J Lipid Atheroscler. 2024 Sep;13(3):292-305
https://doi.org/10.12997/jla.2024.13.3.292
pISSN 2287-2892·eISSN 2288-2561

JLA Journal of Lipid and Atherosclerosis

Review

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Nrf2 Connects Cellular Autophagy and Vascular Senescence in Atherosclerosis: A Mini-Review

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OPEN ACCESS

Received: Dec 9, 2024
Revised: Jan 17, 2024
Accepted: Jan 22, 2024
Published online: Apr 18, 2024

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ABSTRACT

Nuclear factor erythroid 2-related factor 2 (Nrf2), a transcriptional factor that maintains intracellular redox equilibrium, modulates the expression of antioxidant genes, scavenger receptors, and cholesterol efflux transporters, all of which contribute significantly to foam cell development and plaque formation. Nrf2 has recently emerged as a key regulator that connects autophagy and vascular senescence in atherosclerosis. Autophagy, a cellular mechanism involved in the breakdown and recycling of damaged proteins and organelles, and cellular senescence, a state of irreversible growth arrest, are both processes implicated in the pathogenesis of atherosclerosis. The intricate interplay of these processes has received increasing attention, shedding light on their cumulative role in driving the development of atherosclerosis. Recent studies have revealed that Nrf2 plays a critical role in mediating autophagy and senescence in atherosclerosis progression. Nrf2 activation promotes autophagy, which increases lipid clearance and prevents the development of foam cells. Meanwhile, the activation of Nrf2 also inhibits cellular senescence by regulating the expression of senescence markers to preserve cellular homeostasis and function and delay the progression of atherosclerosis. This review provides an overview of the molecular mechanisms through which Nrf2 connects cellular autophagy and vascular senescence in atherosclerosis. Understanding these mechanisms can provide insights into potential therapeutic strategies targeting Nrf2 to modulate cellular autophagy and vascular senescence, thereby preventing the progression of atherosclerosis.

Keywords: Atherosclerosis; Cellular senescence; Autophagy

INTRODUCTION

Atherosclerosis is the leading cause of death worldwide.[1] It is a chronic inflammatory disease characterized by the buildup of lipid plaque, which subsequently leads to the thickening and

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Funding
None.

Conflict of Interest
The authors have no conflicts of interest to declare.

Data Availability Statement
Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

Author Contributions
Conceptualization: Wai KW, Yap WH; Writing - original draft: Wai KW; Writing - review & editing: Low LE, Goh BH, Yap WH.

narrowing of the artery and in turn increases the risk of severe cardiovascular events such as heart attacks or strokes.[2] Atherosclerosis begins with endothelial dysfunction, which increases the expression of adhesion molecules, facilitating monocyte recruitment and foam cell formation, resulting in the development of fatty streaks. This process is accompanied by an increase in the production of inflammatory cytokines and growth factors, which promote the proliferation and migration of smooth muscle cells into the intima layer, further exacerbating the thickening of arterial walls and ultimately the progression of atherosclerotic plaques.[3,4]

Excessive generation of reactive oxygen species (ROS) can lead to the formation of oxidized low-density lipoprotein (oxLDL) species, thereby inducing oxidative stress within cells. This oxidative stress has the potential to trigger cell death and contribute to the progression of cardiovascular diseases.[5] The transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2) plays a crucial role as a regulator of cellular defense against oxidative stress and inflammation, both of which are major factors in the development of atherosclerosis.[6] Nrf2 activation leads to the transcription of genes encoding for various antioxidant enzymes and cytoprotective proteins, such as heme oxygenase-1 (HO-1), NAD(P)H: quinone oxidoreductase-1 (NQO1) and glutathione peroxidase 1.[7,8] However, Nrf2 has been found to exhibit both pro- and anti-atherogenic effects by regulating lipid metabolism, foam cell formation, and the expression of antioxidant proteins and inflammatory markers.[4,9] The actual role of Nrf2 in atherosclerosis progression remains a matter of debate.

Recently, cellular senescence and autophagy-lysosomal pathways have emerged as important mechanisms in the pathogenesis of atherosclerosis. Cellular senescence is a state of irreversible growth arrest characterized by the increased expression of senescence-associated proteins (e.g., p16 and p21), senescence-associated β-galactosidase (SA-β-gal) activity, and levels of adhesion molecules (e.g., intercellular adhesion molecule-1 [ICAM-1], and vascular cell adhesion molecule 1 [VCAM-1]) and proinflammatory cytokines (e.g., interleukin 6 [IL-6] and interleukin 8 [IL-8]).[10,11] Senescence in endothelial cells, smooth muscle cells, and macrophages is associated with various detrimental effects, including vascular dysfunction, foam cell formation, the and upregulation of inflammatory factors, which collectively create a pro-atherosclerotic environment that accelerates the progression of this disease.[10] In contrast, autophagy, a cellular process responsible for maintaining cellular homeostasis and plaque stability, is involved in the accumulation of oxidized lipids, inflammation, and the formation of foam cells, which have been implicated in atherosclerosis.[12,13] Furthermore, the transcription factor Nrf2 has emerged as playing a major role in the complex interplay of cellular activities. Nrf2 has been shown to be involved in regulating autophagy activity by promoting autophagy efflux and is also closely associated with the Keap1/p62 pathway, which mediates proteasomal activity. Induction of Nrf2 has been shown to enhance the expression of autophagy-related genes (e.g., *BECN1,* p62, *ATG5,* and *ATG7*) and autophagic flux, upregulate p62, and decrease Keap1 levels, which attenuate macrophage infiltration, intracellular lipid content, and foam cell formation, as well as protecting against oxidative damage in vascular endothelial cells (VECs) and vascular smooth muscle cells (VSMCs).[14-16] Nrf2 has also been implicated in cellular senescence in atherosclerosis. Nrf2 signaling reduces the effects of senescence by reducing the expression of senescence-related markers (e.g., p21, p16, and β-gal staining), mediating antioxidant gene expression (e.g., *HO-1, GST*, and *NQO1*) and thereby decreasing oxidative stress,[17-19] and modulating inflammatory cytokines (e.g., IL-6, IL-8, and tumor necrosis factor alpha [TNF-α]) to reduce the inflammatory response[20,21] and regulate adhesion molecule expression (ICAM-1, VCAM-1 and E-selectin),[22,23] all of which are associated with monocyte recruitment, endothelial dysfunction, disrupted

vascular homeostasis, and foam cell formation. Taken together, the recent literature has highlighted the potential involvement of Nrf2 in bridging autophagy with senescence in atherosclerosis. The activation of Nrf2 inhibits cellular senescence, while promoting the induction of autophagy, thereby protecting against oxidative stress, alleviating inflammation, and regulating vascular function during atherosclerosis progression. This review emphasizes the role of Nrf2 in connecting cellular senescence and autophagy, focusing on the interplay between autophagy and vascular senescence in atherosclerosis, and providing insights into the molecular mechanisms of Nrf2 in these processes. The involvement of Nrf2 in these processes highlights its potential role in modulating the development of atherosclerosis.

INTERPLAY BETWEEN AUTOPHAGY AND VASCULAR SENESCENCE IN ATHEROSCLEROSIS

Atherosclerosis is initiated by oxidative stress, which leads to the upregulation of proinflammatory cytokines and adhesion molecules that contribute to endothelial dysfunction, foam cell formation, and plaque development.[24] Autophagy plays an important role in atherosclerotic plaque development and stability, which may either exert protective or detrimental effects. The activation of autophagy in atherosclerotic sites may occur due to increased ROS production or metabolic stress.[25] Multiple studies have demonstrated the protective effects of maintaining basal autophagy in atherosclerosis, including inhibiting oxidative stress, preventing excessive lipid accumulation within cells, and delaying the formation of atherosclerotic plaques.[26,27]

Immuno-senescence or inflammaging is associated with impaired autophagy in various immune cells within atherosclerotic lesions.[28] It has been observed that VSMC senescence contributes to the destabilization of atherosclerotic plaque due to lower collagen production and increased secretion of extracellular matrix-degrading proteases, which leads to fibrous cap thinning, necrotic core formation, and calcification.[29] Senescent VSMCs are characterized by changes in phenotype from contractile to synthetic forms, altered replicative potential, and reduced responsiveness to mediators involved in contraction and relaxation.[30] It also promotes the senescence-associated secretory phenotype (SASP) factor secretion, which stimulates the release of cytokines and adhesion molecules.[31] Autophagy has been found to regulate the phenotype and proliferation capacity of VSMCs, which in turn, plays a role in controlling vascular function and the initiation of vascular diseases.[32] Studies have shown that the induction of autophagy can regulate contractile capacity and protect VSMCs from calcification by regulating Ca^{2+} release and influx, as well as the expression of transforming growth factor (TGF)-β1, which modulates cellular osteogenic differentiation.[33,34] The blockage of autophagy by the genetic disruption of essential autophagy proteins (ATG7), as well as autophagy inhibitors (e.g., 3-methyladenine and bafilomycin A1), has been linked to positive staining for SA-β-gal and expression of senescence markers p52, p16 and p21, suggesting that autophagy dysregulation promotes atherosclerotic lesions and exacerbate senescence in VSMCs.[19,35,36] Conversely, the stimulation of autophagy has been found to attenuate oxLDL-induced VSMC senescence by regulating the autophagy-related proteins mammalian target of rapamycin (mTOR)C1, ULK1, and ATG13 and promoting plaque stability.[35] Similarly, the activation of mTOR pathway in VSMCs in the thoracic aorta could inhibit autophagy and induce VSMC senescence with the expression of p53, p21, and p16.[37] These studies have demonstrated that autophagy induction could alleviate atherosclerosis progression by inhibiting VSMC senescence and stabilizing atherosclerotic plaques.

https://doi.org/10.12997/jla.2024.13.3.292

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Numerous studies have also linked the mechanism of autophagy to endothelial cell homeostasis and function. The basal autophagy process is responsible for degrading toxic ROS. However, under stressful conditions, deregulated autophagy may have negative effects on endothelial cell function and contribute to autophagic cell death.[38] Studies have shown that the inhibition of autophagy with bafilomycin or knockdown of autophagy genes (e.g., *ATG3* and *ATG7*) suppressed the phosphorylation of endothelial NO synthase (eNOS), reduced nitric oxide (NO) production, and altered vascular permeability.[39-42] Autophagy has also been found to regulate the inflammatory response in endothelial cells.[43,44] Deficiency in endothelial autophagy showed increased TNF-α–induced inflammation and expression of the senescence marker p16 under high-shear stress (SS) conditions, indicating that the activation of endothelial autophagy by high SS protects against atherosclerotic plaques by preventing senescence and inflammation.[45,46] Endothelial autophagy was found to reduce lipid accumulation in the vessel wall, indicating that lipophagy may be an important mechanism for preventing foam cell formation.[47,48] The impairment of autophagy flux by high concentrations of glucose, palmitic acid, or H_2O_2 increases endothelial cell apoptosis and inflammation, which can be attenuated by regulating the AMPK pathway, which induces autophagy and thereby alleviates the progression of atherosclerosis.[49-51] Furthermore, the induction of autophagy by rapamycin can prevent the elevation of expression of p21, VCAM-1, ICAM-1, and the DNA damage marker γ-H2AX and modulate the production of the vasodilation molecule NO, suggesting that autophagy modulates endothelial senescence and senescence-associated endothelial dysfunction.[52]

Meanwhile, senescent macrophages have been discovered to influence atherosclerosis progression by promoting inflammation and impairing cholesterol efflux.[53,54] The activation of autophagy in macrophages plays an important protective role in atherosclerosis. Autophagy can promote macrophage survival by decreasing macrophage foam cell formation by promoting the hydrolysis of lipid droplets and cholesterol efflux.[47] The activation of autophagy has also been shown to increase the expression of cholesterol transport receptors (ATP-binding membrane cassette transporter; ABCA1 and ABCG1), promote cholesterol efflux, and decrease total cholesterol levels in oxLDL-treated macrophages.[55,56] The promotion of autophagy by quercetin has been found to decrease lipid accumulation, the number of cells staining positive for SA-β-gal, and the expression of p16 and p21 proteins, while the inhibition of autophagy induces opposing effects.[57] The inhibition of autophagy in macrophages was found to lead to the accumulation of p62, which upregulates the activity of mTOR, decreases intracellular cholesterol efflux (mediated by ABCA1 and ABCG1), and accelerates foam cell formation.[56] Studies using *in vivo* atherosclerotic models showed that treatment with an autophagy inhibitor increased aortic plaques, attenuated lipid accumulation, and increased the expression of p53 and p21, while decreasing the LC3 II/I ratio.[58] The findings suggest that autophagy plays an important role in regulating macrophage function in foam cell formation, which influences the progression of atherosclerosis. Overall, these studies have demonstrated that autophagy induction alleviates atherosclerosis progression by inhibiting VEC and VSMC senescence and stabilizing advanced atherosclerotic plaques.

NRF2 SIGNALING, OXIDATIVE STRESS, AND ATHEROSCLEROSIS

Oxidative stress mediated by ROS plays a significant role in the initiation and development of atherosclerosis. Numerous studies have demonstrated that atherosclerotic risk factors such

https://doi.org/10.12997/jla.2024.13.3.292

as hypertension, diabetes, or dyslipidemia are associated with increased ROS production.[59] The imbalance of antioxidant and oxidant systems can lead to an elevation in ROS levels, which further accelerates the advancement of atherosclerosis.[60] Excessive ROS production in the vascular wall causes a decrease in NO bioavailability triggered by eNOS uncoupling, interfering with endothelial permeability by promoting the adhesion of monocytes and leading to endothelial dysfunction.[61] ROS cause the oxidation of low-density lipoproteins and induce foam cell formation and the inflammatory response, thereby further promoting atherogenesis.[62–64] Therefore, neutralizing oxidative stress in cells may provide a solution to atherosclerosis progression. In this context, the transcriptional factor Nrf2 is of interest, since it has been recognized for its role in regulating antioxidant defenses and cellular redox reactions, playing a main role in eliminating oxidative stress.

Nrf2 activates antioxidant response element (ARE)-dependent genes, which regulate the production of proteins involved in the detoxification and removal of ROS and electrophiles. It can also regulate mitochondrial ROS production by controlling substrate availability for mitochondrial respiration and the efficiency of mitochondrial fatty acid oxidation.[65] Under normal conditions, Nrf2 is associated with the Keap1 protein and undergoes polyubiquitination for proteasomal degradation.[66] In contrast, under unfavorable conditions such as oxidative stress, metabolic stress, and oncogenic stress, Nrf2 dissociates from Keap1, translocates, and accumulates in the nucleus. It then binds with the small Maf protein and interacts with the ARE in the nucleus. The interaction of ARE with Nrf2 transcriptionally regulates its target gene, which then activates the expression of several antioxidant enzymes that protect cells from oxidative stress, such as glutathione (GSH) and superoxide dismutase 1, and carry out drug detoxification, such as glutathione S-transferase (GST) and NQO1.

It has been found that Nrf2 regulates multiple pathways in atherosclerosis development. Nrf2 regulates macrophage foam cell formation and oxidative stress redox reactions by modulating the expression of antioxidant genes, scavenger receptors, and cholesterol efflux transporters, thereby exerting protective effects against atherosclerosis development (**Fig. 1**).[62,67–70] Nrf2 signaling was shown to play a role in macrophage lipid uptake by regulating CD36 scavenger receptors.[71–73] In addition, Nrf2 was found to upregulate HO-1 and peroxiredoxin 1 expression, increase ABCA1 levels, and decrease LOX-1 levels, suggesting that Nrf2 can alleviate oxidative stress and inhibit macrophage-derived foam cell formation by increasing cholesterol efflux and decreasing lipid intake.[74,75] It has also been shown that Nrf2 regulates monocyte recruitment and the inflammatory response by modulating the expression of proinflammatory cytokines (IL-6 and IL-1β) and adhesion molecules (e.g., monocyte chemoattractant protein-1 [MCP-1]) that play critical roles in driving atherosclerotic plaque formation.[9,76,77] Studies have also shown that Nrf2 can protect VECs by reducing lipid peroxidation and increasing antioxidant enzyme activity in *Ldlr*$^{-/-}$ mice fed with a high-fat diet.[78] Surprisingly, it was found that Nrf2 deficiency in the aorta and VSMCs in an atherosclerosis model reduced LOX-1 expression and the uptake of oxLDL, which further attenuated the atherosclerotic plaque burden, reduced VSMC migration and proliferation, and inhibited oxidative stress.[79] A study also found that Nrf2 functions as a novel positive regulator of the inflammasome, as its signaling exacerbated atherosclerosis by increasing IL-1 production triggered by cholesterol crystals.[80] The deficiency of Nrf2 in macrophages was found to contribute to foam cell formation by increased lipid uptake and decreased cholesterol efflux and promote a proinflammatory phenotype with elevated expression levels of MCP-1, IL-6, and TNF-α.[9]

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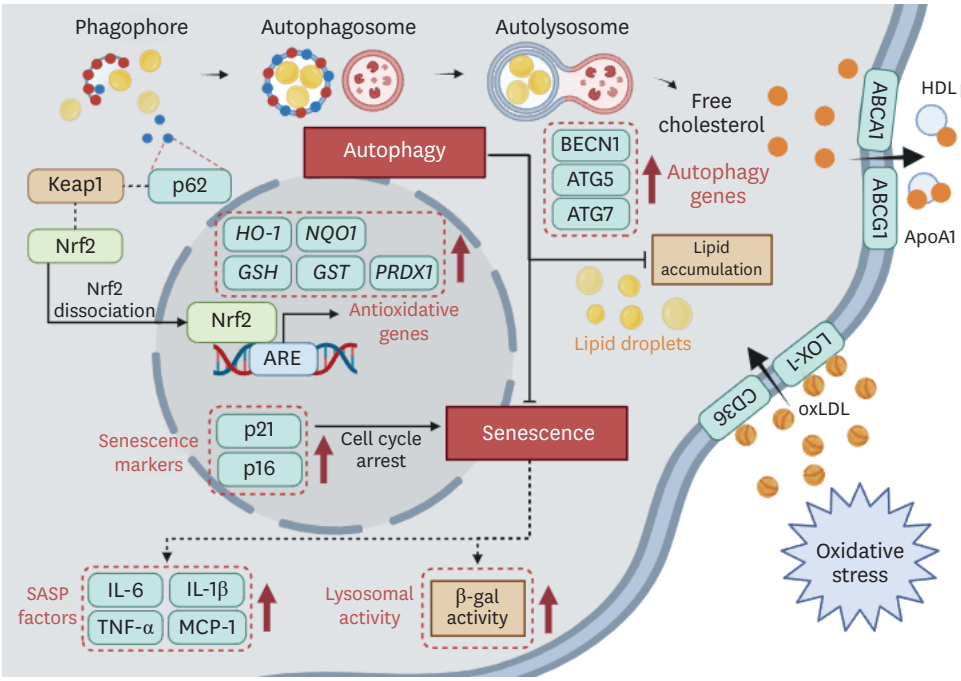

Fig. 1. Role of Nrf2 in regulating the autophagy and senescence pathways that promote foam cell development. In response to oxidative stress, Nrf2 mediates the expression of antioxidative genes (*HO-1*, *NQO1*, *PRDX1*, *GSH*, and *GST*). The activation of Nrf2 has been linked to the upregulation of autophagy genes (including p62), and it is associated with the inhibition of expression of senescence markers, such as p16, p21, IL-1β, TNF-α, MCP-1, IL-6, and β-gal activity. The interplay between autophagy activation and senescence inhibition has been implicated in the processes driving cholesterol efflux and lipid accumulation during the development of foam cells. The diagram was constructed using BioRender.com.
GSH, glutathione; GST, glutathione S-transferase; HO-1, heme oxygenase-1; IL-1β, interleukin-1β; IL-6, interleukin 6; NQO1, NAD(P)H quinone oxidoreductase-1; MCP-1, monocyte chemoattractant protein-1; Nrf2, nuclear factor erythroid 2-related factor 2; Prdx1, peroxiredoxin 1; TNF-α, tumor necrosis factor alpha.

MOLECULAR MECHANISMS OF NRF2 IN MEDIATING AUTOPHAGY AND VASCULAR SENESCENCE

Recent studies have demonstrated that Nrf2 plays a crucial role in modulating the balance between autophagy and senescence in atherosclerosis. The activation of Nrf2 induces antioxidant gene expression, which protects against oxidative stress that can trigger endothelial dysfunction, smooth muscle cell proliferation, and macrophage activation, all of which are critical events in atherosclerosis development.[14,16,81] Nrf2 activation has been shown to reduce the senescence effect and suppress senescence-associated inflammation by inhibiting the secretion of proinflammatory cytokines and matrix metalloproteinases (MMPs).[82-84] It is also involved in the regulation of adhesion factors and efflux transporters, which modulate monocyte recruitment and cholesterol efflux. Furthermore, the activation of Nrf2 can increase autophagic flux, which promotes the removal of oxidized lipids such as oxLDL and prevents the formation of foam cells.[14,19,85,86] The molecular mechanisms by which Nrf2 mediates autophagy and vascular senescence in atherosclerosis are summarized in **Table 1**. Overall, the activation of Nrf2 can increase autophagy and reduce cellular senescence, thereby reducing inflammation and promoting cellular homeostasis within the arterial wall, which influences the development and progression of atherosclerosis.

In an *in vitro* atherosclerosis model induced by ox-LDL, the activation of Nrf2 was found to be associated with increased autophagy.[85] This was accompanied by decreased levels of SASP markers (TNF-α, IL-6, and IL-1β), adhesion factors (ICAM-1 and VCAM-1), and cholesterol efflux regulatory proteins (ABCA1, ABCG1, and SR-BI). Similarly, the activation of Nrf2 by

https://doi.org/10.12997/jla.2024.13.3.292

Table 1. Molecular mechanisms of Nrf2 in mediating autophagy and vascular senescence

Atherosclerosis model	Nrf2 mechanism	Senescence marker	Autophagy marker	Reference
HUVECs and human acute monocytic leukemia cells (THP-1 macrophages) incubated with oxLDL	Treatment with KLF2 activates Nrf2 and promotes autophagy, increases cell viability, and reduces inflammation, adhesion factors, and foam cell formation.	· ↓ TNF-α, IL-1β, and IL-6 expression · ↓ ICAM-1, VCAM-1, and E-selectin · ↑ ABCA1, ABCG1, and SR-BI	· ↑ LC3II/LC3I and Beclin1 · ↓ p62	85
$NaAsO_2$-induced oxidative stress in HUVECs	TTM treatment activates Nrf2 and its target genes (*HMOX1* and *GCLM*), protecting against oxidative stress and promoting autophagy.	· ↓ Cell death (↓ PARP, ↓ γH2AX)	· ↑ Protein levels of LC3B-II and SQSTM1 · Autophagy inhibition suppresses Nrf2 activation	16
CuONP-induced oxidative stress in a mouse model and HUVECs	Nrf2 pathway activation by CuONPs increases HMOX1 and GCLM, providing protection against oxidative stress and cell death, while the Nrf2 signaling pathway is also modulated by autophagy.	· Nrf2 knockout: ↑ γH2AX and ↑ superoxide anions accumulation	· *Atg5*-KO or autophagy inhibition by an inhibitor (3-MA, Wort, and CQ) ↓ Nrf2 and HMOX1 expression	81
THP-1 macrophages incubated with oxLDL	Nrf2 activation by tBHQ treatment in autophagy-blocked macrophages upregulates HO-1, further aggravating autophagy blockage and increasing inflammation	· ↑ IL-1β and IL-18	· ↑ Expression levels of LC3II/I and p62	82
Streptozotocin-induced diabetes in apolipoprotein E-deficient mice	*In vivo*: Treatment of mice with tBHQ induces Nrf2 expression, leading to reduced size and lipid content of atherosclerotic lesions, decreased inflammation, and increased autophagic flux. *In vitro*: Nrf2 activation by tBHQ-induced antioxidant genes (*HMOX1*, *SOD1*, and *CAT*), suppresses cytokine-induced expression of pro-inflammatory and oxidative stress genes and promotes autophagic activity	· ↓ Chemokine (CCL2 and CCL5) expression	· ↑ Expression levels of BECN1, SQSTM1/p62 proteins and conversion of MAP1LC3B-I to MAP1LC3B-II	14
ATP-stimulated NLRP3 inflammasome activation in THP-1 macrophages	Treatment with rapamycin induces autophagy, activates Nrf2 and NQO1, and reduces inflammation	· ↓ IL-1β secretion and caspase-1 cleavage, IL-6, IL-8, MCP-1 and IκBα transcript	· ↓ p62/SQSTM1 protein · ↑ The levels of LC3-II, beclin 1, and ATG5	84
Stress-induced premature senescence in mouse embryonic fibroblasts and replicative senescence in WI38 human cells	Activating Nrf2 with rapamycin increases the expression of target genes (*GST* and *NQO1*), inhibits cellular senescence, and induces autophagy	· ↓ β-gal activity · ↓ levels of p16 and p21 molecular markers	· ↓ Levels of p62 · ↑ LC3B-I to LC3B-II interconversion	83
Deletion of the autophagy gene *Atg7* in murine VSMCs (*atg7*$^{-/-}$ VSMCs)	Defective autophagy activates the Nrf2 signaling pathway, leading to the upregulation of antioxidative enzymes (*GST* and *NQO1*), the induction of senescence, and the promotion of neointima formation.	· G1-cell cycle arrest (atg7$^{-/-}$ nuclei in G1-phase ↑, in G2/M-phase ↓) · ↑ SA-β-gal activity · ↑ collagen deposition and migration (MMP-9, TGFB, and CXCL12)	· ↑ SQSTM1 · ↓ LC3B-II levels	19

Nrf2, nuclear factor, erythroid 2-like 2; HUVEC, human umbilical vein endothelial cells; KLF2, Krüppel like transcription factor 2; TNF-α, tumor necrosis Factor alpha; IL-1β, interleukin 1 beta; IL-6, interleukin 6; ICAM-1, intercellular adhesion molecule 1; VCAM-1, vascular cell adhesion protein 1; ABCA1, ATP binding cassette subfamily A member 1; ABCG1, ATP binding cassette subfamily G member 1; SR-BI, scavenger receptor class B type I; MAP1LC3B, microtubule-associated protein 1 light chain 3 beta; TTM, ammonium tetrathiomolybdate; HMOX1/HO-1, heme oxygenase 1; GCLM, glutamate-cysteine ligase; PARP, poly(ADP-ribose) polymerase; γH2AX, phosphorylated histone H2AX; SQSTM1, sequestosome 1; CuONP, copper oxide nanoparticle; 3-MA, 3-methyladenine; Wort, wortmannin; CQ, chloroquine; oxLDL, oxidized low-density lipoprotein; tBHQ, tertiary butylhydroquinone; IL-18; interleukin 18; SOD1, superoxide dismutase; CAT, catalase; CCL5, C-C motif chemokine ligand 5; BECN1, beclin-1; ATP, adenosine triphosphate; NQO1, NAD(P)H quinone oxidoreductase; IL-8, interleukin 8; MCP-1/CCL2, monocyte chemoattractant protein-1; ATG5, autophagy-related 5; GST, glutathione S-transferase; SA-β-gal, senescence-associated β-galactosidase; ATG7, autophagy-related 7; MMP-9, matrix metalloproteinase 9; TGFB, transforming growth factor beta; CXCL12, CXC motif chemokine 12.

different stimuli, such as ammonium tetrathiomolybdate and copper oxide nanoparticles, has been shown to protect against oxidative stress-induced endothelial cell death.[16,81] Nrf2 activation resulted in the upregulation of antioxidative genes and triggered autophagy flux, as evidenced by increased levels of LC3B-II, SQSTM1, and autophagosome formation. The disruption of autophagy genes, including *ATG5* and *SQSTM1*, has been shown to suppress Nrf2 and inhibit the transcription of antioxidant genes, which promote oxidative stress and cell death.[16] Furthermore, it has been shown that autophagy plays a crucial role in stabilizing Nrf2 and its downstream genes by inhibiting the ubiquitin–proteasome machinery,

https://doi.org/10.12997/jla.2024.13.3.292

preventing the proteasome-dependent degradation of Nrf2 and resulting in the activation of Nrf2 antioxidant pathways. Conversely, autophagy inhibition promotes the proteasome-dependent degradation of Nrf2, activating the Nrf2 antioxidant pathway and exacerbating vascular injury, as shown by upregulated inflammatory factor expression. These findings emphasize the interconnected roles of Nrf2 and autophagy in vascular health.[81]

In a diabetes-driven atherosclerosis model, tBHQ-induced Nrf2 activation in macrophages showed an atheroprotective effect by reducing the size and lipid content of atherosclerotic lesions while decreasing inflammation and oxidative stress.[14] This is correlated with upregulated expression of autophagy-related markers and enhanced autophagic flux. Meanwhile, Nrf2 activation by tBHQ in an *in vitro* model was also found to suppress the cytokine-induced expression of proinflammatory and oxidative stress genes and promoted autophagic activity, highlighting Nrf2 activation as a potential atheroprotective approach against inflammation and oxidative stress, while restoring autophagy.[14] Studies have also explored the effect of rapamycin-induced autophagy induction, which suppressed inflammation in macrophages, as demonstrated by the reduced production of MCP-1, IL-6, IL-1β, and IL-18. This also involved the activation of Nrf2 through upregulation of p62/SQSTM1, which contributed to the reduction of oxidative stress.[84] Moreover, the activation of Nrf2 by rapamycin was also found to delay cell senescence, as evidenced by decreased β-gal activity and lower levels of the molecular markers p16 and p21. This effect appears to be related to the activation of autophagy, as suggested by decreased levels of p62 and increased conversion of LC3B-I to LC3B-II.[83] The dual effect of reducing oxidative stress and decreasing senescence and inflammatory markers highlights the complex interplay between autophagy, Nrf2, and cellular senescence.

In contrast, defective autophagy resulting from deletion of the autophagy gene *Atg7* has been found to lead to the accumulation of p62 and accelerate the development of stress-induced premature senescence, characterized by increased CDKN2A-RB-mediated G1-cell cycle arrest, and SA-β-gal activity.[19] This is also associated with increased Nrf2 activation, resulting in resistance to oxidative stress-induced cell death. Impaired autophagy is associated with increased expression of SASP markers (MMP9, TGFB, and CXCL12) and CDKN2A, promoting postinjury neointima formation and atherogenesis in murine VSMCs with tissue-specific deletion of the essential autophagy gene *Atg7* (*Atg7*$^{-/-}$ VSMCs). Furthermore, the inhibition of autophagy by chloroquine in ox-LDL-treated macrophages not only reduced cell viability but also triggered inflammation, increasing the expression levels of LC3II/I and Nrf2, which was likely to have been achieved through the accumulation of p62.[82] Thus, these studies collectively highlight the complex interconnections between Nrf2, autophagy, and cellular senescence, and their impact on atherosclerosis.

THERAPEUTIC STRATEGIES TARGETING CELLULAR AUTOPHAGY AND VASCULAR SENESCENCE IN THE DEVELOPMENT OF CARDIOVASCULAR ATHEROSCLEROSIS

Cellular autophagy and vascular senescence are two critical pathophysiological processes that play a vital role in the development and progression of atherosclerosis. Therefore, therapeutic strategies targeting cellular autophagy and vascular senescence may have a significant impact on atherosclerosis prevention and treatment.

https://doi.org/10.12997/jla.2024.13.3.292

Several pharmacological agents have been shown to regulate autophagy and vascular senescence and attenuate atherosclerosis progression. Resveratrol, a polyphenolic compound found in grapes and red wine, has been shown to decrease the formation of atherosclerotic plaques, lipid accumulation, and levels of inflammatory cytokines through decreased PI3K/AKT/mTOR signaling.[87] It was also found to inhibit ICAM-1 expression via transcriptional regulation of the FERM-kinase and Nrf2/ARE interaction, thereby blocking monocyte adhesion and thus yielding an anti-atherogenic effect.[88] Studies also found that resveratrol exerts its antioxidative vascular protective effects by increasing Nrf2 expression, which affects γ-glutamylcysteine synthetase, HO-1, and NQO-1 in arterial coronary endothelial cells.[89] Resveratrol has also been linked to SIRT1 expression, which exerts anti-inflammatory and antioxidative stress activities and suppresses TNF-α-triggered ROS generation.[90]

Additionally, rapamycin, an mTOR inhibitor that is known to induce autophagy, has been studied regarding its ability to regulate the stabilization of atherosclerotic plaques.[91] It was found to inhibit cell senescence, as demonstrated by decreased SA-β-gal activity and the downregulation of senescence-associated molecular markers such as SASP, p16, and p21, which are correlated with increased levels of Nrf2 and the activation of autophagy.[83] Treatment with rapamycin was found to activate autophagy and suppress the production of SASP factors, as well as reducing the number of SA-β-gal-positive senescent cells.[92] Another study also found that the activation of the mTOR pathway in VSMCs led to reduced levels of signal-associated autophagy proteins (LC3II and beclin-1), and the inhibition of mTOR pathway resulted in a reduced number of SA-β-gal-stained cells and regulated the expression of senescence markers (p53/p21/p16).[37]

Several natural compounds, including sulforaphane and curcumin, have demonstrated their potential to attenuate the progression of atherosclerosis in animal models. Notably, sulforaphane was found to have the ability to slow the advancement of atherosclerotic lesions induced by a high-fat diet, as well as to mitigate vascular dysfunction. This effect is likely attributed to its ability to lower cholesterol levels, reduce vascular oxidation (via increasing vascular GSH and preventing lipid peroxidation), and suppress inflammation mediated by nuclear factor-κB (NF-κB).[93] Similarly, curcumin appears to exert its effects by modulating lipid metabolism, macrophage infiltration, and inflammation through the suppression of molecules such as CD36, VCAM-1, ICAM, NF-κB, IL-1β, and TNF-α.[55,94] These findings show the protective potential of natural compounds against atherosclerosis, particularly through antioxidant and anti-inflammatory properties.

CONCLUSION

In summary, Nrf2 plays a critical role in regulating both autophagy and vascular senescence, which are key processes involved in the development and progression of atherosclerosis. Overall, these findings suggest that Nrf2 could be a promising target for the development of therapeutic strategies aimed at combatting atherosclerosis and other age-related diseases. The molecular mechanisms by which Nrf2 regulates these processes are complicated and involve multiple signaling pathways, emphasizing the need for further in-depth research to unravel the precise signaling pathways and molecular interactions to facilitate the development of more targeted and effective interventions. Further studies are also needed to explore the potential side effects of Nrf2 activation in various tissues and cell types. In conclusion, our review underscores the significance of Nrf2 in the context of autophagy

https://doi.org/10.12997/jla.2024.13.3.292

and vascular senescence in atherosclerosis development. Further investigation of this intricate interplay and its therapeutic potential promises to strengthen our ability to prevent atherosclerosis, ultimately alleviating the burden of cardiovascular disease.

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