Functions and Mechanisms of Arginase in Age-Associated Cardiovascular Diseases

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Abstract Advanced age is a prominent risk factor of cardiovascular and metabolic diseases, such as atherosclerosis and type II diabetes. The underlying mechanisms are complex and not fully understood. Recent studies provide accumulating evidence demonstrating a potential role of arginases including arginase-I and arginase-II, the isoenzymes involved in L-arginine metabolism, in cardiovascular aging and metabolic diseases by affecting the functions of vascular endothelial cells, smooth muscle cells, and macrophages. At the molecular level, a positive interaction between the arginase II and mTOR-S6K1 pathway has been now demonstrated to play an important role in promoting oxidative stress, inflammation, cellular proliferation, senescence and death, which ultimately leads to accelerated vascular diseases and metabolic disorders. This article reviews the most recent advances in understanding the functional roles and mechanisms of arginase isoforms in cardiovascular aging and the potential of novel therapies to targeting arginases as a means to moderate cardiovascular and metabolic diseases associated with aging.

Keywords Advanced age · Hypertension · Atherosclerosis · Type II diabetes · Arginase · Oxidative stress · Inflammation · Cellular proliferation · Senescence · Isoforms · Cardiovascular disease · Metabolic disease

Introduction

Advanced age is highly associated with increased incidence and prevalence of cardiovascular and metabolic diseases including hypertension, atherosclerotic heart disease, stroke, obesity and

obesity-related type II diabetes mellitus [1]. The prediction that advanced age population of our society will increase steadily in the future decades due to advancement of medical care system and therapeutic modalities raises an important challenging issue for our society [2], i.e., how to better moderate the high susceptibility of older adults to cardiovascular disease and metabolic disorders. Prior literature suggests that chronic low-grade inflammation and excessive oxidative stress are the key mechanisms linking cellular aging and age-associated diseases [3, 4]. However, it remains largely mysterious how inflammation and oxidative stress occur in aging and disease. In this article, we will briefly review the functional roles and molecular mechanisms of the enzyme arginase in promoting oxidative stress and inflammation that are involved in cellular aging and age-associated cardiovascular and metabolic diseases.

The Arginase Isoforms and Tissue/Cellular Distribution

Arginase is a hydrolase that converts L-arginine to urea and L-ornithine. Two major isoforms of arginase encoded by two different genes, i.e., cytoplasmic arginase-I (Arg-I) and mitochondrial arginase-II (Arg-II), have been identified [5]. The human Arg-I gene maps to chromosome 6q23 and encodes a 322 amino acid protein [6, 7], while the human Arg-II gene maps to chromosome 14q24.1 and encodes a 354 amino acid protein [8, 9]. They show similar structure and share approximately 92 % homology within the conserved regions while they share approximately 42 % homology outside the conserved regions [8].

Arg-I and Arg-II are confined to distinct subcellular compartments and tissues. Arg-I is a cytosolic enzyme which is abundantly and constitutively expressed in liver [6] and also expressed at low levels in some of extrahepatic tissues including stomach, pancreas and lung [10•]. Recent study demonstrates that it is also highly expressed in the red blood cells of

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humans and rodents [11]. Arg-II is a mitochondrial enzyme and abundantly expressed in kidney and widely expressed in extrahepatic tissues such as brain, prostate, intestine and pancreas [8, 10•] as well as in cells that are importantly involved in pathogenesis of cardiovascular diseases such as macrophages, vascular endothelial and smooth muscle cells [12•, 13•, 14••].

Hepatic and Extrahepatic Functions of Arginase Isoforms

Both isoforms of arginase hydrolyze L-arginine to urea and L-ornithine [5]. In the liver, Arg-I is the sixth and final enzyme of the urea cycle responsible for elimination of excessive nitrogen generated primarily by the metabolism of amino acids derived from the food intake or from endogenous protein catabolism [15]. This function of Arg-I in the hepatic urea cycle is vital. Arg-I knockout mice exhibited severe symptoms of hyperammonemia, and died between postnatal days 10 and 14 [16]. Arg-I deficiency due to gene mutation in humans reveals hyperargininemia and exhibits neurologically based clinical symptoms in early childhood, including progressive neurologic impairment, development retardation, and hepatic dysfunction associated with cirrhosis and carcinoma [15, 17].

In extrahepatic tissues where there is no functioning urea cycle, both isoforms are believed to exert their functions through metabolizing L-arginine resulting in reduction of L-arginine and formation of L-ornithine with downstream production of polyamines and L-proline. By metabolizing L-arginine, arginases compete with nitric oxide synthase (NOS), including endothelial, inducible, and neuronal NOS (eNOS, iNOS and nNOS, respectively), for their common substrate L-arginine, leading to decreased NO production in endothelial cells, macrophages and in the neural tissue [18–20]. In addition, arginases also exert their biological effects through the downstream metabolites L-proline and polyamines. While L-proline is a precursor, along with vitamin C, of collagen, polyamines are important for cell proliferation, anti-inflammatory effects in macrophages and neuroregeneration [18, 21, 22]. It has been hypothesized that an increased arginase gene expression and/or activity therefore play an important role in the pathogenesis of cardiovascular diseases through decreasing eNOS activity to produce NO as well as promoting smooth muscle cell proliferation and collagen production [18]. In supporting this notion, enhanced Arg-I in rats and Arg-II in mouse and humans have been shown to associate with decreased vasoprotective endothelial NO production linking to atherosclerosis, hypertension, diabetic endothelial dysfunction, diabetic nephropathy and age-associated cardiovascular dysfunction [23–27]. Moreover, a recent study reports that selective endothelial overexpression of Arg-II induces endothelial dysfunction and hypertension and enhances atherosclerosis in mice [28•]. Furthermore, inhibition of arginase pharmaceutically in rats and mice as well as genetic ablation of Arg-II in mice restores

endothelial function in various disease conditions including atherosclerosis, hypertension and aging [23, 24, 26, 29].

Intriguingly, functions that are independent of L-arginine ureahydrolase activity for Arg-II in vascular smooth muscle cells have been recently shown to occur by our most recent study [14••], which will be discussed later in this review.

Does Arginase Cause Endothelial Dysfunction Through L-arginine Deficiency?

The hypothesis that increased arginase activity causes L-arginine deficiency leading to eNOS-uncoupling, a situation that eNOS produces O_2^- instead NO (Regarding detailed mechanisms of eNOS-uncoupling, please refer to the article [30]), vascular endothelial dysfunction is under ongoing debate. Though several groups demonstrated that supplementation of L-arginine improved endothelium-dependent vasodilation in disease animal models or patients with cardiovascular diseases [31, 32], numerous studies with supplemental L-arginine therapy either in animal models or humans do not show consistent results. No effect or no sustained effects on endothelial function [33–35], and even harmful effects on atherosclerotic lesion formation in Apolipoprotein E (ApoE)/iNOS double knockout mice have been reported with L-arginine supplementation [36]. Most importantly, a randomized, double-blinded, placebo-controlled study in patients with acute myocardial infarction, the VINTAGE MI study, demonstrates that 6-month oral L-arginine supplementation (3 g 3 times a day on top of standard postinfarction therapy) does not have any benefits on vascular stiffness and left ventricular ejection fraction, but increases mortality [37]. A second clinical trial in patients with peripheral artery disease, the so-called NO-PAIN study, shows decreased NO[•] production and shortened walking distance in patients receiving L-arginine supplementation as compared to the placebo group [38]. The impact of L-arginine supplementation, particularly chronic supplementation for treatment of cardiovascular diseases seems detrimental. These results have challenged the hypothesis whether L-arginine deficiency exists under disease conditions and whether arginase impairs endothelial function through depleting L-arginine bioavailability for endothelial NO production.

In fact, the concentration of L-arginine in adult human and mouse plasma is around 100 $\mu\text{mol/L}$ and intracellular L-arginine concentration is between 50–200 $\mu\text{mol/L}$, well above that required to support maximum eNOS activity ($K_m=2\text{--}20\ \mu\text{mol/L}$) [39]. A real L-arginine deficiency does not seem to occur. Yet, despite the sufficiently high intracellular concentrations of L-arginine, increased extracellular L-arginine in cultured endothelial cells or acute L-arginine supplementation in patients and animals enhances NO[•] production and endothelium-dependent relaxation, a phenomenon called “arginine paradox” [40]. Several hypotheses have been proposed to explain the “arginine paradox”, which include the hypothesis of distinct intracellular

L-arginine pools for NO[•] production [41, 42]. In this hypothesis, one presumes that the exogenous L-arginine seems channeled to eNOS to produce NO[•], while the intracellular L-arginine pool is not freely exchangeable with the extracellular L-arginine; however, it is accessible to both eNOS and arginase. This model could explain the “L-arginine paradox” and the observation that inhibition of arginase stimulates NO[•] production while overexpression of Arg-I or -II suppresses NO[•] production in the endothelial cells, which is associated with only a mild reduction in intracellular L-arginine concentration (11 to 25 % decrease) even in the presence of high extracellular concentration of L-arginine (0.4 mmol/L) [43].

The “distinct L-arginine pool” hypothesis has been however challenged by a recent study by Elms and colleagues [44•]. In this study, the investigators show that inhibition of eNOS activity by Arg-I cannot be modified by targeting eNOS or Arg-I to disparate intracellular compartments in the presence of L-arginine supplementation. A different explanation for the “arginine paradox” is the “ADMA hypothesis”. ADMA is the endogenous eNOS inhibitor, which blocks intracellular L-arginine utility by eNOS to produce NO[•] [45]. It is assumed that an increase in arginase activity in the presence of ADMA in endothelial cells would further significantly limit intracellular L-arginine bioavailability for eNOS to produce NO[•], although the intracellular L-arginine concentration is only mildly decreased. Whether L-arginine supplementation under the condition of “relative L-arginine deficiency” causes detrimental effects due to production of other undesired metabolites from L-arginine via arginase such as urea and L-ornithine which is further metabolized to polyamines, is not fully clear [18, 21, 22]. Results available from the literature do not seem to support this hypothesis. One study reports that polyamines stimulate autophagy process preventing cellular aging [46]. Moreover, urea and L-ornithine do not show any effects on eNOS activity [44•]. There is also no linear relationship between arginase activity and eNOS inhibition [44•], suggesting that the inhibitory effect of arginase on eNOS activity is not entirely due to L-arginine depletion. Consistent with these findings, our most recently published study demonstrates that Arg-II exhibits detrimental vascular effects in atherosclerosis also through some of its L-arginine ureahydrolase activity-independent mechanisms [14•].

L-arginine Ureahydrolase Activity Independent Effects of Arginase

Since enzymes may have pleiotropic or off-target effects, we would like to address the question whether Arg-II exhibits its biological functions only through its L-arginine metabolizing effect or through regulation of the L-arginine/NO pathway. For this purpose, we have recently generated a catalytically inactive Arg-II mutant with a point mutation of histidine to phenylalanine

at position 160, referred to as H160F, and investigated the functions of this mutant in the vascular smooth muscle cells (VSMC) that do not produce NO [13•, 14•]. We found that in VSMC, the long term overexpressing wild type Arg-II gene as well as the enzymatically inactive H160F mutant, promote VSMC apoptosis and senescence, whereas only the wild type Arg-II (not the H160F inactive mutant) is able to enhance the cell proliferation [13•, 14•]. This intriguing result demonstrates that Arg-II on the one hand promotes VSMC proliferation and on the other hand causes VSMC apoptosis and senescence. While the cell proliferation-stimulating effect of Arg-II is dependent on its L-arginine ureahydrolase activity via synthesis of ornithine and polyamines as shown by previous reports [22], the cell apoptosis/senescence-promoting effect is independent of its enzymatic activity. Further experiments show that this L-arginine ureahydrolase activity-independent effect is mediated through the protein kinases S6K1 and ERK1/2, that in turn activate p66Shc leading to H₂O₂ production and mitochondrial dysfunction, cellular apoptosis and senescence [13•, 14•]. In parallel to these signaling pathways, p53 is also activated by Arg-II independently of its L-arginine ureahydrolase activity, contributing to the cell senescence of the apoptosis process [13•, 14•]. Importantly, expression of Arg-II and activities of S6K1, ERK1/2, p66Shc, and p53 are all augmented in senescent VSMC, and genetic inhibition or ablation of Arg-II not only reduces these signaling pathways and VSMC senescence/apoptosis in vitro, but also in atherosclerosis-prone ApoE^{−/−} mice in vivo, which at least in part accounts for the reduced plaque lesion formation and a more stable plaque characteristics in aortic roots in Arg-II-deficient ApoE^{−/−} mice [13•, 14•]. Moreover, our recent studies provide evidence that Arg-II promotes vascular dysfunction and atherosclerosis also through activation of macrophage inflammation, an effect that seems unlikely mediated by its L-arginine metabolizing mechanism (unpublished data) [12•]. How Arg-II, independently of its L-arginine ureahydrolase activity, promotes VSMC senescence/apoptosis requires further investigation. Whether these enzymatic dependent and independent effects also exist for Arg-I awaits investigation.

Mechanisms of Regulation of Arginase Expression and Activity

While Arg-I is the isoform expressed in the liver across different species including rodents and humans, the isoforms expressed in the cardiovascular tissues/cells seem to exhibit some species-specific differences. Although vascular endothelial and smooth muscle cells express both Arg-I and Arg-II, the relative level of the two isozymes seems different depending on species [18]. In human and mouse vasculature, Arg-II seems to be the predominant isoenzyme [23, 47], whereas in rats and pigs, Arg-I seems to play an important role in regulation of vascular functions [29]. Arg-II expression/activity in the

vascular cells are usually very low; it is, however, significantly induced under several physiological, particularly in pathological conditions, including aging, atherosclerosis, and diabetes mellitus [13•, 23, 24, 26, 27].

Several signal transduction pathways have been explored being involved in the regulation of expression and activity of arginases. In macrophages, up-regulation of Arg-I is associated with M2 anti-inflammatory phenotype [48], which is mediated by cAMP, IL-4 and TGF- β [49]. In the vascular cells including endothelial cells and smooth muscle cells, IL-13 is reported to upregulate both Arg-I and Arg-II in the lung causing pulmonary arteriole remodeling and subsequently pulmonary hypertension in mouse [50]. In human pulmonary arterial smooth muscle cells, hypoxia is capable of inducing Arg-II expression through cAMP [51]. However, another study shows that cAMP, besides JAK/STAT6 upregulates Arg-I (not Arg-II) in

rat aortic smooth muscle cells upon stimulation by IL-4 or IL-13 [52]. It is not clear whether this could be explained by the different biological properties of smooth muscle cells of different origins or species or the different stimuli used in their experimental settings.

Other important regulatory signals for arginases are the small G-protein RhoA and its downstream kinase ROCK [23, 53], p38mapk [54] as well as S6K1 as demonstrated by our most recent studies [13•, 14••]. There is emerging evidence suggesting that oxidative stress, hyperglycaemia, and aging may upregulate arginase activity and expression through these signaling pathways [55–59]. Our recent study demonstrates a positive cross talk between S6K1 and Arg-II in endothelial cells, which is importantly involved in vascular endothelial aging, endothelial-monocyte interaction due to enhanced endothelial expression of adhesion molecules VCAM-1 and ICAM-

Fig. 1 Summary of the multiple roles of arginases in age-associated cardiovascular diseases. 1) In endothelial cells, enhanced expression/activity of Arg-I or Arg-II that positively cross talks with S6K1 causes eNOS-uncoupling, leading to endothelial senescence and inflammation. 2) In macrophages, enhanced Arg-I is associated with anti-inflammatory M2 phenotype, whereas enhanced Arg-II has been shown to promote macrophage pro-inflammatory responses through mitochondrial ROS. 3) In VSMC, enhanced Arg-I or Arg-II activity promotes cell proliferation

and collagen production through their downstream metabolites derived from L-arginine, polyamine and L-proline, respectively. On the other hand, enhanced Arg-II gene expression, which positively cross talks with S6K1, induces mitochondrial dysfunction leading to cell senescence and apoptosis independently of its L-arginine ureahydrolase activity. All the effects exerted by arginases promote cardiovascular aging, insulin resistance and atherosclerosis. The meaning of the colors of the arrow is indicated in the figure

1 through uncoupling of eNOS. Moreover, our most recent study also demonstrates a positive cross talk among ERK1/2-S6K1-p66Shc and Arg-II in VSMC [14••].

Conclusion/Perspectives

Increasing evidence demonstrates the potential role of arginase, particularly Arg-II as a therapeutic target in cardiovascular diseases associated with aging such as atherosclerosis and type-II diabetes. A deficiency of Arg-II reduces macrophage inflammatory responses, vascular inflammation and oxidative stress, and VSMC apoptosis in mice fed high cholesterol diet and high fat diet, and improves endothelial function in aging, reduces atherosclerosis and improves insulin sensitivity and glucose (Fig. 1) [12••, 13•, 14••]. The role of Arg-II in vascular diseases is further confirmed by a study showing that endothelial specific Arg-II transgenic mice on ApoE^{-/-} background reveal accelerated atherosclerosis [28•]. Although some studies implicate that targeting Arg-I is also of therapeutic relevance in cardiovascular diseases, firm evidence is still lacking, which is due to the fact that systemic Arg-I deficient mouse died between postnatal days 10 and 14 [16], and an endothelial specific Arg-I knockout mouse is not yet available.

The therapeutic potential of targeting arginases with inhibitors has been demonstrated in a number of experimental models of cardiovascular disease with the improved endothelial function as a primary end point. This has been demonstrated for a range of human studies, including elderly [60], hypertensive [61], and coronary artery disease and type 2 diabetic [62] populations. While it remains of concern that these inhibitors could theoretically inhibit liver Arg-I and lead to hyperammonemia, this side effect has not been reported. All the studies are solely dependent on the pharmacological inhibitors which inhibit both isoforms of arginases. Development of isoform-specific arginase inhibitors is desirable.

Since Arg-II exhibits a L-arginine ureahydrolase activity independent effect, inhibitors that only target its enzymatic activity may not fully achieve our goals of treatment of cardiovascular disease. Pharmacological drugs that are capable of reducing the Arg-II protein level in aging and age-associated diseases such as atherosclerosis and diabetes would be more efficient and desirable. Pharmacological agents that target signaling transduction pathways regulating arginase gene expression such as rapamycin or resveratrol that inhibit the mTOR/S6K1 pathway involved in upregulation of Arg-II levels recouples eNOS function in aging animal models [13•, 63], and also inhibit smooth muscle cell apoptosis and senescence [14••]. One of the major challenges of further research is to understand the regulatory mechanisms of gene expression of arginase including Arg-I and Arg-II in different cells and under different physiological and pathological conditions, which will

allow development of strategies that specifically target isoforms of the enzyme in specific tissues or cells.

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Compliance with Ethics Guidelines

Conflict of Interest Xiu-Fen Ming and Zhihong Yang declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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