IRF3 and IRF7 require SIRT1 for liquid–liquid phase separation and transactivation of IFN-1

Deacetylation of specific lysine residues in the DNA-binding domain of the transcription factors IRF3 and IRF7 by SIRT1 is necessary for liquid–liquid phase separation and transactivation of type I interferons. SIRT1 agonists partially restored the impaired innate immune responses in senescent cells and aged mice, suggestive of a possible strategy for preventing innate immunosenescence.

This is a summary of:
Qin, Z. et al. Deactylation by SIRT1 enables liquid–liquid phase separation of IRF3/IRF7 in innate antiviral immunity. *Nat. Immunol.* https://doi.org/10.1038/s41590-022-01269-0 (2022).

Published online:
25 July 2022

The question

In both the 1918–1919 influenza pandemic and the current COVID-19 pandemic, diminished and delayed induction of type I interferon (IFN-I) responses and much higher rates of hospitalization and death were observed in patients of >60 years of age than in people of <60 years of age[1,2]. These observations suggest that innate antiviral immunity deteriorates substantially with aging. However, the mechanisms responsible for aging-associated degeneration of interferon signaling and innate immunosenescence remain largely unknown. Identifying which crucial molecules in interferon signaling are dysfunctional in aged people and the underlying mechanisms could provide insights into viral diseases in an aging society and strategies to combat them.

The discovery

Interferon-regulatory factor 3 (IRF3) and IRF7 are the two pivotal transcriptional factors that directly control the production of IFN-α and IFN-β, determining the antiviral state of the host. We first demonstrated that IRF3 and IRF7 underwent liquid–liquid phase separation (LLPS), a general regulatory mechanism that enables the creation of intracellular droplets to compartmentalize biochemical reactions[3]. Then, we screened a small-molecule inhibitor library for key factors that control IRF3 and IRF7 LLPS and immune-response activation and found that activity of the NAD-dependent protein deacetylase sirtuin-1 (SIRT1) was required for the transactivation of interferon-encoding genes by IRF3 and IRF7. Next, we determined cellular and host innate antiviral immune response and viral load in control and *Sirt1*-deficient myeloid cells and mice. By performing mass-spectrometry analysis of IRF3 and IRF7 isolated from cells treated with a SIRT1 inhibitor, we identified SIRT1-specific lysine-deacetylation sites and confirmed this result by using antibodies to site-specific acetyl-lysine in SIRT1-overexpressing or SIRT1-deficient cells. Moreover, we investigated the role of site-specific acetylation on IRF3 and IRF7 LLPS and binding to interferon-stimulated response element (ISRE). Genetic-code-expansion orthogonal translation systems enable the incorporation of amino acids carrying post-translational modifications. We used these systems to integrate acetyl-lysine into recombinant IRF3 and IRF7 to create single or double site-specific acetylated proteins in vitro and in vivo and examined the role of these modified IRF3 and IRF7 proteins in ISRE binding, droplet formation and transactivation of IFN-α and IFN-β. We used human peripheral blood mononuclear cells, mouse primary macrophages, mouse embryonic fibroblasts and mouse lung fibroblasts to demonstrate the correlation between SIRT1 and IRF3 and IRF7 transactivation of IFN-α and IFN-β and the links of these mechanisms to age-related innate immunosenescence.

We discovered that SIRT1 was required for maximal induction of IFN-I, which reduced viral load and mortality in mice infected with vesicular stomatitis virus or herpes simplex virus type 1. Furthermore, we identified specific acetylated lysine residues — K39 and K77 on IRF3, and K45 and K92 on IRF7 — that are located in the DNA-binding domain and are important for ISRE binding. Single or double site-specific acetylation of IRF3 and IRF7 inhibited the LLPS function of IRF3 and IRF7 in vitro (Fig. 1a,b). This phenotype was rescued by ectopic expression of SIRT1. In addition, repressed SIRT1 activity and impaired LLPS and transactivation of IRF3 and IRF7 were observed in samples from aged patients and mice, which revealed that SIRT1 loss of function is an important determinant of innate immunosenescence (Fig. 1c).

The implications

We showed that the deacetylation of specific lysine residues in the DNA-binding domain of IRF3 and IRF7 by SIRT1 is a previously unknown prerequisite step for the LLPS and activation of IRF3 and IRF7. As SIRT1 is an NAD-dependent deacetylase, the decline in NAD^+ levels contributes to low SIRT1 activity during senescence and aging[4,5]. Our study using SIRT1 agonists revealed that enhanced SIRT1 activity partially rescued senescent cells from innate immunosenescence and alleviated signs of viral infection in aged mice, which indicates that stimulating SIRT1 activity might be a promising way to improve innate immunity in aged people.

The experiments with the SIRT1 agonist were all performed in mice or mouse cells; thus, whether the results are applicable to humans remains unknown. Moreover, whether SIRT1 agonists could also affect other viral infection-related immune responses in addition to interferon production and/or contribute to innate immune promotion needs to be further investigated.

Future work will determine whether SIRT1 agonists can potentiate innate immunity in senescent human cells, and whether SIRT1 agonists target other components of the host's antiviral immunity.

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EXPERT OPINION

"This work not only identifies a novel mechanism by which SIRT1 regulates interferon signaling by affecting IRF3 and IRF7 LLPS, but also provides a possible strategy to boost innate immunity in aged population." **Gaya Amarasinghe, Washington University in St. Louis, St. Louis, MO, USA.**

FIGURE

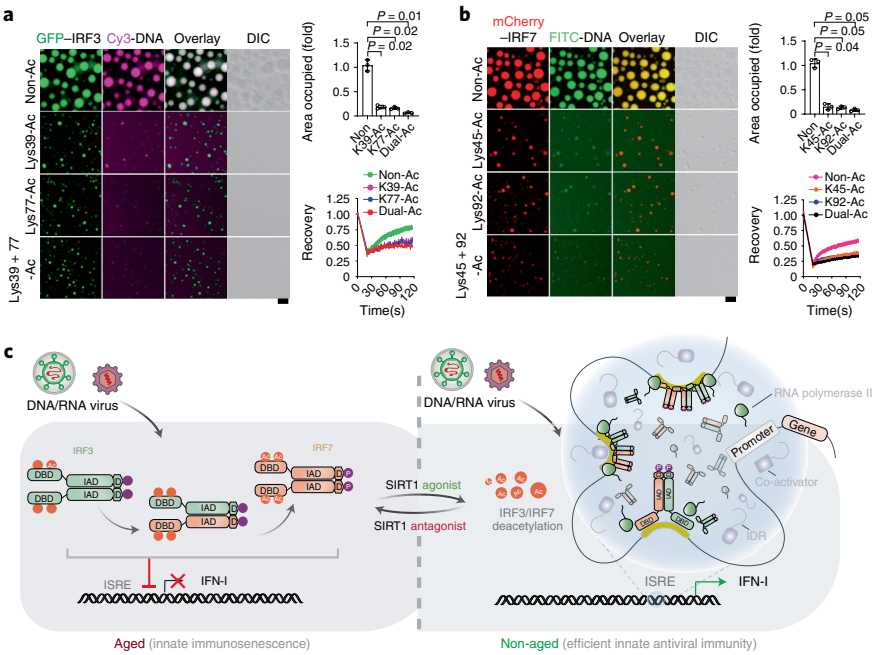

Fig. 1 | SIRT1-mediated deacetylation of specific residues in the DNA-binding domain of IRF3 and IRF7 is required for interferon transactivation and antiviral innate immune activation. a,b, Droplet formation (LLPS) obtained by mixing IRF3 (**a**) or IRF7 (**b**) with single, double (Dual) or no (Non) site-specific acetylated (Ac) lysines with cyanine (Cy3)-labeled or fluorescein isothiocyanate (FITC)-labeled ISRE DNA. Right, quantification of droplet area (top) and fluorescence recovery after photobleaching (bottom). DIC, differential interference contrast; GFP, green fluorescent protein. **c**, Working model for the role of deacetylation-dependent LLPS of IRF3 and IRF7 in controlling IFN-I transcription and innate antiviral immunity. DBD, DNA-binding domain; IAD, IRF-associated domain; IDR, intrinsically disordered region. © 2022, Qin, Z. et al.

BEHIND THE PAPER

A traditional method for determining the role of a specific acetyl-lysine post-transcriptional modification is to express lysine-to-arginine and lysine-to-glutamine substitution mutants in cells or animals and examine the relevant biological events. However, this approach cannot unambiguously determine the effects of acetylation. To overcome this limitation, we developed the genetic-code-expansion orthogonal translation system in vivo. We engineered a chimeric ribozyme from two archaea to increase the insertion efficiency of acetyl-lysine and used four repeats of tRNA under control of the U6 promoter to strengthen the binding of tRNA to the stop codon. For the first time, this system enabled us to express site-specific acetylated proteins in cells and functionally evaluate acetylated IRF3 and IRF7. **L.Z.**

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FROM THE EDITOR

"Although we know a lot about antiviral interferon signaling and the role of IRF3 and IRF7 as intermediaries in this signaling, this paper shows some of the finer mechanistic details — crucially, the importance of LLPS. Moreover, I was somewhat blown away by the attention to detail and the extent that these authors went to here with their experiments." **Nick Bernard, Senior Editor, *Nature Immunology***