

Polyamines reverse immune senescence via the translational control of autophagy

Hanlin Zhang^a, and Anna Katharina Simon^a

^aThe Kennedy Institute of Rheumatology, University of Oxford, Roosevelt Drive, Oxford, OX3 7FY, UK

Correspondence: hanlin.zhang@kennedy.ox.ac.uk

Abstract

Organismal aging is associated with compromised cellular function, which can be partially attributed to accumulation of cellular damage. Being the major, if not only, cellular bulk-degradation mechanism, autophagy declines with age in multiple tissues and organisms. Spermidine is an endogenous polyamine metabolite that also declines with age. It prolongs lifespan and improves tissue functions of model organisms in an autophagy-dependent manner. We report that autophagic flux is significantly reduced in B cells from old mice. Spermidine induces autophagy and improves the function of both old mouse and old human B cells. Mechanistically, spermidine post-translationally modifies (hypusinates) the translation factor eIF5A. Hypusinated eIF5A specifically regulates the synthesis of the master autophagy and lysosome transcription factor TFEB. This pathway declines with age in both mice and humans, which may eventually lead to declining autophagy and impaired tissue functions in old individuals.

Keywords

spermidine, eIF5A, hypusine, translation, TFEB, autophagy, aging, B cells

Aging of the immune system, termed immune senescence, is characterized by altered ratios of different immune cell types and their reduced function. This causes failure of efficient clearance of pathogens and tissue damage, which directly increases the infectious disease burden in old individuals and also generally aggravates the aging process of other tissues. B cells are the only known antibody-secreting cell in the body and are key in the fight against infections and for effective vaccinations. The antibody responses against pathogens in old individuals are severely compromised, rendering them highly vulnerable to infectious diseases. Despite the long recognized change in B cell phenotypes (by surface markers and function) that occurs during aging, the molecular mechanism underlying B cell senescence is still poorly understood. Efficient ways to improve B cell responses in the elderly are also lacking.

Using multiple LC3-II based readouts, we confirm that autophagic flux is significantly reduced in B cells from old mice [1]. More specifically, old B cells show higher LC3-II protein levels, which are not further increased by the lysosomal inhibitor bafilomycin

A1, indicating that autophagic flux is blocked at the lysosomal level. Autophagy deficiency in B cells leads to defective memory responses, mimicking the ageing phenotype. Spermidine administration increases autophagic flux and improves B cell responses in old mice, but not in mice with an autophagy deficiency in B cells.

Spermidine is an essential substrate of the hypusination process, in which it donates an aminobutyl moiety to a specific lysine on the eukaryotic Translation Initiation Factor 5A (eIF5A) to form hypusine. eIF5A is a translation factor with the well-defined function of facilitating the elongation step during protein synthesis with its hypusine residue. Hypusine is an unusual amino acid modification so far only known to exist in eIF5A. We show that depleting cellular spermidine leads to reduced hypusination of eIF5A, and that inhibiting eIF5A expression or its hypusination reduces autophagic flux in both mammalian cell lines and *ex vivo* activated primary B cells. To identify how eIF5A regulates autophagy, protein mass spectrometry was used. Interestingly, transcription factor EB (TFEB) protein expression is repetitively found reduced upon inhibiting hypusination in several proteomic approaches, which is confirmed by Western blot. Mechanistically, TFEB contains a polyproline-containing motif (...SPPPVPG...) at its N-terminus predicted to be a translation-stalling motif. This motif is sufficient to cause reduced translation as shown using a mCherry/GFP reporter system. Moreover, mutating this motif alone renders the expression of TFEB less dependent of hypusinated eIF5A while its function is unaltered. Therefore, eIF5A promotes TFEB synthesis at least partially via facilitating translation of hard-to-read regions. However, not all polyproline-containing proteins are down-regulated upon inhibition of hypusination. The specificity of TFEB might be attributed to its long stalling motif and short protein half-life.

Spermidine levels decline with age in multiple model organisms and as confirmed by us also in human peripheral blood mononuclear cells (PBMCs). We find that hypusination of eIF5A, overall eIF5A protein levels, and TFEB expression is reduced in B cells from old mice, which can be fully or partially rescued by spermidine administration *in vivo*. A similar improvement is observed in *ex vivo* cultured old human B cells. Spermidine treatment induces the pathway and improves the antibody production of old human B cells in a hypusination-dependent manner. Next it would be exciting to test whether spermidine improves immune responses in the elderly in a clinical trial. Moreover, the amount of spermidine, the expression of hypusinated eIF5A and TFEB, as well as the autophagic flux in peripheral blood could be used as biomarkers to assess the biological aging status and the efficiency of other anti-aging drugs. It would be also interesting to see whether this pathway operates in other tissues.

Being a major catabolic process, autophagy can be induced by various cellular stresses. The mammalian target of rapamycin (mTOR) integrates cellular stress and when conditions are favourable, it phosphorylates multiple targets to promote signalling proteins that inhibit autophagy while activating anabolism and cell growth, including translating the protein

synthesis machinery. However, here we observe that activation of immune cells is associated with up-regulation of both anabolic and catabolic pathways, protein synthesis and autophagy. In this case therefore translation and autophagy are induced in a coordinated fashion, and autophagy induction relies on optimal translation. It is likely that a cross talk between catabolism and anabolism exists: Autophagy provides building blocks and energy resources to support translation, while the translation machinery maintains high levels of short-lived autophagy proteins such as TFEB (Figure 1). Although TFEB has been identified as a target of eIF5A, we postulate that more eIF5A targets may exist that regulate autophagy and other processes. In addition to this novel translational regulation of autophagy involving eIF5A, other autophagy-regulatory mechanisms that operate at the translational level are likely to be discovered.

Disclosure statement

There is no conflict of interest.

Funding resources

H.Z. is funded by the China Scholarship Council-Nuffield Department of Medicine Scholarship and the Oxford-Elysium Prize Fellowship. A.K.S. lab is funded by a Wellcome Trust Investigator Award (103830/Z/14/Z).

Reference

1. Zhang H, Alsaleh G, Feltham J, Sun Y, Napolitano G, Riffelmacher T, et al. Polyamines Control eIF5A Hypusination, TFEB Translation, and Autophagy to Reverse B Cell Senescence. *Molecular cell* 2019; 76:110-25.

