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Review

The roles of autophagy in vascular smooth muscle cells

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ABSTRACT

Autophagy, which is an evolutionarily conserved mechanism and links to several cellular pathways, impacts vascular smooth muscle cells (VSMCs) survival and function. Activation of autophagy by intercellular and/or extracellular stimuli has protective effects on VSMCs against cell death, while on the contrary, overloading autophagy has been recognized as a deleterious process by excessive self-digestion. Alterations in autophagy has been documented in VSMC in response to various stimuli, resulting in modulation of VSMC functions, including proliferation, migration, matrix secretion, contraction/relaxation, and differentiation. Each of these changes in VSMC functions plays a critical role in the development of vascular diseases. Importantly, emerging evidence demonstrates that autophagy deficiency in VSMCs would contribute to atherosclerosis and restenosis, shedding novel light on therapeutic target of the vascular disorders. Herein, this review summarizes the recent progress associated with the roles of autophagy in VSMC and offers the perspectives to several challenges and future directions for further studies.

1. Introduction

Vascular smooth muscle cells (VSMCs) are critical in maintaining the physiological functions of blood vessel wall. In response to environmental changes, modulation of VSMC survival and function plays a crucial role in the development of vascular disease [1].

In the vasculature, changes in autophagy have been documented in numerous vascular diseases, including hypertension [2,3], vascular aging [4,5], atherosclerosis [6], and restenosis [7]. Several review articles have summarized the role of autophagy in SMC or vascular biology [8,9], indicative of the importance of autophagy in the progress of vascular diseases. Recently, emerging evidence demonstrated that defective autophagy in VSMCs accelerates senescence and promotes neointima formation and atherogenesis [7], further suggesting a critical role of autophagy in vascular diseases through regulating the VSMC functions. This review will focus on the following questions: what the role of autophagy is in the survival of VSMCs, and how autophagy regulates VSMC function. We also summarize the findings related to the effects of autophagy on VSMCs in vascular diseases.

2. Autophagy in VSMCs

Autophagy exists in 3 separate forms: microautophagy, chaperone-mediated autophagy, and macroautophagy [8]. The term autophagy usually refers to macroautophagy, which is the most prevalent and best studied form of autophagy. In this review, we will focus exclusively on macroautophagy, further cited as autophagy.

Autophagy, as an important biological process that contributes to cellular homeostasis, has been recognized as an important mediator of survival and function in vascular cells including VSMCs, endothelial cells (ECs), and macrophages [8,10]. It protects cells against potential damage associated with the exposure to stress and thereby helps to defend cells and, subsequently, the vessel against dysfunction [11,12]. Specifically, autophagy is activated or inhibited in VSMCs by multiple stimuli and stressors including metabolic stress, reactive species, cytokines and drugs [9,13]. Under these conditions, autophagy-initiating UNC-5 like autophagy activating kinase 1 (ULK1), acts as a convergence point for multiple signals that control autophagy initiation [14], subsequently triggers downstream events, leading to the assembly of a semi-circular double-membrane vesicle called the phagophore and ultimately maturating into a double membrane vesicle called the autophagosome. The autophagosome then fuses with the lysosome leading to the lytic degradation of autophagosomal contents. Protein degradation yields amino acids and other building blocks that can be reutilized by the cell for the biosynthesis of essential macromolecules or energy production [9,15].

It would be of importance to highlight autophagy could interact with other cellular pathways in response to external and internal stimuli. The autophagy protein-6 [16–19] (ATG6, also known as Beclin1)

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was recognized as important signal pathways between autophagy and apoptosis. The essential autophagy gene product Atg7, which modulates p53 activity to regulate cell cycle [20], may be a potential link between the autophagy and cell cycle. In addition, autophagy proteins AMBRA1 and Beclin 1 were recognized to play a role in the crosstalk between autophagy and cell proliferation by regulation of c-Myc [21,22]. In particular, the consequences from VSMCs in response to the changes in autophagy program are different. Hence, it is worthy to systematically review the current literature regarding the roles of autophagy in vascular diseases through regulation of VSMC survival and function.

3. Autophagy effects on the survival of VSMCs in vascular disease

For decades, the effects of autophagy on VSMC survival in response to stimuli in the pathogenesis of vascular disorders have been investigated in numerous studies (Table 1), which provide a concept the autophagy may exert multiple effects on VSMCs in the different conditions.

The initial studies of autophagy involvement in the regulation of VSMC function appear to suggest its promotion of cell death [23]. In atherosclerotic lesions, autophagy can be modulated by cytokines in plaque VSMCs, such as tumor necrosis factor-alpha (TNF-α) and insulin-like growth factor-1 (IGF-1) [23]. TNF-α, present in atheromas and secreted by inflammatory cells as well as VSMCs [24], stimulates apoptosis and autophagy of VSMCs [23]. TNF-α actions are through inducing microtubule-associated protein 1 light chain 3 (LC-3) mRNA expression via *c-Jun* N-terminal kinase and protein kinase B pathways and increasing Beclin-1 protein expression through *c-Jun* N-terminal kinase. In contrast, IGF-1 can promote cell survival through inhibiting autophagy in plaque VSMCs by reducing LC-3 mRNA expression via the Akt pathway [23]. IGF can also prevent serum withdrawal-induced autophagy of mitochondria [25]. These studies have triggered further investigations to elucidate the precise roles of autophagy in the viability of VSMCs in certain conditions. Similarly, autophagy induced by osteopontin (OPN), which has multifunctional properties in promoting cell adhesion or apoptosis shown by in vitro studies [26,27], was found to contribute to VSMC death [28]. Moreover, angiotensin II (Ang II)-induced autophagy in VSMCs may also play a detrimental role in the onset of vascular injury [29]. These results suggest that activation of autophagy, under some conditions, detrimentally contributes to the viability of VSMCs. Exactly how autophagy promotes cell death programs remains to be elucidated. Given that autophagy could utilize different strategies to target the cargo including protein and organelle for degradation, it may discreetly propose that excessive self-digestion might occur in VSMCs in response to certain severe stimuli, leading to cell death.

Conversely, the protective effects of autophagy on VSMCs have also been observed. For example, activation of autophagy by stimuli such as hypoxia [30], 4-hydroxynonenal (NHE) [31], 1-palmytoyl-2-(5-oxovaleroyl)-sn-glycero-3-phosphocholine (POVPC) [32], or 7-ketocholesterol [33], which promoted endoplasmic reticulum (ER) stress or mitochondrial dysfunction, protected VSMCs against cell death. Moreover, enhanced autophagy triggered by an overload of free cholesterol, as illustrated by increased formation of autophagic vacuoles and conversion of LC3-I into the autophagosome-specific LC3-II, might be involved in a pro-survival mechanism to prevent the death of VSMCs [34]. On the contrary, inhibition of autophagy by treatment with 3-methyladenine (3-MA) increased endoplasmic ER stress and mitochondrial depolarization, thereby leading to apoptosis and cell necrosis. In the same study, treatment with rapamycin, an autophagy inducer attenuating ER stress, inhibited cell death resulting from cholesterol overloading. In this scenario, it appears that autophagy is activated as a survival mechanism in VSMCs by removing damaged protein and defective mitochondria, thereby preventing the cell death. Another pathological stimulus, β-amyloid, has been shown to activate autophagy in cerebral vascular dysfunction and disease [35]. Indeed, the degree of endothelial autophagic activation seems to be correlated with the distance from β-amyloid deposition (for review, see [8]). However, it remains to be answered whether autophagy in VSMCs could be activated by β-amyloid and provide beneficial effects.

More recently, studies have demonstrated that defective autophagy promotes senescence and prevents oxidative stress-induced cell death in VSMCs [7], shedding new light on the roles of autophagy in VSMCs. Specifically, SMC-specific knockout of the essential autophagy gene, Atg7, in murine VSMCs resulted in acceleration of the development of senescence, as suggested by cellular and nuclear hypertrophy and senescence-associated β-galactosidase activity. The mechanism underlying Atg7-knockout induced-senescence would be due to the accumulation of SQSTM1, an ubiquitin binding protein involved in cell signaling, oxidative stress, and autophagy, since transfection of SQSTM1-encoding plasmid DNA in normal VSMCs was shown to induce similar features. Interestingly, autophagy deficiency enhances VSMC resistance to oxidative stress-induced cell death. The underlying mechanism may involve the nuclear translocation of the transcription factor NFE2L2, resulting in upregulation of several anti-oxidative enzymes. These findings suggest maintenance of physiological levels of autophagy is indispensable for normal homeostasis in VSMCs. The absence of autophagy exacerbates proteotoxicity in the VSMCs by an accumulation of damaged protein and defective organelle, which, in turn, causes the senescence of VSMCs. As to the effects of resistance to oxidative stress-induced cell death in the absence of autophagy, the causal role for defective autophagy in this scenario remains to be investigated.

Given that multiple cell types including VSMCs, ECs, and macrophages are determinants in the process of vascular diseases, it is worth to note that autophagy has diverse effects on different vascular cell types in vascular diseases [10,13]. In atherosclerotic plaques, macrophage-specific deletion of ATG5 in low-density lipoprotein receptor knockout ($LDLR^{-/-}$) mice exacerbates atherosclerotic plaque progression by increasing macrophage apoptosis and necrosis [36]. In addition, defective autophagy in macrophages is associated with hyperactivation of the inflammasome, and stimulating atherosclerotic plaque development [37]. Hence, defective autophagy in macrophages may promote atherosclerotic plaque formation and destabilization. However, according to the report by M. Grootaert et al. [7,10], SMC-specific deletion of ATG7 in apolipoprotein E-deficient ($ApoE^{-/-}$) mice accelerates atherosclerotic plaque development as characterized by an increase in fibrous cap thickness and collagen, suggesting that defective autophagy

Table 1
Autophagy effects on the survival of VSMCs.

	Agents	Cell types	Effects	Consequences	Ref.
Cytokines	Tumor necrosis factor-α	VSMC from human atherosclerotic lesions	Activation	Cell death	[23]
	Insulin and insulin-like growth factor-1	VSMC from human atherosclerotic lesions	Inhibition	Cell survival	[23]
	Osteopontin	Human VSMCs in culture	Activation	Cell death	[28]
	Angiotensin II	Rat aortic SMCs in culture	Activation	Cell death	[29]
Metabolic stress	Hypoxia	Human pulmonary artery SMCs in culture	Activation	Cell survival	[30]
Reactive species	4-Hydroxynonenal	Rat aortic SMCs in culture	Activation	Cell survival	[31]
	POVPC	Rat aortic SMCs in culture	Activation	Cell survival	[32]
	7-Ketocholesterol	Human aortic SMCs in culture	Activation	Cell survival	[33]
	Free cholesterol	Rat aortic SMCs in culture	Activation	Cell survival	[34]