muscle weakness in a Mas-dependent manner. Furthermore, we found that ACE2 KO mice exhibit accelerated aging traits not only in skeletal muscle but also in skin and adipose tissue [50]. Which physiological function of ACE2 contributes to anti-aging will be discussed from what is known thus far.

ACE2, a multifunctional molecule that has recently attracted attention as a receptor for SARS-CoV-2, is known to regulate integrin signaling by binding to integrins β1 and α5, which are cell adhesion molecules and are used by many viruses for cell entry [62, 63].

Regarding the function of ACE2 as a peptidase, in addition to RAS components, dynorphin A, apelin, and des-Arg9 bradykinin can be substrates [23, 46, 64].

Among the ACE2 substrates, apelin is known as an exerkine, which is secreted by muscle upon exercise-induced stimulation [65]. APJ, the receptor of apelin, is known to inhibit the AII-AT1 system by forming a heterodimer with AT1 [66], and the binding of apelin and APJ has a hypotensive effect through NO production in vascular endothelial cells [67, 68]. Additionally, the expression of apelin was reported to decrease with aging, and muscle mass and muscle function were improved in aged mice by supplementation or gene introduction of apelin into skeletal muscle [65]. ACE2 decomposes apelin as a substrate, while apelin also upregulates ACE2 mRNA expression, suggesting that ACE2 and apelin have a mutually regulating relationship [69]. However, since catalytic activity toward apelin is also thought to be negatively regulated under ACE2-deficient conditions, catalytic activity toward apelin is unlikely to be a mechanism for the antiaging function of ACE2. On the other hand, des-Arg9 bradykinin, which is produced by the degradation of bradykinin and is a substrate for the ACE2 enzyme reaction, acts as an agonist for the B1 (BK type 1) receptor. B1 receptors are rarely expressed in healthy tissues but are induced by tissue damage and inflammatory stimuli and engage in chronic pain and inflammation [70]. It is possible that the antiaging function of ACE2 depends on the degradative function of des-Arg9 bradykinin, but it is not yet clear how the binding reaction between des-Arg9 bradykinin and B1 receptors affects aging, so future studies are needed.

On the other hand, ACE2 is highly expressed in intestinal tissues, and the function of ACE2 in the small intestine is to regulate tryptophan absorption and other neutral amino acids through its conjugation with the amino acid transporter B0AT1 in the small intestinal epithelium, to regulate the expression of antibacterial peptides through tryptophan absorption and to contribute to the maintenance of intestinal microflora homeostasis [71–74]. ACE2 KO mice are known to have lower tryptophan levels in their blood, brain and skeletal muscle than WT mice [73, 75]. In addition, another study reported that mice on a tryptophan-deficient diet showed skeletal muscle atrophy and recovery after tryptophan administration, suggesting that tryptophan plays an important role in the maintenance of skeletal muscle [76]. The major metabolic pathways for tryptophan include the kynurenine pathway, the serotonin pathway, and the indole pathway involving the gut microbiota. Serotonin in the brain and blood is known to be decreased in ACE2 KO mice, while peripheral serotonin has been reported to be important for muscle adaptation to endurance training [77]. There are a number of previous reports on its relationship to aging that support the possibility that tryptophan and its metabolites may contribute to the regulation of aging [78]. The degradation of tryptophan via the kynurenine pathway, the main metabolic pathway of tryptophan, is thought to be accelerated with aging [79, 80], and blood tryptophan levels are lower in elderly people with frailty and cognitive decline than in normal elderly people [81]. In addition, nicotinamide adenine dinucleotide (NAD^+), the final metabolite of the kynurenine metabolic pathway, decreases in various organs with aging and is thought to be involved in the development of diseases such as obesity, diabetes and Alzheimer's disease via impaired energy metabolism and Sirtuin function [82]. Taken together, the antiaging function of ACE2 may depend on its ability to absorb tryptophan, but this has not yet been investigated in detail.

Prospects

RAS research began with the discovery of renin by Dr. Tigerstedt in 1898, and even more recently, alamandine and its receptor, Mas-related G protein-coupled receptor D (MrgD), have been recognized as new RAS players, as the concept of the RAS continues to expand [83–85]. In terms of the expansion of the RAS concept, as described in this review, regulatory mechanisms of aging have become indispensable when discussing the physiological functions of the RAS.

Our previous results showed that Mas KO mice did not show accelerated senescence traits, but simultaneous deletion of the Mas receptor and the AT2 receptor, a receptor for AII and A1-7, caused accelerated senescence not seen in mice lacking each receptor alone [86].

In mice with double deficiency of AT2 and Mas receptors, activation of the ACE-AII-AT1 axis and promotion of inflammatory pathology were clearly observed, indicating that the two receptors functionally complement each other, again making us reconsider the influence of A1-7 on the regulation of aging.

On the other hand, as described in the review, no activation of the ACE-AII-AT1 axis or promotion of inflammatory pathology was observed in ACE2 KO mice, suggesting the existence of a RAS-independent antiaging function for ACE2 and providing a new direction for research on the regulatory function of the RAS in aging.

Thus, research on the regulatory function of the RAS in aging is still expanding and will continue to be a topic of interest as the aging society becomes a global issue.

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

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