Published in final edited form as:

Curr Opin Nephrol Hypertens. 2010 March ; 19(2): 201–207. doi:10.1097/MNH.0b013e3283361c0b.

Arterial Aging: a Journey into Subclinical Arterial Disease

Mingyi Wang, MD, PhD, Robert E. Monticone, MS, and Edward G. Lakatta, MD

Laboratory of Cardiovascular Science, Intramural Research Program, National Institute on Aging,
National Institutes of Health, Baltimore, MD

Abstract

Purpose—Age-associated arterial alterations in cells, matrix, and biomolecules are the foundation for the initiation and progression of cardiovascular diseases in older persons. This review focuses on the latest advances on the intertwining of aging and disease within the arterial wall at the cell and molecular levels.

Recent findings—Endothelial dysfunction, VSMC proliferation/invasion/secretion, matrix fragmentation, collagenization and glycation are characteristics of an age-associated arterial phenotype that creates a microenvironment enriched with reactive oxygen species (ROS) for the pathogenesis of arterial disease. This niche creates an age-associated arterial secretory phenotype (AAASP), which is orchestrated by the concerted effects of numerous age-modified Ang II signaling molecules. Most of these biomolecular, cell, and matrix modifications that comprise the AAASP can be elicited by experimental hypertension or atherosclerosis at a younger age. The arterial AAASP also shares features of a senescence associated secretory phenotype (SASP) identified in other mesenchymocytes, i.e. fibroblasts.

Summary—A subclinical AAASP evolves during aging. Targeting this subclinical AAASP may reduce the incidence and progression of the quintessential age-associated arterial diseases, i.e. hypertension and atherosclerosis.

Keywords

angiotensin II; arterial aging; arterial disease

Introduction

Morbidity and mortality due to the quintessential cardiovascular diseases, i.e., hypertension and atherosclerosis, leading to stroke, and heart failure increase exponentially with advancing age [1]. The number of Americans over 65 will more than double, from 34.8 million in 2000 (12 percent of the population) to 70.3 million in 2030 (20 percent of the total population) [1, 2]. Thus, the incidence and prevalence of arterial diseases will increase dramatically within this time-frame.

Age-associated arterial proinflammation is an early molecular manifestation of a chronic stress response i.e. overloading of ROS, of vascular endothelial and smooth muscle cells. That is orchestrating by an age-associated arterial secretory phenotype (AAASP) that involves the concerted effects of numerous Ang II biosignaling networks (Table 1) [3^{**},4,5^{*},6,7^{**} 8].

Address for Correspondence: Edward G. Lakatta, M.D., Laboratory of Cardiovascular Science, Intramural Research Program, National Institute on Aging, National Institutes of Health, 5600 Nathan Shock Drive, Baltimore, MD 21224, (410) 558-8202, Fax (410) 558-8150, lakattae@grc.nia.nih.gov.

Conflicts of interest

None

Proinflammation fosters a biological “battlefield” that nurtures arterial diseases (Table 1) [3**,4,5*,6,7** 8]. Arterial dilatation capacity declines after middle age, while arterial stiffness becomes markedly increased with aging [6]. Large arterial walls, in particular the intima, remodel with aging. Age-associated changes in cells and matrix magnify susceptibility to macro-and micro-environmental insults, rendering the arterial wall a fertile soil in which diseases, like hypertension or atherosclerosis flourish.

This review focuses on evidence to support the idea that arterial aging and arterial disease are interwoven at the cellular and molecular levels vis a vis an AAASP, and that realization of this intertwining of age-disease mechanisms may afford novel approaches to the prevention and treatment of atherosclerosis, hypertension, heart failure, and stroke.

Arterial wall aging

The arterial wall is remodeled by the joint effects of numerous protein alterations, in particular, Ang II signaling molecules, with advancing age.

Arterial remodeling

Age-associated arterial remodeling, particularly diffused intimal thickening, is evolutionarily conserved in various species, including rats, rabbits, nonhuman primates, and humans (Table 1). Thus, findings from experimental arterial aging studies in animals provide insights into the molecular and cellular mechanisms of arterial aging in humans. Age dramatically alters the volume and contents of the arterial intima in rats, nonhuman primates and humans [9,10**, 11,12,13,14,15,16]. Small disoriented vascular smooth muscle cells (VSMC), and collagen types I and type III markedly increase within the thickened intima of old rats [9,13]. Old monkeys (~20 yrs) have a thickened intima containing cells and matrix beneath an intact endothelium, and nearly all of these cells stain positively for α -smooth muscle actin [11,17].

Ang II Molecular signaling

Molecular elements of the angiotensin II (ang II) signaling cascade are upregulated both in aged arterial walls and play a causal role in arterial aging, and in the diseased vessel (Table 1). Ang II, angiotensin converting enzyme (ACE), Ang II receptor AT1 receptor are markedly increased with increasing age within the arterial wall, particularly, within the thickened intima in several species, including humans [10**,11,12]. Interestingly, chymase, another angiotensin convertase, has also been detected in the arterial wall in aged nonhuman and human primates [11,12].

Milk fat globule EGF-8 (MFG-E8)—Transcription and translation of arterial MFG-E8, a novel molecule is substantially increased with aging in several mammalian species, including humans [7**]. MFG-E8 expression is stimulated by Ang II and MFG-E8 colocalizes with Ang II and MCP-1 within aged aortic wall and [7**].

Monocyte chemoattractant protein-1 (MCP-1)—Transcription, translation, activity of MCP-1, a powerful chemoattractant and a potential molecular event of intimal hypercellularity is increased within the aged arterial wall [15]. Interestingly, the MCP-1 gradient across the arterial wall from intima to media is substantially increased with aging [12,15].

Calpain-1—Ang II induces calpain-1 expression in the aortic walls [10**]. The transcription, translation, and activity of calpain-1 are significantly up-regulated in aortae from old compared to young rats [10**].

Transforming growth factor-beta 1 (TGF- β 1)—TGF- β 1 transcription, translation, and activity are increased within the aorta of old compared to young rats [14]. Latent associated protein (LAP)-bound TGF- β 1 (~75 kDa) and the latent TGF binding protein-1 (LTBP-1)-bound to precursor TGF- β 1 (190–250 kDa) also increase within the aged aortic wall, particularly within the thickened intima [14].

Matrix metalloproteinase type II (MMP-2)—Levels of transcription, translation, and activity of MMP-2 are enhanced within the arterial wall, with aging [9,10**,11,12,13,14,16]. Enhanced arterial wall MMP-2 activity *in situ* is also observed of several species, including humans [9,11,12,13,14]. Furthermore, an increase of MMP-2 activity is attributable, not only to an enhanced transcription and translation, but also an imbalance activating and inhibiting factors, e.g., membrane-type1 matrix metalloproteinase (MT1-MMP) and tissue inhibitor of MMP-2 (TIMP2), respectively [11,13].

ROS and NO bioavailability—Arterial NAD(P)H oxidase activation, a major source of ROS, increases with age [3**,4,18**,19*,20,21,22], while Cu/Zn SOD, Mg SOD, and extracellular matrix superoxide dismutase decrease in the arterial wall with increasing age [18**]. This imbalance of oxidase and dismutase consequently results in the increased *in situ* superoxide and hydrogen peroxides in the arterial wall with aging [18**,19*].

ROS are major modulators of NO availability in the aging process [19*,22]. The decrease in endothelial NO bioavailability with aging occurs concurrent with an increase in O²⁻ production, both in the aorta and in the carotid artery [22]. The interaction of NO and O²⁻ radicals will result in subsequent formation of peroxynitrite (ONOO-) [22]. Interventional studies indicate that increase breakdown of NO due to an augmented production of O²⁻ is an important key mechanism leading to the decline of endothelium-dependent vasorelaxation with increasing age [22].

Arterial aging proteomics

A combined 2-D gel and isobaric tag for relative and absolute quantitation (iTRAQ) proteome approaches has identified 981 arterial proteins from young and old rats, of which, 50 (~5%) were shown to have a significantly different abundance with aging (Table 2) [7**]. Of these, 27 proteins are linked to the Ang II signaling pathway [7**].

Specific age-associated effects on vascular cells and matrix

A concurrent remodeling of cells and matrix is the cornerstone of the aging vascular wall.

Endothelial cells (EC)

Aging impairs the structure, functions, and regeneration of the endothelium (Figure 1), which is tightly controlled by the NF- κ B system [18**]. NF- κ B is a key transcription factor that mediates Ang II signaling [18**]. Recent human studies show that NF- κ B p65 is increased in EC from older subjects [20]. Interestingly, the NF- κ B inhibitor, salsalate, efficiently increases expression of the NF- κ B inhibitor, I κ B and reduces NF- κ B in endothelial cells, reduces expression of the p47phox, nitrotyrosine from old subjects, and improves brachial artery flow-mediated dilatation in old subjects [21].

Endothelial precursor cells (EPC)

Aging reduces EPC availability and impairs function, including homing, adhesion, spreading, proliferation and migration [23**]. Interestingly, the AT1 antagonist, valsartan, reduces Ang II accelerated senescence of EPC by upregulation of telomerase activity [24]; and polyphenol resveratrol inhibits EPC senescence to preserve the integrity of the arterial wall [25].

Furthermore, young rat serum is able to rescue the age-associated decline in the regenerative capability of EPC in vitro and in vivo [26**]. These findings suggest that a young niche could rescue aged EPC.

VSMC

The heterogeneity of VSMC phenotype substantially increases with aging.

Proliferation—Cultured VSMC from old rat aortae display accelerated growth rates compared to VSMC from young rats [27]. Additionally, compared to early passage VSMC from young rat aortae, old VSMC from old rat aortae have a greater percentage of their population in the S phase, and a lower percentage in the G0/G1 phase [28]. These differences are mediated by dysregulation of the cell cycle, impart, via ERK1/2 signaling that occurs during aging [29].

Invasiveness—Early passage aortic VSMC of old rats exhibit an exaggerated chemotactic PDGF-BB response, whereas cells from young aortae require several additional passages in culture to generate an equivalent response [30]. In response to a chemoattractant gradient of PDGF-BB and MCP-1 VSMC isolated from old aortae also exhibit increased invasion relative to young VSMC [12,16]. This age difference is abolished or substantially reduced by Losartan, an AT1 antagonist, vCCI, an inhibitor of MCP-1/CCR2 signaling, GM 6001, an MMP inhibitor, and Ci 1, a calpain inhibitor, or by silencing MFG-E8 [7**,8,9,10 **,12,15]. Thus, the increased age-associated VSMC invasion/migration is modulated by concurrent increases in elements of Ang II biosignaling networks.

Senescent proteins—Senescence-associated β -gal activity in the old rat arterial wall is detected in VSMC enriched with p16 and NOX4 [31,32*]. Some aspects of VSMC senescence may be due to telomere shortening (replicative senescence) or to DNA damage response (DDR) to age-associated stress (chronic accumulation of ROS), known as stress-induced premature senescence (SIPS)(Figure 1) [33,34*,35**,36**,37]. Telomere shortening and DDR results in the phosphorylation of histones such as H2AX, the association of DNA repair enzymes and cofactors and activation of the transducer protein CHK2 [36**,37]. These subsequently result in activation of effector molecules such as the breast cancer susceptibility gene BRCA1, E2F transcription factors, p53, p21, and p16 and in inactivation of effector molecules such as Rb and CDK4, and force VSMCs into G1 growth arrest [36**,37].

Comparative analysis of the transcriptome profiles of early and late passage human arterial VSMC found a total of 327 differentially expressed probesets [38*]. These include IL-1 β , IL-8, ICAM-1, and MCP-1 [38*]. Furthermore, senescent VSMC also secrete IL-1, IL-6/8, MCP-1, PAI-1, MMP-2 [33,36**,37], similar to a phenomenon found in senescent fibroblasts, referred to as the senescence associated secretory phenotype (SASP) by Campisi J et al [34**,35**]. Thus, senescent VSMC become “non-conventional” inflammation response (AAASP) cells. Like the SASP, the AAASP also likely allows damaged cells to communicate with the surrounding tissue as providing a complementary signaling role in arterial aging.

Extracellular matrix

Repeated interactions of EC and VSMC with extracellular matrix (ECM) via AASP including Ang II signaling molecules occurs within the arterial wall initiating a journey into subclinical arterial pathology. With aging, ECM becomes fragmented, collagenized calcified, and glycated (Figure 1) ([3**,4]. Emerging evidence show that arterial advanced glycation end products (AGEs) accumulate in arteries with age in rodents, nonhuman primates, and humans [39,40, 41]. Treatment with alagebrium (formerly named ALT7-111), a thiazolium derivative, can

break established AGE crosslinks, reversing age-related increases in arterial stiffness in rats, nonhuman and human primates [39,40,41].

Links between arterial aging and hypertension

The aging process and essential hypertension cause similar functional and/or structural, and molecular modifications (Table 1). For example, sustained hypertension leads to functional and structural changes of the arterial wall in young subjects, similar to normotensive old ones [3**,42,43]. These alterations include the endothelial-dependent vascular dilatation, pulse wave velocity (PWV), and intimal medial thickness (IMT) [3**,42,43]. Further, hypertension accelerates and intensifies age-associated remodeling of the arterial wall and modifications of its mechanical properties and complications including atherosclerosis, stroke, and heart failure [44,45,46].

Findings from experimental studies with animals provide insights into the molecular and cellular mechanisms of the interaction of aging and hypertension in humans. In vivo chronic infusion of FXBN rats with Ang II at a subpressor doses increases MMP2 expression and activity, and increases collagen production within the arterial wall [9]; at higher concentrations sufficient to elicit an increase in arterial pressure of a magnitude similar to that of old, and an increases of MMP2, calpain-1, and TGF- β 1 activity [9]. Ang II infusion also imparts structural and molecular characteristics of arteries of old, untreated rats to the central arteries of young rats [9,10**]. Chronic infusion of phenylephrine (PE), an adrenergic transmitter (α 1-AR) known to increase with age [9,47], into young rats, increases arterial wall Ang II levels, and reproduces Ang II effects [9]. Thus, Ang II signaling can indeed mediate structural, biochemical, and functional features of aging within the arterial wall of young rats and produce hypertension. These results complement those of other studies that have demonstrated that chronic ACE inhibition or AT1 receptor blockade at an early age markedly delays the progression of age-associated arterial remodeling in rodents [48,49]. In addition, the production of downstream Ang II signaling molecules, TGF- β 1 and ROS, and blood pressure increase concomitantly in SHR compared to WKY rats with aging [50,51,52]. These findings suggest that Ang II signaling plays a magnified role in the pathogenesis of hypertension disorders with aging, which may reduce lifespan. Indeed, disruption of the *Agtr 1a* gene that encodes AT1, markedly increases life span in mice via an enhanced number of mitochondria and upregulation of the prosurvival genes nicotinamide phosphoribosyltransferase and sirtuin 3 [53**]. Further, lifelong treatment with the AT1 blocker, fonsartan, in young stroke-prone spontaneously hypertensive rats (SHR-sp) doubles the lifespan to 30 months by alleviating complications of hypertension, compared to the normotensive Wistar-Kyoto (WKY) rats [54].

Atherosclerosis with aging

The aging process and inflammatory components of pre-lipid deposition aspects of atherosclerosis cause similar functional and/or structural, and molecular modifications (Table 1). At autopsy, an advanced grade of arterial atherosclerosis is observed in older compared to younger patients [55]. Animal studies show that although similar age alteration in plasma lipid profile, more prevalent and extensive fatty lesions occur in old mice, rabbit, nonhuman primates compared to young [56,57,58]. Recently, findings from proteomic studies reveal insights into similar mechanisms involved in arterial aging and atherosclerosis [7**,59]. In a proteomic analysis of 35 human coronary atherosclerotic plaques that identified a total of 806 proteins [59], many of the abundant proteins in the atherosclerotic intimae are those that increase in the aged arterial wall (Table 2), including TGF- β 1, periostin, MFG-E8, and PDGF- β [59], providing additional support for the idea that the aged arterial wall with enhanced ang II signaling is fertile soil that nurtures atherosclerosis. Further, AT1a deficiency, which effectively protects ApoE $^{-/-}$ mice from the initiation and progression of atherogenesis,

exhibits the inhibition of age-associated increases in expression or activation of collagen, 22phox, and MMP-2/9 [60]. Endothelium restricted inhibition of NF- κ B activation, achieved by ablation of NEMO/IKKg, or by expression of dominant-negative IkBa specifically in endothelial cells, potentially reduces atherosclerotic plaque formation in ApoE $^{-/-}$ mice fed with a cholesterol rich diet with aging. [61**]. The accelerated vascular injury in older LDLR $^{-/-}$ mice is closely associated with the profound inability to mount an antioxidant response [62*].

Conclusion

The novel AAASP concept provides an explanation for observation that the arterial wall of younger animals in response to experimental induction of low-grade chronic inflammation, e.g., by hypertension or early atherosclerosis, is transformed into a phenotype that is strikingly similar to that which develops during aging [3**,4,63]. Thus, “aging”-associated arterial changes and those associated with hypertension and atherosclerosis are fundamentally intertwined at the cellular and molecular levels (Table 1) [3**]. In humans, other well-known “life-style” risk factors (e.g., altered lipid metabolism due to dietary indiscretion, smoking, and lack of exercise) likely interact with this arterial substrate that has been altered during aging, rendering the aging artery a fertile soil that facilitates the initiation and magnified progression of arterial diseases [3**,4]. Lifestyle and pharmacologic interventions have already proved to be effective in preventing or ameliorating vascular disease associated with aging [3**,4]. The subclinical AAASP affords novel targets to reduce the incidence and progression of the quintessential age-associated arterial diseases, i.e. hypertension and atherosclerosis.

Acknowledgments

Funding

This research was supported by the Intramural Research Program of the National Institute on Aging, National Institutes of Health.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

* of special interest

** of outstanding interest

1. Aging-dynamics of cancer. at <http://www.cdc.gov/nchs/>
2. Humes K. The population 65 years and older: Aging in America. The book of the States 2005:464–470.
- 3**. Lakatta EG, Wang M, Najjar SS. Arterial aging and subclinical arterial disease are fundamentally intertwined at macroscopic and molecular levels. *Med Clin North Am* 2009;93:583–604. This is a comprehensive overview on the current knowledge on the intertwining of arterial aging and disease. [PubMed: 19427493]
4. Lakatta EG. Central arterial aging and the epidemic of systolic hypertension and atherosclerosis. *J Am Soc Hypertens* 2007;1:302–340. [PubMed: 20409863]
- 5*. Wang M, Lakatta EG. The salted artery and angiotensin II signaling: A deadly duo in arterial disease. *J Hypertens* 2009;27:19–21. This editorial commentary provides a brief introduction to high salt diets, ang II signaling, aging, and arterial disease. [PubMed: 19050444]
6. Celermajer DS, Sorensen KE, Spiegelhalter DJ, et al. Aging is associated with endothelial dysfunction in healthy men years before the age-related decline in women. *J Am Coll Cardiol* 1994;24:471–476. [PubMed: 8034885]

- 7**. Fu Z, Wang M, Gucek M, et al. Milk fat globule protein epidermal growth factor-8: a pivotal relay element within the angiotensin II and monocyte chemoattractant protein-1 signaling cascade mediating vascular smooth muscle cells invasion. *Circ Res* 2009;104:1337–1346. This is the first comprehensive study of the arterial proteome with aging and presents the discovery of a novel Ang II/MFG-E8/VSMC invasive signaling pathway. [PubMed: 19443842]
8. Li Z, Froehlich J, Galis ZS, et al. Increased expression of matrix metalloproteinase-2 in the thickened intima of aged rats. *Hypertension* 1999;33:116–123. [PubMed: 9931091]
9. Wang M, Zhang J, Spinetti G, et al. Angiotensin II activates matrix metalloproteinase type II and mimics age-associated carotid arterial remodeling in young rats. *Am J Pathol* 2005;167:1429–1442.
- 10**. Jiang L, Wang M, Zhang J, et al. Increased aortic calpain-1 activity mediates age-associated angiotensin II signaling of vascular smooth muscle cells. *PLoS ONE* 2008;3:e2231. This is the first article on Ang II signaling, calpain-1 activation, and arterial aging. [PubMed: 18493299]
11. Wang M, Takagi G, Asai K, et al. Aging increases aortic MMP-2 activity and angiotensin II in nonhuman primates. *Hypertension* 2003;41:1308–1316. [PubMed: 12743015]
12. Wang M, Zhang J, Jiang LQ, et al. Proinflammatory profile within the grossly normal aged human aortic wall. *Hypertension* 2007;50:219–227. [PubMed: 17452499]
13. Wang M, Lakatta EG. Altered regulation of matrix metalloproteinase-2 in aortic remodeling during aging. *Hypertension* 2002;39:865–873. [PubMed: 11967241]
14. Wang M, Zhao D, Spinetti G, et al. Matrix metalloproteinase 2 activation of transforming growth factor-beta1 (TGF-beta1) and TGF-beta1-type II receptor signaling within the aged arterial wall. *Arterioscler Thromb Vasc Biol* 2006;26:1503–1509. [PubMed: 16690877]
15. Spinetti G, Wang M, Monticone R, et al. Rat aortic MCP-1 and its receptor CCR2 increase with age and alter vascular smooth muscle cell function. *Arterioscler Thromb Vasc Biol* 2004;24:1397–1402. [PubMed: 15178559]
16. Wang M, Zhang J, de Cabo R, et al. Calorie restriction reduces MMP-2 activity and retards age-associated aortic restructuring in rats. *Circulation* 2006;114(Suppl II):II-335.
17. Asai K, Kudej RK, Shen YT, et al. Peripheral vascular endothelial dysfunction and apoptosis in old monkeys. *Arterioscler Thromb Vasc Biol* 2000;20:1493–1499. [PubMed: 10845863]
- 18**. Csizsar A, Wang M, Lakatta EG, et al. Inflammation and endothelial dysfunction during aging: role of NF- κ B. *J Appl Physiol* 2008;105:1333–1341. This is a comprehensive review of age-associated proinflammation and endothelial dysfunction. [PubMed: 18599677]
- 19*. Zhou X, Bohlen HG, Unthank JL, et al. Abnormal Nitric Oxide Production in Aged Rat Mesenteric Arteries is Mediated by NAD(P)H Oxidase-derived Peroxide. *Am J Physiol Heart Circ Physiol*. 2009 Sep 25; This study shows that elevated baseline and suppressed flow-mediated NO production in rats with aging mediated by NAD(P)H oxidase-derived H₂O₂.
20. Donato AJ, Eskurza I, Silver AE, et al. Direct evidence of endothelial oxidative stress with aging in humans: relation to impaired endothelium-dependent dilation and upregulation of nuclear factor- κ B. *Circ Res* 2007;100:1659–1666. [PubMed: 17478731]
21. Pierce GL, Lesniewski LA, Lawson BR, et al. Nuclear factor- κ B activation contributes to vascular endothelial dysfunction via oxidative stress in overweight/obese middle-aged and older humans. *Circulation* 2009;119:1284–1292. [PubMed: 19237660]
22. Van der Loo B, Labugger R, Skepper JN, Bachschmid M, Kilo J, Powell JM, et al. Enhanced Peroxynitrite Formation Is Associated with Vascular Aging. *J Exp Med* 2000;192:1731–1744. [PubMed: 11120770]
23. Rauscher FM, Goldschmidt-Clermont PJ, Davis BH, et al. Aging, progenitor cell exhaustion, and atherosclerosis. *Circulation* 2003;108:457–463. [PubMed: 12860902]
24. Imanishi T, Hano T, Nishio I. Angiotensin II accelerates endothelial progenitor cell senescence through induction of oxidative stress. *J Hypertens* 2005;23:97–104. [PubMed: 15643130]
25. Balestrieri ML, Schiano C, Felice F, et al. Effect of low doses of red wine and pure resveratrol on circulating endothelial progenitor cells. *J Biochem* 2008;143:179–186. [PubMed: 17984121]
- 26**. Zhu G, Song M, Wang H, et al. Young environment reverses the declined activity of aged rat-derived endothelial progenitor cells: involvement of the phosphatidylinositol 3-kinase/Akt signaling pathway. *Ann Vasc Surg* 2009;23:519–534. This study demonstrates that creation of a

niche of youth in old EPC is able to rescue the declining function of old EPC both in vivo and in vitro. [PubMed: 19540437]

27. Li Z, Cheng H, Lederer WJ, et al. Enhanced proliferation and migration and altered cytoskeletal proteins in early passage smooth muscle cells from young and old rat aortic explants. *Exp Mol Pathol* 1997;64:1–11. [PubMed: 9203504]
28. Hariri RJ, Hajjar DP, Coletti D, et al. Aging and arteriosclerosis. Cell cycle kinetics of young and old arterial smooth muscle cells. *Am J Pathol* 1988;131:132–136. [PubMed: 3354639]
29. Gennaro G, Ménard C, Giasson E, et al. Role of p44/p42 MAP Kinase in the Age-Dependent Increase in Vascular Smooth Muscle Cell Proliferation and Neointimal Formation. *Arterioscler Thromb Vasc Biol* 2003;23:204–210. [PubMed: 12588760]
30. Pauly RR, Passaniti A, Crow M, et al. Experimental models that mimic the differentiation and dedifferentiation of vascular cells. *Circulation* 1992;86(Suppl):III68–73. [PubMed: 1330366]
31. Yang D, McCrann DJ, Nguyen H, et al. Increased polyploidy in aortic vascular smooth muscle cells during aging is marked by cellular senescence. *Aging Cell* 2007;6:257–260. [PubMed: 17291294]
- 32*. McCrann DJ, Yang D, Chen H, et al. Upregulation of Nox4 in the aging vasculature and its association with smooth muscle cell polyploidy. *Cell Cycle* 2009;8:902–908. This article reports on ROS, VSMC polyploidy, and senescence. [PubMed: 19221493]
33. Kunieda T, Minamino T, Nishi J, et al. Angiotensin II induces premature senescence of vascular smooth muscle cells and accelerates the development of atherosclerosis via a p21-dependent pathway. *Circulation* 2006;114:953–960. [PubMed: 16908765]
- 34*. Rodier F, Coppé JP, Patil CK, et al. Persistent DNA damage signalling triggers senescence-associated inflammatory cytokine secretion. *Nat Cell Biol* 2009;11:973–979. This article addresses DDR, SASP, and inflammation. [PubMed: 19597488]
- 35*. Coppe J, Patil CK, Rodier K, et al. senescence-associated secretory phenotypes Reveal cell-nonautonomous functions. *Plos Biology* 2008;6:e301. This article for first time defines the concept of senescence-associated secretory phenotypes/SASP.
- 36*. Mahmoudi M, Gorenne I, Mercer J, et al. Statins use a novel Nijmegen breakage syndrome-1-dependent pathway to accelerate DNA repair in vascular smooth muscle cells. *Circ Res* 2008;103:717–725. This is the first report in detail on ROS, DDR, VSMC senescence, and atherogenesis. [PubMed: 18723444]
37. Costopoulos C, Liew TV, Bennett M. Ageing and atherosclerosis: Mechanisms and therapeutic options. *Biochemical Biochemistry* 2008;75:1251–1261.
- 38*. Burton DG, Giles PJ, Sheerin AN, et al. Microarray analysis of senescent vascular smooth muscle cells: A link to atherosclerosis and vascular calcification. *Exp Gerontol*. 2009 Jul 23; [Epub ahead of print] This is the first microarray report of senescing VSMC.
39. Susic D, Varagic J, Ahn J, et al. Cardiovascular and renal effects of a collagen cross-link breaker (ALT 711) in adult and aged spontaneously hypertensive rats. *American J Hypertens* 2004;17:328–333.
40. Kass DA, Shapiro EP, Kawaguchi M, et al. Improved arterial compliance by a novel advanced glycation end-product crosslink breaker. *Circulation* 2001;104:1464–1470. [PubMed: 11571237]
41. Vaitkevicius PV, Lane M, Spurgeon H, et al. A cross-link breaker has sustained effects on arterial and ventricular properties in older rhesus monkeys. *Proc Natl Acad Sci U S A* 2001;98:1171–1175. [PubMed: 11158613]
42. Taddei S, Virdis A, Mattei P, et al. Hypertension causes premature aging of endothelial function in humans. *Hypertension* 1997;29:736–743. [PubMed: 9052889]
43. Scuteri A, Najjar SS, Muller DC, et al. Metabolic syndrome amplifies the age-associated increases in vascular thickness and stiffness. *J Am Coll Cardiol* 2004;43:1388–1395. [PubMed: 15093872]
44. Franklin SS, Larson MG, Khan SA, et al. Does the relationship of blood pressure to coronary heart disease risk change with aging? The Framingham Heart Study. *Circulation* 2001;103:1245–1249. [PubMed: 11238268]
45. Scuteri A, Chen CH, Yin FC, et al. Functional correlates of central arterial geometric phenotypes. *Hypertension* 2001;38:1471–1475. [PubMed: 11751738]
46. Perticone F, Ceravolo R, Pujia A, et al. Prognostic significance of endothelial dysfunction in hypertensive patients. *Circulation* 2001;104:191–196.

47. Rudner XL, Berkowitz DE, Booth JV, et al. Subtype specific regulation of human vascular alpha (1)-adrenergic receptors by vessel bed and age. *Circulation* 1999;100:2336–2343. [PubMed: 10587338]
48. Michel JB, Heudes D, Michel O, et al. Effect of chronic ANGI-converting enzyme inhibition on aging processes: II: Large arteries. *Am J Physiol* 1994;267:R124–R135. [PubMed: 8048614]
49. Basso N, Cini R, Pietrelli A, et al. Protective effect of long-term angiotensin II inhibition. *Am J Physiol Heart Circ Physiol* 2007;293:H1351–H1358. [PubMed: 17557916]
50. Uddin M, Yang H, Shi M, et al. Elevation of oxidative stress in the aorta of genetically hypertensive mice. *Mech Ageing Dev* 2003;124:811–817. [PubMed: 12875744]
51. Wu R, Millette E, Wu L, et al. Enhanced superoxide anion formation in vascular tissues from spontaneously hypertensive and desoxycorticosterone acetate-salt hypertensive rats. *J Hypertens* 2001;19:741–748. [PubMed: 11330877]
52. Sarzani R, Arnaldi G, Takasaki I, et al. Effects of hypertension and aging on platelet-derived growth factor and platelet-derived growth factor receptor expression in rat aorta and heart. *Hypertension* 1991;18(5 Suppl):III93–III99. [PubMed: 1657776]
- 53*. Benigni A, Corna D, Zoja C, et al. Disruption of the Ang II type 1 receptor promotes longevity in mice. *J Clin Invest* 2009;119:524–530. The first study showing longer lifespan sans AT1 receptor through increasing mitochondria biogenesis and expression of a longevity gene, Sirt3. [PubMed: 19197138]
54. Linz W, Heitsch H, Schölkens BA, et al. Long-term angiotensin II type 1 receptor blockade with fonsartan doubles lifespan of hypertensive rats. *Hypertension* 2000;35:908–913. [PubMed: 10775560]
55. Virmani R, Avolio AP, Mergner WJ, et al. Effect of aging on aortic morphology in populations with high and low prevalence of hypertension and atherosclerosis. Comparison between occidental and Chinese communities. *Am J Pathol* 1991;139:1119–1129. [PubMed: 1951629]
56. Fenton M, Huang HL, Hong Y, et al. Early atherogenesis in senescence-accelerated mice. *Experimental gerontology* 2004;39:115–122. [PubMed: 14724071]
57. Spagnoli LG, Orlandi A, Mauriello A, Santeusano G, de Angelis C, Lucreziotti R, Ramacci MT. Aging and atherosclerosis in the rabbit. 1. Distribution, prevalence and morphology of atherosclerotic lesions. *Atherosclerosis* 1991;89:11–24. [PubMed: 1772469]
58. Clarkson, TB.; Adams, MR.; Weingand, KW.; Miller, LC.; Heydrick, S. Effect of age on atherosclerosis progression in nonhuman primates. In: Bates, SR.; Ganghoff, EC., editors. *Atherogenesis and Aging*. NY: Springer-Verlag; 1987. p. 57-71.
59. Bagnato C, Thumar J, Mayya V, et al. Proteomics analysis of human coronary atherosclerotic plaque: a feasibility study of direct tissue proteomics by liquid chromatography and tandem mass spectrometry. *Mol Cell Proteomics* 2007;6:1088–1102. [PubMed: 17339633]
60. Eto H, Miyata M, Shirasawa T, et al. The long-term effect of angiotensin II type 1a receptor deficiency on hypercholesterolemia-induced atherosclerosis. *Hypertens Res* 2008;31:1631–1642. [PubMed: 18971539]
- 61*. Gareus R, Kotsaki E, Xanthouleas S, et al. Endothelial cell-specific NF-kappaB inhibition protects mice from atherosclerosis. *Cell Metab* 2008;8:372–383. The discovery that an inhibition of NF-κB activity in endothelial cells slows age-associated atherogenesis. [PubMed: 19046569]
- 62*. Collins AR, Lyon CJ, Xia X, et al. Age-accelerated atherosclerosis correlates with failure to upregulate antioxidant genes. *Circ Res* 2009;104:e42–e54. This is the first report on age-accelerated atherosclerosis due to a downregulation of antioxidant genes. [PubMed: 19265038]
63. Smith BJ, Lightfoot SA, Lerner MR, et al. Induction of cardiovascular pathology in a novel model of low-grade chronic inflammation. *Cardiovasc Pathol* 2009;18:1–10. [PubMed: 18402801]

Figure 1. Illustration of potential molecular and cellular mechanisms for arterial proinflammation in the aging-to-disease journey.

Table 1

Arterial Remodeling with Aging, Hypertension, and Atherosclerosis.

	Aging					Hypertension	Atherosclerosis	Ang II -signaling
	Humans >65 yrs	Monkeys 15-20 yrs	Rats 24-30 mo	Rabbits 2-6 yrs				
Luminal dilation	+	+	+	+	±	±	±	±
↑Stiffness	+	+	+	+	+	+	+	+
Endothelial dysfunction	+	+	+	+	+	+	+	+
Diffuse Intimal Thickening	+	+	+	+	+	+	+	+
Lipid involvement	-	-	-	-	±	+	+	+
↑VSMC number	+	+	+	+	+	+	+	+
Macrophages	+	-	-	-	+	+	+	+
T-cell	+	-	-	-	+	+	+	+
↑Matrix	+	+	+	+	+	+	+	+
↑Local ANGII-ACE	+	+	+	+	+	+	+	+
MMP dysregulation	+	+	+	?	+	+	+	+
↑MCP-1/CCR2	+	+	+	+	+	+	+	+
↑ICAM	+	+	+	?	+	+	+	+
↑TGF-β	+	+	+	+	+	+	+	+
↑NADPH Oxidase	+	+	+	+	+	+	+	+
↓VEGF	+	?	?	+	+	+	+	+
↓Nitric Oxide Bioavailability	+	+	+	+	+	+	+	+
HYPERTENSION	±	±	±	±	+	±	±	±
ATHEROSCLEROSIS	±	-	-	-	±	+	+	+

? Information unknown

Table 2

Differentially Abundant Proteins Identified by 2-D DIGE and iTRAQ with Aging

Protein Class and Protein Name	Accession from SwissProt Database	iTRAQ O/Y (fold)
Apoptosis/cell cycle/proliferation		
MFG-E8	MFGM_RAT	2.31
Serine protease HTRA1	HTRA1_MOUSE	2.03
Integral membrane protein 2B	ITM2B_RAT	2.28
Cysteine and glycine-rich protein 2	CSRP2_RAT	1.56
Platelet factor 4 (CXCL4)	PLF4_RAT	3.09
Insulin-like growth factor-binding protein 7	IBP7_MOUSE	1.40
Cell Metabolism		
Creatine kinase M-type	KCRM_RAT	0.58
Glycerol 3-P dehydrogenase 1	GPDA_RAT	0.58
Cytochrome c oxidase subunit 5B	COX5B_RAT	0.66
Glutamine synthetase	GLNA_RAT	0.58
2-oxoglutarate dehydrogenase E1 component	ODO1_RAT	0.67
2,4-dienoyl-CoA reductase	DECR_RAT	0.63
3-ketoacyl-CoA thiolase	THIM_RAT	0.66
Hydroxyacyl-coenzyme A dehydrogenase	HCDH_RAT	0.61
Trifunctional enzyme subunit β	ECHA_RAT	0.66
Long-chain-fatty-acid-CoA ligase 1	ACSL1_RAT	0.63
Isocitrate dehydrogenase [NADP]	IDHP_RAT	0.75
Pyruvate carboxylase	PYC_RAT	0.93
Cytoskeleton/invasion/migration		
RalA-binding protein 1	RBP1_RAT	1.49
Actin-related protein 2/3 complex subunit 4A	RPC4_MOUSE	1.54
STE20-like serine/threonine-protein kinase	SLK_RAT	0.59
Calponin-1	CNN1_RAT	1.42
Calponin-3	CNN3_RAT	1.45
Extracellular matrix/cell adhesion		
Transforming growth factor β -3	TGFB3_RAT	2.22
Clusterin	CLUS_RAT	1.89
72 kDa type IV collagenase (MMP-2)	MMP2_RAT	1.40
Biglycan	PGS1_RAT	1.56
Collagen alpha-1(I) chain	CO1A1_RAT	0.39
Collagen alpha-2(I) chain	CO1A2_RAT	0.54
Fibronectin 1	FINC_RAT	1.68
SPARC-like protein 1	SPRL1_RAT	1.47
Vitronectin	VTNC_MOUSE	1.91
Apolipoprotein A-I	APOA1_RAT	0.68
Kininogen 1	KNT1_RAT	1.67
Periostin (OSF-2)	POSTN_MOUSE	2.43
Apolipoprotein E	APOE_RAT	1.55

Protein Class and Protein Name	Accession drom SwissProt Database	iTRAQ O/Y (fold)
Fetuin-A; Alpha-2-HS-glycoprotein	FETUA_RAT	0.61
Fetuin-B	FETUB_RAT	0.66
Miscellaneous		
Adenylate cyclase type 5	ADCY5_RAT	1.54
a -1-antiproteinase	A1AT_RAT	0.66
a -1-inhibitor III	A1I3_RAT	0.67
Carbonic anhydrase 3	CAH3_RAT	0.63
Carboxylesterase 1	EST2_RAT	0.49
Contrapsin-like protease inhibitor 1	SPA3K_RAT	0.66
Contrapsin-like protease inhibitor 3	SPA3L_RAT	0.62
Ferritin light chain	FRIL1_RAT	1.56
Major urinary protein	MUP_RAT	0.46
Facilitated glucose transporter member 4	GTR4_RAT	2.01
Transthyretin	TTHY_RAT	0.59
Tripartite motif-containing protein 47	TRI47_MOUSE	1.97

iTRAQ= isobaric tag for relative and absolute quantitation; Y =young; and O=old.