Autophagy 6:1, 1-3; January 1, 2010; © 2010 Landes Bioscience

The life span-prolonging effect of sirtuin-1 is mediated by autophagy

Eugenia Morselli,[1-3] Maria Chiara Maiuri,[1-3] Maria Markaki,[4] Evgenia Megalou,[4] Angela Pasparaki,[4] Konstantinos Palikaras,[4] Alfredo Criollo,[1-3] Lorenzo Galluzzi,[1-3] Shoaib Ahmad Malik,[1-3] Ilio Vitale,[1-3] Mickael Michaud,[1-3] Frank Madeo,[5,†] Nektarios Tavernarakis[4,†,*] and Guido Kroemer[1-3,†,*]

[1]INSERM; U848; Villejuif, France; [2]Institut Gustave Roussy; Villejuif, France; [3]Université Paris Sud-XI; Villejuif, France; [4]Institute of Molecular Biology and Biotechnology; Foundation for Research and Technology; Vasilika Vouton; Heraklion, Crete Greece; [5]Institute of Molecular Biosciences; University of Graz; Graz, Austria

[†]These authors share senior co-authorship

The life span of various model organisms can be extended by caloric restriction as well as by autophagy-inducing pharmacological agents. Life span-prolonging effects have also been observed in yeast cells, nematodes and flies upon the overexpression of the deacetylase Sirtuin-1. Intrigued by these observations and by the established link between caloric restriction and Sirtuin-1 activation, we decided to investigate the putative implication of Sirtuin-1 in the response of human cancer cells and *Caenorhabditis elegans* to multiple triggers of autophagy. Our data indicate that the activation of Sirtuin-1 (by the pharmacological agent resveratrol and/or genetic means) per se ignites autophagy, and that Sirtuin-1 is required for the autophagic response to nutrient deprivation, in both human and nematode cells, but not for autophagy triggered by downstream signals such as the inhibition of mTOR or p53. Since the life span-extending effects of Sirtuin-1 activators are lost in autophagy-deficient *C. elegans*, our results suggest that caloric restriction and resveratrol extend longevity, at least in experimental settings, by activating autophagy.

Key words: aging, beclin 1, rapamycin, senescence, spermidine, starvation

Abbreviations: *atg*, autophagy-related gene; *bec-1*, *beclin 1*; mTOR, mammalian target of rapamycin; NAD, nicotinamide adenine dinucleotide; RNAi, RNA interference

Submitted: 11/22/09

Revised: 11/26/09

Accepted: 12/02/09

Previously published online: www.landesbioscience.com/journals/autophagy/article/10817

*Correspondence to: Nektarios Tavernarakis and Guido Kroemer; Email: tavernarakis@imbb.forth.gr and kroemer@igr.fr

Punctum to: Morselli E, Maiuri MC, Markaki M, Megalou E, Pasparaki A, Palikaras K, et al. Caloric restriction and resveratrol prolong longevity via the sirtuin-1 dependent induction of autophagy. Cell Death Disease 2010; In press.

Stressed and dying cells often accumulate autophagic vacuoles, an observation that has been (mis)interpreted as suggesting that autophagy would contribute to cellular suicide. Today, it has become clear that autophagy constitutes—in most if not all cases—a (sometimes futile) mechanism of cellular defense that facilitates the adaptation of cells to adverse conditions including deprivation of nutrients, growth factors and/or oxygen as well as sublethal damage to various organelles. The systemic administration of pharmacological agents that induce autophagy, such as rapamycin or spermidine, can prolong the life span of model animals including the nematode *Caenorhabditis elegans*, the fruit fly *Drosophila melanogaster* and mice. The longevity-extending effect of rapamycin in nematodes is lost when *bec-1*, the worm ortholog of the mammalian gene *Atg6/beclin 1* (which codes for an essential autophagic modulator), is knocked down. Similarly, spermidine does not exert anti-aging activities in nematodes or fruit flies subjected to the knockdown or knockout of essential *atg* genes, respectively. Altogether, these results demonstrate that the whole-body induction of autophagy by pharmacological agents may prolong life span, at least in laboratory conditions, indicating that autophagy does not only mediate cytoprotection but has beneficial anti-aging effects at the organism level as well.

Driven by these considerations, we formulated the working hypothesis that autophagy might constitute the mechanism through which life span-prolonging manipulations operate. The first protein shown to extend longevity in a model organism, yeast (*Saccharomyces cerevisiae*), is Sirtuin-1. Transgene-enforced overexpression of Sirtuin-1 augments the life span of yeast cells as well as that of

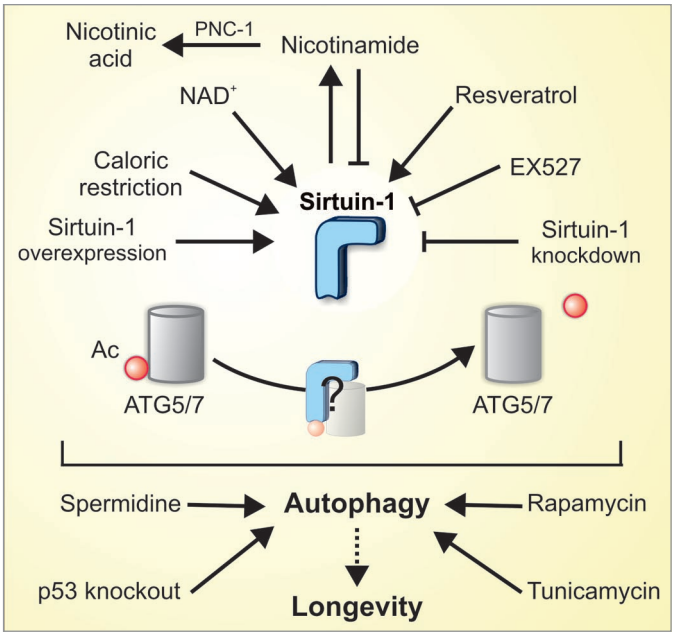

Figure 1. Schematic representation of the role of Sirtuin-1 in the regulation of autophagy and longevity. Please consult the main text for further details. Ac, acetyl; PNC-1, pyrazinamidase/nicotinamidase (*C. elegans*).

animals including *C. elegans* and flies. Sirtuin-1 is a nicotinamide adenine dinucleotide (NAD^+)-dependent deacetylase that can be activated by caloric restriction (presumably through increased NAD^+ concentrations), by depletion of its negative regulators (such as nicotinamide, the end-product of NAD breakdown) or by pharmacological activators, in particular resveratrol, which is now used by the cosmetic industry as an anti-aging measure. Sirtuin-1 induces autophagy, an effect presumably mediated by the deacetylation of essential autophagy proteins such as ATG5 and ATG7. Therefore, we explored the possibility that transgenic expression of Sirtuin-1 might trigger autophagy in human cancer cells in vitro and in *C. elegans* in vivo. We found that Sirtuin-1 overexpression promotes the autophagic flux in human cancer cells, and that this effect can be prevented by the pharmacological Sirtuin-1 inhibitor EX527. Similarly, transgene-mediated overexpression of *sir-2.1* (the *C. elegans* ortholog of human *Sirtuin-1*) causes autophagy in nematodes, suggesting that the link between the activation of Sirtuin-1 and autophagy is phylogenetically ancient.

Next, we investigated whether Sirtuin-1 is required for autophagy triggered by physiological and pharmacological stimuli. Human cancer cells were depleted of Sirtuin-1 through RNA interference (RNAi) or cultured in the presence of EX527, and were then stimulated with different inducers of autophagy. Thus, while the depletion or inhibition of Sirtuin-1 does not affect autophagy driven by the mTOR inhibitor rapamycin, the p53 inhibitor pifithrin-α, and the endoplasmic reticulum stressor tunicamycin, it does abolish the pro-autophagic effect of nutrient (amino acid and glucose) and growth factor (serum) deprivation. Similar results are obtained in *C. elegans*, in which a loss-of-function mutation of *sir-2.1* abolishes autophagy induced by caloric restriction but not the autophagic response to rapamycin or tunicamycin. Thus, Sirtuin-1 is an evolutionarily conserved link between nutrient deprivation-dependent stress and autophagy.

Autophagy is a mechanism through which cells can adapt to declining resources. Therefore, the failure to mount an autophagic response should reduce the fitness of cells that are cultured in the absence of nutrients and oxygen (metabolic stress). In line with this notion, we observe that the knockdown or inhibition of Sirtuin-1 augments the mortality of human cancer cells cultured in conditions of metabolic stress. This phenomenon can be partially prevented by the addition of rapamycin, suggesting that it is indeed failing autophagy that accounts for the increased susceptibility of Sirtuin-1-incompetent cells to metabolic stress.

In a next step, we investigated whether autophagy contributes to the increase in life span mediated by the *C. elegans* Sirtuin-1 ortholog SIR-2.1. Transgenic overexpression of *sir-2.1* augments the median and maximum life span of nematodes as compared to wild-type strains. This gain in longevity is lost when the essential autophagy gene *bec-1* is knocked down by feeding worms with bacteria producing a *bec-1*-specific small interfering RNA. Transgenic overexpression of *pnc-1*, which encodes a pyrazinamidase/nicotinamidase that activates Sirtuin-1 by depleting nicotinamide, also enhances life span, an effect that is abolished by the concomitant knockdown of *sir-2.1* or by that of either of the two essential autophagy genes *bec-1* or *atg-5*. Similarly, the addition of resveratrol to the culture medium reduces the age-associated mortality of *C. elegans*, and this benefit also is prevented by the knockdown of *sir-2.1* or by the knockdown of *bec-1*. Knockdown of the *C. elegans* p53 ortholog *cep-1*, which increases longevity by inducing autophagy, does not further augment the gain in longevity conferred by *sir-2.1* overexpression. The results of this epistatic analysis are in agreement with the hypothesis that SIR-2.1 accumulation and CEP-1 depletion enhance longevity through a common pathway that implicates autophagy. Altogether, these results suggest that activation of the *C. elegans* Sirtuin-1 ortholog by three distinct procedures (overexpression, pharmacological activation with resveratrol, or removal of its negative regulator nicotinamide) augments longevity by stimulating autophagy (**Fig. 1**).

Our data underscore the notion that the induction of autophagy is universally required for life span extension by caloric restriction and by a variety of pharmacological agents including rapamycin, spermidine or resveratrol. Based on these results, it will be important to investigate which (if any) genetic manipulations designed to increase longevity do so

independently of autophagy. In addition, our work generates two questions that must be answered by future investigation. What are the precise mechanisms though which the activation of Sirtuin-1 stimulates autophagy? And how does autophagy mediate its cytoprotective and life span-prolonging effects in molecular terms?

Acknowledgements

N.T. is supported by grants from EMBO, the European Research Council (ERC) and the European Commission Coordination Action ENINET (contract number LSHM-CT-2005-19063). G.K. is supported by the Ligue Nationale contre le Cancer (Equipes labellisée), Agence Nationale pour la Recherche (ANR), European Commission (ApoSys, ChemoRes, ApopTrain, Active p53), Fondation pour la Recherche Médicale (FRM), Institut National du Cancer (INCa) and Cancéropôle Ile-de-France. E.M. was founded by a PhD student grant from ApopTrain.