

## Arginase II: Atherogenesis Beyond Enzyme Activity

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It is now well established that endothelial cells (EC) and vascular smooth muscle cells (VSMC) are two specialized and interacting components of the vascular wall that orchestrate the pathogenesis of atherosclerosis through complex signaling cascades. Endothelial dysfunction is an early and critical event that initiates atherogenesis. This is mostly mediated through impaired regulation of endothelial nitric oxide synthase (eNOS) with a resultant decrease in vasoprotective nitric oxide (NO) and increased reactive oxygen species (ROS) production that promotes vascular injury. Emerging data support upregulation of Arginase II (ArgII) in the pathobiology of atherogenesis, and most investigations have focused on deleterious effects of ArgII in EC, where it competes with eNOS for the common substrate L-arginine. Depletion of L-arginine by elevated ArgII activity reciprocally regulates eNOS and results in increased production of harmful oxidants and decreased NO availability. Apoptosis and senescence of VSMC promote plaque rupture in late-stage atherosclerotic vessels.<sup>1</sup> The mechanisms involved in this process are still unclear and are under active investigation.

In an interesting article in this issue of *JAHA* entitled “Arginase II Induces Vascular Smooth Muscle Cell Senescence and Apoptosis Through p66Shc and p53 Independently of Its L-Arginine Ureahydrolase Activity: Implications for Atherosclerotic Plaque Vulnerability,” Xiong et al<sup>2</sup> report novel findings implicating ArgII as an agent of VSMC senescence and apoptosis during late-stage atherosclerosis. Their evidence is based primarily on experiments using a catalytically inactive ArgII mutant with a point mutation of

histidine to phenylalanine at position 160—abrogating the ability of ArgII to degrade L-arginine into urea and ornithine. The authors demonstrate that increased expression of catalytically inactive ArgII in robust, early-passage VSMC leads to apoptosis and senescence via activation of S6K1-p66Shc and p53, with subsequently augmented H<sub>2</sub>O<sub>2</sub> production and mitochondrial dysfunction.

Importantly, the current study occurs in the context of previous reports of the role of ArgII in EC dysfunction during atherogenesis,<sup>3</sup> interactions between S6K1 and p53 signaling cascade,<sup>4</sup> the key place of mitochondrial ROS in apoptosis,<sup>5</sup> and the central roles that S6K1, P66Shc and P53 each have in intracellular redox regulation through ROS and NO.<sup>6–8</sup> Further, p66Shc has been studied as a putative direct impetus for remodeling of mitochondrial cristae with subsequent pore opening and initiation of cell death.<sup>9</sup> Novelty in the current study lies in the fact that the observed changes in integrative cellular signaling were triggered by overexpression of the catalytically inactive ArgII mutant, and that S6K1 mediated the activation of p66Shc by ArgII. The observed ArgII-mediated changes in S6K1, p53, and p66Shc activity appear to be regulated via activation of separate sets of signaling cascades that will require further investigation to elucidate. The possibility that ArgII serves as a scaffold for an integrated VSMC injury response needs to be explored.

Regulation of a multifaceted cellular injury response by a urea cycle enzyme is indeed intriguing. Interestingly, this is not the first time that an enzyme involved in the urea cycle has been reported to have an important role in vascular function that is independent of its catalytic activity. Argininosuccinate Lyase (ASL) has been shown to have a scaffolding function that contributes to the formation of a multiprotein complex with eNOS that is required for NO production.<sup>10</sup> Similar interactions between ArgII and S6K1 could explain some of the findings in the work of Xiong et al.

The majority of the meticulously executed experiments in the current study utilized adenoviral-mediated overexpression of catalytically active and inactive mutants of ArgII. An important additional consideration is that overexpression of an inactive mutant may have precipitated a dominant negative signaling scenario for endogenous VSMC ArgII activity with subsequently enhanced atherogenic activity of iNOS.<sup>11,12</sup> This

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can occur through the formation of functional oligomers, and has been reported for other enzymes.<sup>13</sup>

An interesting yet enigmatic component of the current work is that overexpression of the inactive ArgII mutant in VSMC led to H<sub>2</sub>O<sub>2</sub> production without apparent effects on total superoxide production, since H<sub>2</sub>O<sub>2</sub> is usually produced in cells by dismutation of superoxide. P66Shc has been reported to regulate NADPH Oxidase 4 (Nox4) expression in AGE-induced tissue injury.<sup>14</sup> Nox4 is the only known Nox homolog that directly produces H<sub>2</sub>O<sub>2</sub> in cells.<sup>15,16</sup> These findings collectively suggest that ArgII can activate Nox4 in VSMC via p66Shc.

The relative importance of the Arginase isotypes I and II in VSMC and elsewhere in the vascular wall remains controversial. In the current study the authors claim that ArgII is the dominant isoform in VSMC, and focus their experiments on the use of an ArgII overexpression system. However, given the differences in the subcellular localization between ArgI and ArgII, experiments with ArgI overexpression in VSMC would also be interesting, and could shed light on mechanisms of crosstalk between Arginase and p66Shc activation. The rationale for the current study seems partly based upon previous data showing that p66Shc and ArgII are both localized to mitochondria where they are known to mediate cellular dysfunction.<sup>17,18</sup> A study of the effects of overexpression of ArgI, which is localized to cytosol, on p66Shc activity would clarify whether mitochondrial localization of Arginase is required for the activation of p66Shc.

The authors believe that VSMC senescence and apoptosis are due to increased ArgII expression, and that ArgII contributes to atherosclerotic plaque instability. Another important yet confounding finding of this study, however, is that ArgII activity is upregulated in senescent VSMC. There are several published reports that have shown that ArgII promotes endothelial and smooth muscle cell proliferation via synthesis of ornithine and polyamines. Interestingly, silencing ArgII further reduced the proliferation marker PCNA in senescent cells, and overexpression of ArgII increased PCNA levels in young cells. These findings support the original finding that ArgII promotes cell proliferation. However, this function of ArgII would seem to require enzymatic activity.

Previous studies have demonstrated a protective role for Arg II deletion with regard to the formation of atherosclerotic lesions in hypercholesterolemic mice. This occurs through inhibition of ArgII-mediated uncoupling of eNOS in endothelial cells<sup>3</sup> and macrophages.<sup>18</sup> ApoE<sup>-/-</sup>, ArgII<sup>-/-</sup> double knockout mice fed a high-fat diet exhibited reduced atherosclerotic lesion formation when compared with ApoE<sup>-/-</sup>, ArgII<sup>+/+</sup> controls.<sup>3</sup> Although there are no direct measurements to support improved atherosclerotic plaque stability in the current study, reduced VSMC apoptosis and impaired S6K1-

P66Shc and p53 signaling were observed in atherosclerotic plaque of ApoE<sup>-/-</sup>, ArgII<sup>-/-</sup> mice, as compared with their wild-type littermates. Elsewhere, inactivation of the p66Shc gene provided protection against ROS-mediated endothelial dysfunction in old mice<sup>19</sup> and shRNA-mediated suppression of p66Shc enhanced endothelium/NO-dependent vasorelaxation.<sup>7</sup> These findings emphasize that the athero-protective actions of p66Shc are mainly mediated through EC. This is also the case for ArgII.<sup>3</sup> Hence, we await the use of VSMC-specific ArgII KO mice to understand whether the improved plaque phenotype in global ArgII knockout mice is mediated via the endothelium or by vascular smooth muscle.

In conclusion, this is an important and interesting study that opens new vistas in our developing understanding of the critical place for Arginase in atherogenesis.

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## Disclosures

None.

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