S-nitrosylation-impaired autophagy: An alternative mechanism underlying aging?

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Abstract

Aging is mysterious with unknown managing patterns. A surprising finding on the tune mode of autophagy by S-nitrosylation is a distinctive step towards the interpretation of the mechanism underlying aging and age-related diseases. This commentary article will discuss, in a wider sense, the implications of S-nitrosylation- and nitration-switched dysfunction of proteins/enzymes in neurodegenerative disorders including Alzheimer's disease (AD), Huntington's diseases (HD) and Parkinson's disease (PD).

Autophagy, or autophagocytosis, is a lysosomal clearance process that recycles cellular components and reallocates nutritional intermediates to ensure the homeostasis between anabolism and catabolism within a living cell. Upon response to diverse environmental signals such as growth factors, amino acids, energy currency, and starvation, autophagy is deeply implicated in many physiological and pathological aspects. Longevity has been attributed to the controlled autophagy in model organisms and mammalian tissues, in which knockdown of the autophagy inhibitor p53 induces degenerative alterations that resemble those seen during aging, and the aging process is often associated with reduced autophagy. While extension of life span triggers autophagy, impaired autophagy can accompany with the loss of longevity-promoting effects conferred by rapamycin, resveratrol, and caloric restriction (CR) (*1*). Perhaps the most primordial function of autophagy is adaptation to nutrient deprivation, but new evidence also reveals a crucial role for autophagy in immunity and inflammation because autophagy protects against infectious, autoimmune and inflammatory diseases (*2*).

Autophagy is dually tuned by two critical enzymes in mammals: the silent mating type information regulation 2 homolog 1 (SIRT1) and the mechanistic target of rapamycin complex 1 (mTORC1). Knockin of SIRT1 induces autophagy, whereas knockout or knockdown of SIRT1 prevents the induction of autophagy by resveratrol and nutrient deprivation in human cells (*3*). Autophagy is negatively regulated by mTORC1, and mTORC1 activity can be inhibited by rapamycin or starvation. Under glucose starvation, AMP-activated protein kinase (AMPK) promotes autophagy by directly activating the mammalian autophagy-initiating kinase Ulk1 through phosphorylation of serine 317 and serine 777. Under nutrient sufficiency, high mTOR activity prevents Ulk1 activation by phosphorylating Ulk1 at serine 757 and disrupting the interaction of Ulk1 with AMPK (*4*). SIRT1 can negatively regulate mTOR signaling because SIRT1 deficiency results in elevated mTOR signaling, and the SIRT1 activator resveratrol reduces mTOR activity in a SIRT1-dependent manner. SIRT1 interacts with TSC2, a component of the mTOR inhibitory complex upstream to mTORC1, and regulates mTOR signaling in a TSC2-dependent manner (*5*). Paradoxically, rapamycin extends the life span and insulin sensitivity of yeast, fruit

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flies and mice, but chronic administration of rapamycin causes glucose intolerance and insulin resistance in mice and humans. This phenomenon has been now deciphered by a finding that rapamycin disrupts a second mTOR complex, mTORC2, which is required for the insulin-mediated suppression of gluconeogenesis. Suppression of mTORC1 signaling is sufficient to extend life span independently from glucose homeostasis, so the differential effects of rapamycin on glucose homeostasis and longevity can be uncoupled (*6*).

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are known to regulate autophagy, but how nitric oxide (NO), the major kind of RNA, is involved in autophagy remains obscure. Surprisingly, Sarkar *et al*. (*7*) have recently revealed an unexpected autophagy-modulating pattern by NO-mediated S-nitrosylation of target proteins in mammalian cells. NO inhibits autophagic flux by S-nitrosylation of the cysteine residue of JNK1 or IKKβ to abrogating phosphorylation, which subsequently decreases JNK1 activity and abrogates Bcl-2 phosphorylation, or activates mTORC1 upon inactivation of IKKβ, AMPK and TSC2. While overexpression of nitric oxide synthase (NOS) impairs autophagic flux, inhibition of NO synthesis induces autophagy. Nevertheless, the NOS inhibitor *Nω*-nitro-L-arginine methyl ester hydrochloride (L-NAME) induces autophagy independent on mTORC1 activity or Bcl-2 phosphorylation. Interestingly, deprival of NO by L-NAME or the endogenous dominant-negative NOS regulator NOS4 reduces mutant huntingtin aggregation and neurodegeneration in a fruit fly model of Huntington disease (HD). Although S-nitrosylation is able to interpret NO-enhanced autophagy and alleviated neurodegeneration, it is still uncertain whether S-nitrosylation is a general mechanism of NO-impaired autophagy or only represents an accidentally randomized event. Cho *et al.* (*8*) have demonstrated that S-nitrosylation of dynamin-related protein 1 (DRP1) rich in brains of Alzheimer's disease (AD) patients increases GTPase activity and mediates β-amyloid-related mitochondrial fission and neuronal injury. However, Bossy *et al.* (*9*) have refuted that S-nitrosylation of DRP1 does not affect GTPase activity and is not specific to AD. This discrepancy, as an assumption, may be attributed to the reversible feature of S-nitrosylation although another possibility of alternative modifications by S-nitrosylation of downstream proteins also exists.

Except for S-nitrosylation of cysteine, NO can also nitrate tyrosine and other amino acid residues through peroxynitrite ($ONOO^-$), which is formed from reaction of NO with superoxide (O_2^-). Pilon *et al*. (*10*) have uncovered that treatment of rats or mice by the endotoxin lipopolysaccharide (LPS) *in vivo* or $ONOO^-$ *in vitro* significantly promotes the nitration of insulin receptor substrate 1 (IRS-1) and reduces insulin-dependent tyrosine phosphorylation, eventually leading to skeletal muscle insulin resistance. Lam *et al*. (*11*) detected the increased nitration of mitochondrial proteins with aging, in which the nitration of F1-ATPase at tyrosine 269 leads to deficient ADP binding to the active site of the enzyme, and is associated with a moderate impairment of mitochondrial functions, as indicated by the decreased

respiratory control ratio as a function of age and by the release of mitochondrial cytochrome *c* to the cytosol. If the nitration of tyrosine occurs globally, it seems reasonable to interpret the results of Sarkar *et al*. (*7*) by the nitrosative inactivation of separated signaling proteins of the autophagy machinery. Besides, L-NAME abrogates neurodegeneration perhaps through directly blocking the mis-folding and aggregation of proteins such as Huntingtin with S-nitrosylation or tyrosine nitration. Indeed, S-nitrosylation of protein-disulfide isomerase or the E3 ubiquitin ligase parkin has been found to initiate protein mis-folding and aggregation in Parkinson's disease (PD) (*12*).

In discussion regarding the implication of NO in longevity, we should distinguish the bioavailable NO from the biounavailable $ONOO^-$, a major class of RNS. Due to reaction with O_2^- for generation of $ONOO^-$, available NO levels in blood vessels are extremely low although all isoforms of NOS, including the endothelial NOS (eNOS) and the neuronal NOS (nNOS) are constitutively expressed. Indeed, senescent endothelial cells display higher mTORC1 activity, increased O_2^- production and decreased bioactive NO levels than young endothelial cells, which is contributed by eNOS "uncoupling" that no longer produces NO but O_2^-. Silencing mTORC1 in senescent cells reduces O_2^- generation and enhances NO production, whereas overexpression of a constitutively active mTORC1 mutant in young endothelial cells mimics endothelial dysfunction of senescent cells through eNOS uncoupling and induces premature cellular senescence. Rapamycin and resveratrol, by inhibiting mTORC1 signaling, result in decreased O_2^- generation and enhanced NO levels in the senescent cells as well as in the aortas of old rats (*13*). Likewise, short-term CR initiated in old age reverses age-associated vascular endothelial dysfunction by restoring NO bioavailability, reducing NADPH oxidase-mediated O_2^- production, stimulating anti-oxidant enzyme activity, and upregulating SIRT1 (*14*).

Mitochondrial biogenesis has been suggested as a hallmark of a long life span in model animals. Branched-chain amino acid (BCAA) supplementation increases SIRT1 expression and mitochondria biogenesis accompanying by enhanced physical endurance in primary skeletal myocytes and in cardiac and skeletal muscle of middle-aged mice (*15*). Enhanced mitochondrial biogenesis driven by the eNOS-derived NO burst plays a central role in playing a beneficial effect of CR on health and lifespan, which can be simulated by the treatment of mice with the mitochondrial uncoupler dinitrophenol (DNP). CR and DNP increase the expression of the peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α), cytochrome *c* oxidase and mitofusin-2 (*16*). How to associate autophagy with mitochondrial biogenesis to propose a unified longevity-promoting theory? Cumulative evidence suggests that PGC-1α that is activated by SIRT1 plays a pivotal role in mitochondrial biogenesis (*17*). By linking SIRT1 to PGC-1α, autophagy can be relevant to mitochondrial biogenesis. So it can be said that longevity is conferred by SIRT1-mediated autophagy and mitochondrial biogenesis. From current knowledge,

an integrated autophagy-controlling network comprising both mTORC1 and SIRT1 signaling pathways can be summarized in Fig.1.

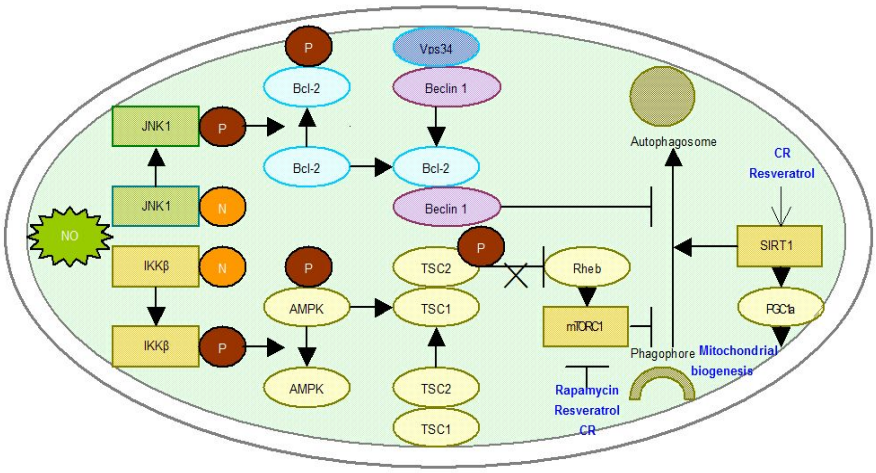

Fig.1. NO inhibits autophagy by S-nitrosylation and inhibition of JNK1 phosphorylation, thereby reducing phospho-Bcl-2 and increasing Bcl-2-Beclin1 interaction, which disrupts Vps34-Beclin1 association. NO also allows S-nitrosylation and inhibition of IKKb phosphorylation, leading to reduced phospho-AMPK and TSC2 activity, which alleviates the inhibitory effect of TSC1/2 on Rheb (denoted by "x"), thereby allowing Rheb to activate mTORC1 and inhibit autophagy. Rapamycin induces autophagy by inhibiting mTORC1, whereas resveratrol and CR induces autophagy by activating SIRT1 or inactivating mTORC1. SIRT1 accelerates mitochondrial biogenesis by activating PGC1α.

Finally, it is worthy noting that rapamycin can downregulate pro-inflammatory cytokines to exert an immunosuppressive effect, and also compromises collagen-triggered NO burst in collagen-induced arthritis mice (*18*), which may imply that rapamycin can modulate autophagy via either directly inhibiting mTORC1 or through NO-mediated S-nitrosylation/nitration. Because autophagy is implicated in lifespan extension, S-nitrosylation/nitration-impaired autophagy may be responsible for aging and age-related diseases. Accumulation of amyloid-β (Aβ) and tau is an invariant feature of Alzheimer disease (AD), but rapamycin-induced autophagy allows a significant reduction in Aβ levels (*19*). As a prospect, I urge that future studies should be focused on buildup of the generality of NO-impaired autophagy in aging and age-related diseases, especially in neurodegenerative AD, HD and PD, which should pave an avenue towards the exploration of a common strategy dealing with those diseases occurring during aging.

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