









A surge in endogenous spermidine is essential for rapamycin-induced autophagy and longevity

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ABSTRACT

Acute nutrient deprivation (fasting) causes an immediate increase in spermidine biosynthesis in yeast, flies, mice and humans, as corroborated in four independent clinical studies. This fasting-induced surge in spermidine constitutes the critical first step of a phylogenetically conserved biochemical cascade that leads to spermidine-dependent hypusination of EIF5A (eukaryotic translation initiation factor 5A), which favors the translation of the pro-macroautophagic/autophagic TFEB (transcription factor EB), and hence an increase in autophagic flux. We observed that genetic or pharmacological inhibition of the spermidine increase by inhibition of ODC1 (ornithine decarboxylase 1) prevents the pro-autophagic and antiaging effects of fasting in yeast, nematodes, flies and mice. Moreover, knockout or knockdown of the enzymes required for EIF5A hypusination abolish fasting-mediated autophagy enhancement and longevity extension in these organisms. Of note, autophagy and longevity induced by rapamycin obey the same rule, meaning that they are tied to an increase in spermidine synthesis. These findings indicate that spermidine is not only a “caloric restriction mimetic” in the sense that its supplementation mimics the beneficial effects of nutrient deprivation on organismal health but that it is also an obligatory downstream effector of the antiaging effects of fasting and rapamycin.

Abbreviation: EIF5A: eukaryotic translation initiation factor 5A; IGF1: insulin like growth factor 1; MTOR: mechanistic target of rapamycin kinase; ODC1: ornithine decarboxylase 1; TFEB: transcription factor EB.

ARTICLE HISTORY

Received 21 August 2024
Accepted 22 August 2024









KEYWORDS

Aging; autophagy; lifespan; rapamycin; spermidine; MTOR

Polyamines, including putrescine, spermidine, and spermine, as well as their precursors and regulatory enzymes, are highly conserved across species. Our previous work has highlighted the multifaceted consequences of spermidine supplementation, which exerts cardio- and neuroprotective effects, stimulates autophagy and mitochondrial function, and extends lifespan in a variety of laboratory models. These findings are particularly salient given that polyamine metabolism, predominantly regulated by the pacemaker enzyme ODC1 (ornithine decarboxylase 1), is a critical driver of cellular growth. The concordant activity of polyamines, stimulation of cell growth and induction of autophagy, differs from the

discordant action of MTOR (mechanistic target of rapamycin kinase), which stimulates cell growth but represses autophagy.

Rapamycin, a potent and selective inhibitor of MTOR, has long been recognized for its ability to extend longevity across species, including yeast and worms. Our recent data demonstrate that rapamycin treatment in yeast is accompanied by a concomitant increase in endogenous spermidine levels [1]. Notably, the inhibition of endogenous spermidine synthesis significantly attenuates the autophagy-inducing and longevity-promoting effects of rapamycin in yeast, human cell lines and worms, underscoring the essential role of polyamine metabolism in these processes. Accordingly, our study pro-

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vides further compelling evidence that the pro-autophagic and lifespan-extending effects of dietary restriction and intermittent fasting – physiological triggers that shut down TOR signaling – are largely dependent on functional endogenous polyamine metabolism (Figure 1). Acute fasting is associated with an increase in polyamine levels across multiple species and tissues, supporting our hypothesis that this rise in polyamines is necessary to trigger the autophagic cascade. Moreover, genetic perturbation of MTOR activity in transgenic mice further corroborates our findings, as changes in spermidine levels align with expected autophagic outcomes. Notably, our previous work has shown that spermidine can effectively counteract the downstream effects of hyperactive INS (insulin)-IGF1 (insulin like growth factor 1) signaling during cardiac aging in mice.

In yeast, proteomic analyses reveal that TOR complex 1 and TOR complex 2-associated factors exhibit altered responses to starvation when endogenous polyamine synthesis is disrupted. Specifically, the loss of polyamine synthesis results in a delayed deactivation of early TOR activity during nitrogen starvation in *odc1* knockout strains – a defect that can be rescued by polyamine supplementation. This suggests a potential checkpoint mechanism to fine-tune autophagy regulation. Interestingly, excess polyamines do not influence TOR activity in wild-type cells, indicating a tightly regulated interplay between these pathways under nutrient-rich conditions. The precise mechanisms through

which polyamines affect MTOR signaling, and vice versa, remain incompletely understood and likely operate on multiple levels. In the short term, polyamine-controlled translational events are unlikely to explain altered MTOR signaling, given the minute scale of events. At later stages such as spermidine-modulated, EIF5A hypusination-dependent translational processes, may modulate MTOR signaling. Additionally, polyamines might exert their influence on MTOR components or signaling factors indirectly through metabolic alterations. Because MTOR senses amino acid concentrations, including those of arginine and methionine, shifts in such amino acids, as they are found in polyamine-deficient cells, could contribute to the dysregulated MTOR activity.

Furthermore, we observed that blocking endogenous polyamine synthesis diminishes rapamycin-induced autophagy in yeast, human cell lines, and *C. elegans*. This finding suggests that the well-documented lifespan-extending effects of rapamycin are, at least in part, contingent upon the polyamine pathway. This hypothesis has been corroborated in yeast and nematodes. Thus, inhibition of ODC1 prevents rapamycin-induced lifespan extension in these model organisms.

Importantly, spermidine supplementation enhances immune responses in aged mice, improving tumor cell surveillance and vaccine efficacy. While rapamycin is one of the first pharmacological interventions shown to extend the lifespan of mice, concerns about immunosuppressive side effects of rapamycin have been raised. Indeed, the first clinical indication for rapamycin was immunosuppression in the context of organ transplantation. It is tempting to speculate, yet remains to be demonstrated, that such immunosuppressive effects can be mitigated by co-administering rapamycin with spermidine, by using rapamycin in a discontinuous fashion, or by a combination of both approaches.

In conclusion, our research uncovers a reciprocal dependency between MTOR signaling and polyamine metabolism, adding a new layer of complexity to the regulation of autophagy in response to nutrient availability. We speculate that the cross-regulation of MTOR and polyamine pathways evolved as a mechanism to fine-tune cellular growth and ensure cellular survival under varying environmental conditions. Future investigations should aim at elucidating the interplay between these pathways across different cell types and stages of the lifespan. This knowledge could aid in therapeutically exploiting the MTOR-polyamine axis. The strategic combination of rapamycin with spermidine in a sophisticated rhythmic fashion holds promise as a novel approach to maximizing therapeutic efficacy while minimizing adverse effects. We hypothesize that the negative side effects of continuous rapamycin treatment could be effectively counterbalanced by supplementary spermidine administration, paving the way for ever more efficient geroprotective interventions.

In sum, we propose that the MTOR and polyamine pathways function within a finely tuned regulatory network that orchestrates cell growth in response to nutrient availability and metabolic demands. MTOR inhibition upregulates polyamine synthesis, possibly to maintain essential cellular

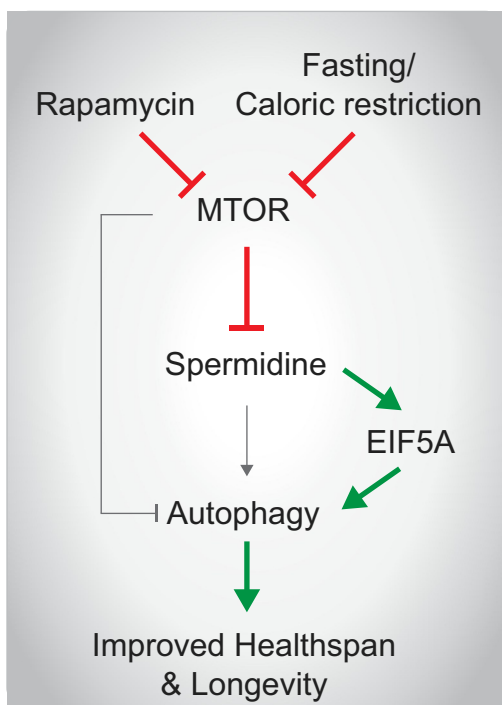


Figure 1. MTOR and the polyamine pathway during nutrient deprivation. In our recent work, we described a novel mechanism by which fasting and caloric restriction lead to the induction of polyamine metabolism and the increased activation of the translation factor EIF5A via the spermidine-dependent post-translational hypusination modification. Activated EIF5A is required for the full autophagy-inducing and longevity-promoting properties of fasting. MTOR functions as a negative regulator of spermidine synthesis and autophagy in our settings. Consequently, pharmacologically inhibiting MTOR with rapamycin also elicits spermidine-dependent effects on autophagic flux and lifespan regulation.

functions, including autophagy, and to prevent compromised cellular growth. We surmise that a detailed mechanistic exploration of this network may yield further clues for anti-aging therapies.

Disclosure statement









FM has equity interests in The Longevity Labs (TLL) and Samsara Therapeutics. GK has been holding research contracts with Daiichi Sankyo, Eleor, Kaleido, Lytix Pharma, PharmaMar, Osasuna Therapeutics, Samsara Therapeutics, Sanofi, Sutro, Tollys, and Vascage. GK is on the Board of Directors of the Bristol Myers Squibb Foundation France. GK is a scientific co-founder of everImmune, Osasuna Therapeutics, Samsara Therapeutics and Therafast Bio. GK is in the scientific advisory boards of Hevolution, Institut Servier, Longevity Vision Funds and Rejuveron Life Sciences. GK is the inventor of patents covering therapeutic targeting of aging, cancer, cystic fibrosis and metabolic disorders. GK's brother, Romano Kroemer, was an employee of Sanofi and now consults for Boehringer-Ingelheim. GK's wife, Laurence Zitvogel, has held research contracts with Glaxo Smyth Kline, Incyte, Lytix, Kaleido, Innovate Pharma, Daiichi Sankyo, Pilege, Merus, Transgene, 9 m, Tusk and Roche, was on the on the Board of Directors of Transgene, is a cofounder of everImmune, and holds patents covering the treatment of cancer and the therapeutic manipulation of the microbiota. The funders had no role in the design of the study; in the writing of the manuscript, or in the decision to publish the results. MA, SS, FM and GK hold patent(s) dealing with the cardiometabolic effects of spermidine. All other authors declare no competing interests.

Funding

This research was funded in whole, or in part, by the Austrian Science Fund (FWF) [10.55776/P37278, 10.55776/P37016, 10.55776/P31727, 10.55776/W1226]. For the purpose of open access, the author has applied a CC BY public copyright licence to any Author Accepted Manuscript version arising from this submission. SJH is grateful for support from the Styrian Government for the grant "AutoKomm." SS and MA acknowledge funding received from the Medical University of Graz (Flagship Project VASC-HEALTH). MA and GK acknowledge funding received from the FWF (DOI: 10.55776/I6931) and ANR under the umbrella of the Partnership Fostering a European Research Area for Health (ERA4Health) (GA N° 101095426 of the EU Horizon Europe Research and Innovation Programme). MA acknowledges additional support from the FWF (DOI: 10.55776/P34926), and BioTechMed-Graz (Young Researcher Group). SS acknowledges the support from FWF through grants P27637-B28 and I3301, BioTechMed-Graz (Flagship project INTERACD+), and Medical University of Graz (Flagship project VASC-HEALTH). NT acknowledges support from the European Research Council (grant ERC-GA695190-MANNA), the European Commission (FETOPEN project Dynamic-GA-863203, and Research Excellence Hub CHAngeing-GA-101087071), the Greek National Research Infrastructure project BIOIMAGING-GR-MIS5002755, the Hellenic Foundation for Research and Innovation and the General Secretariat for Research and Innovation. GK is supported by the Ligue contre le Cancer (équipe labellisée); Agence

National de la Recherche (ANR-22-CE14-0066 VIVORUSH, ANR-23-CE44-0030 COPPERMAC, ANR-23-R4HC-0006 Ener-LIGHT); Association pour la recherche sur le cancer (ARC); Cancéropôle Ile-de-France; Fondation pour la Recherche Médicale (FRM); a donation by Elior; European Joint Programme on Rare Diseases (EJPRD); Wilsonmed; European Research Council Advanced Investigator Award (ERC-2021-ADG, Grant No. 101052444; project acronym: ICD-Cancer, project title: Immunogenic cell death (ICD) in the cancer-immune dialogue); The ERA4 Health Cardinoff Grant Ener-LIGHT; European Union Horizon 2020 research and innovation programmes Oncobiome (grant agreement number: 825410, Project Acronym: ONCOBIOME, Project title: Gut OncoMicrobiome Signatures [GOMS] associated with cancer incidence, prognosis and prediction of treatment response, Prevalung (grant agreement number 101095604, Project Acronym: PREVALUNG EU, project title: Biomarkers affecting the transition from cardiovascular disease to lung cancer: towards stratified interception), Neutrocure (grant agreement number 861878 : Project Acronym: Neutrocure; project title: Development of "smart" amplifiers of reactive oxygen species specific to aberrant polymorphonuclear neutrophils for treatment of inflammatory and autoimmune diseases, cancer and myeloablation); National support managed by the Agence Nationale de la Recherche under the France 2030 programme (reference number 21-ESRE-0028, ESR/Equipex+ OncoPheno-Screen); Hevolution Network on Senescence in Aging (reference HF-E Einstein Network); Institut National du Cancer (INCa); Institut Universitaire de France; LabEx Immuno-Oncology ANR-18-IDEX-0001; a Cancer Research ASPIRE Award from the Mark Foundation; PAIR-Obésité INCa_1873, the RHUs Immunolife and LUCA-pi (ANR-21-RHUS-0017 and ANR-23-RHUS-0010, both dedicated to France Relance 2030); Seerave Foundation; SIRIC Cancer Research and Personalized Medicine (CARPEM, SIRIC CARPEM INCa-DGOS-Inserm-ITMO Cancer_18006 supported by Institut National du Cancer, Ministère des Solidarités et de la Santé and INSERM). This study contributes to the IdEx Université de Paris Cité ANR-18-IDEX-0001. FM is grateful for support from the University of Graz for the grant "Fast4Health," the Austrian Science Fund (FWF) for the grants P 37278-B, P 37016-B, P 31727-B21, W 1226, as well as BioTechMed-Graz (Flagship project EPIAge) and the University of Graz Field of Excellence BioHealth.

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