Cell Research

www.nature.com/cr
www.cell-research.com

RESEARCH HIGHLIGHT

hnRNPA2B1: a nuclear DNA sensor in antiviral immunity

Xingli Zhang[1,2], Richard A. Flavell[2,3,4] and Hua-Bing Li[1,2]

Cell Research (2019) 29:879–880; https://doi.org/10.1038/s41422-019-0226-8

Pattern recognition receptors (PRRs) have critical roles in mediating innate immune responses. In a recent study published in *Science*, Cao and colleagues identified hnRNPA2B1 as a new nuclear DNA sensor that initiated type I interferon (IFN-I) production upon DNA virus infection and amplified IFN-I responses by directly enhancing STING-dependent cytosolic DNA sensing pathways.

The innate immune system is activated by the recognition of pathogen-associated molecular patterns (PAMPs) through pattern recognition receptors (PRRs), which leads to the production of proinflammatory cytokines and type I interferons (IFN-I).[1,2] PRRs that recognize viral DNA in cells include TLR9 in endosomes and several cytosolic sensors such as cyclic GMP-AMP synthase (cGAS), DNA-dependent activator of IFN-regulatory factors (DAI), DEAH box protein 9 (DHX9), DEAH box protein 36 (DHX36), absent in melanoma 2 (AIM2) and IFNγ-inducible protein 16 (IFI16).[3–5] Viral DNA replication mostly occurs in the nucleus of infected cells, where the recognition of viral DNA could potentially happen. However, innate immune response sensors in the nucleus to recognize nuclear pathogen-derived DNA remain unclear. Given that virus-induced IFN-I expression mainly depends on TBK1-IRF3 activation in the cytoplasm,[6] Cao and colleagues sought to identify new nuclear viral DNA sensors that could recognize viral DNA in the nucleus and then translocate to the cytoplasm to activate the TBK1-IRF3 pathway.[7]

To identify potential nuclear DNA sensors, Wang et al. searched the proteins that were precipitated by the biotinylated genomic DNA of HSV-1 (F strain) and translocated from the nucleus to the cytoplasm 2 h after HSV-1 infection, using 2D SDS-PAGE and mass spectrometry. This screen allowed them to identify 23 potential nuclear viral DNA sensors, among which hnRNPA2B1 was validated to bind both self- and pathogen-derived DNA, but not native nucleosomes. hnRNPA2B1, an RNA-binding protein, has been reported to function as an m^{6}A reader and is involved in RNA transport, processing or splicing.[8] Wang et al. found that *Hnrnpa2b1* knockdown in vitro or knockout in mice in vivo impaired DNA virus- but not RNA virus-induced IFN-I production, thus promoting viral replication without affecting the expression of pro-inflammatory cytokines such as TNF-α and IL-6. These results suggested that hnRNPA2B1 was a bona fide IFN-I-inducing nuclear DNA sensor.

How does hnRNPA2B1 initiate the DNA virus-induced IFN-I response? Wang et al. found that upon binding viral DNA in the cell nucleus, hnRNPA2B1 could translocate to the cytoplasm and activate TBK1 through the tyrosine kinase Src and endoplasmic reticulum adaptor STING. *Hnrnpa2b1* deficiency impaired the phosphorylation of TBK1 and IRF3, thus decreasing the kinase activity of TBK1 after HSV-1 infection. Next, they investigated the mechanisms of hnRNPA2B1 nucleocytoplasmic translocation and found that HSV-1 infection induced the homodimerization of hnRNPA2B1. Mutation of the dimer interface in the RRM (RNA recognition motif) domain abrogated hnRNPA2B1 dimerization and nucleocytoplasmic translocation upon HSV-1 infection.

In addition, through mutational screening of arginine, serine, and threonine of hnRNPA2B1, they found that a mutation of Arg226 (R226A) within the arginine-glycine-glycine (RGG) domain significantly enhanced *Ifnb1* expression compared to wild-type hnRNPA2B1. It was previously reported that hnRNPA2B1 could be methylated at arginine residues within the RGG domain.[9] Indeed, arginine methylation assay revealed that R226 was the key site for arginine mono-methylation of hnRNPA2B1, which was decreased after HSV-1 infection. Wang et al. further demonstrated that the demethylation of hnRNPA2B1 at Arg226 was mediated by the arginine demethylase JMJD6. hnRNPA2B1 with dimer interface mutation was unable to associate with JMJD6 after HSV-1 infection and showed increased arginine methylation levels compared to full-length hnRNPA2B1. Thus, dimerization of hnRNPA2B1 upon recognizing viral DNA is required for its demethylation, activation, nucleocytoplasmic translocation and the subsequent initiation of IFN-β expression (Fig. 1).

Are there cross-talks between this new nuclear DNA hnRNPA2B1 sensor pathway and other well-known cytoplasmic DNA sensor pathways? Wang et al. found that hnRNPA2B1 overexpression increased HSV-1-induced TBK1 activation and *Ifnb1* expression in *cgas*$^{-/-}$ L929 cells, suggesting that hnRNPA2B1 was able to induce IFN activation in a cGAS-independent manner. *Hnrnpa2b1* deficiency attenuated IFN-I production induced by HSV-1 and Vaccinia virus (VACV), another DNA virus, replicating in the cytoplasm. They also found that hnRNPA2B1 bound *cgas*, *Ifi16* and *Sting* mRNAs as an RNA-binding protein to transport those mRNAs from nucleus to cytoplasm. Interestingly, hnRNPA2B1 was constitutively associated with RNA m^{6}A demethylases FTO at steady state to keep the m^{6}A levels of those mRNAs at low levels. This association was abrogated after HSV-1 infection, thus increasing the m^{6}A levels and promoting nucleocytoplasmic trafficking to enhance the translation of these mRNAs without affecting their transcription and stability. They demonstrated that *Hnrnpa2b1* deficiency significantly decreased the m^{6}A levels of *cgas*, *Ifi16* and *Sting* mRNAs, and led to the nuclear retention of *cgas*, *Ifi16* and *Sting* mRNAs. Thus, hnRNPA2B1 can also enhance the efficient induction of antiviral IFN-I production mediated by cGAS, IFI16, and STING through m^{6}A machinery (Fig. 1).

[1]Shanghai Institute of Immunology, Key Laboratory of Cell Differentiation and Apoptosis of Ministry of Education of China, Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China; [2]Shanghai Jiao Tong University School of Medicine - Yale Institute for Immune Metabolism, Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China; [3]Department of Immunobiology, Yale University School of Medicine, New Haven, CT 06520-8055, USA and [4]Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, CT 06520-8055, USA
Correspondence: Richard A. Flavell (richard.flavell@yale.edu) or Hua-Bing Li (huabing.li@shsmu.edu.cn)

Published online: 30 August 2019

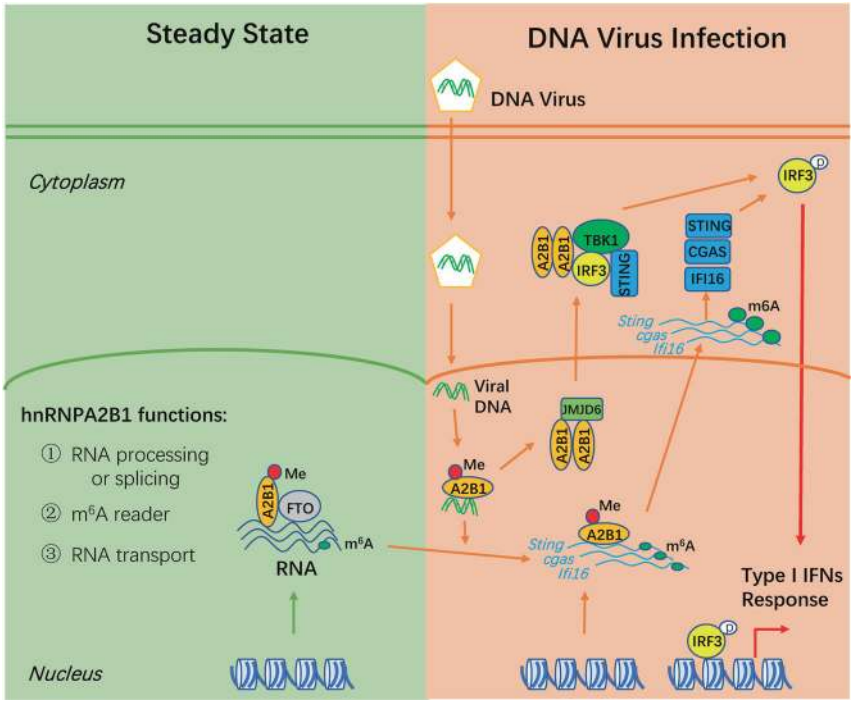

Fig. 1 hnRNPA2B1, an RNA-binding protein, can function as an m^{6}A reader and is involved in RNA transport, processing or splicing. Upon recognition of viral DNA, hnRNPA2B1 dimerizes and then is demethylated by JMJD6, which results in nucleocytoplasmic translocation of hnRNPA2B1 and consequently the activation of TBK1-IRF3 pathway. In parallel, hnRNPA2B1 promotes RNA m^{6}A modification, nucleocytoplasmic trafficking, and translation of *cgas, Ifi16* and *Sting* mRNAs to amplify the antiviral innate immune response

Several questions remain to be explored. hnRNPA2B1 was previously identified as an m^{6}A reader and mediated primary microRNA processing and alternative splicing.[8] It is interesting to speculate whether hnRNPA2B1 can function as a nuclear reader of m^{6}A-methylated *cgas*, *Ifi16* and *Sting* mRNAs to mediate their nucleocytoplasmic trafficking. As an RNA-binding protein, hnRNPA2B1 plays various roles in the nuclear retention of the HIV-1 genomic RNA.[10] It is unclear why hnRNPA2B1 acts as a viral DNA sensor but not viral RNA sensor mediating antiviral innate immune response, and whether other RNA-binding proteins of the hnRNP A/B family that share similar RRM domains can recognize viral DNA or RNA and participate in antiviral immunity. As Cao and colleagues pointed out, hnRNPA2B1 bound both viral and self DNAs, implying that hnRNPA2B1 may play a role in autoimmune diseases. Investigation of the function of hnRNPA2B1 in physiological and pathological condition would be of interest in future studies. In conclusion, Cao and colleagues identified hnRNPA2B1 as a new innate sensor initiating and amplifying the antiviral innate immune response from within the nucleus, providing potential therapeutic target for the treatment of virus infection (Fig. 1).

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (91753141 to HBL), the Program for Professor of Special Appointment (Eastern Scholar) at Shanghai Institutions of Higher Learning (HBL), the start-up fund from the Shanghai Jiao Tong University School of Medicine (HBL & RAF), and the Howard Hughes Medical Institute (RAF).

ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

REFERENCES

1. Kawai, T. & Akira, S. *Nat. Immunol.* **11**, 373–384 (2010).
2. Paludan., S. R. et al. *Nat. Rev. Immunol.* **11**, 143–154 (2011).
3. Wu., J. et al. *Annu Rev. Immunol.* **32**, 461–488 (2014).
4. Kerur., N. et al. *Cell Host Microbe* **9**, 363–375 (2011).
5. Orzalli., M. H. et al. *Proc. Natl Acad. Sci. USA* **109**, E3008–E3017 (2012).
6. Honda., K. et al. *Nat. Rev. Immunol.* **6**, 644–658 (2006).
7. L. Wang. et al. *Science*. https://doi.org/10.1126/science.aav0758 (2019).
8. Tong, J. et al. *Front. Med* **12**, 481–489 (2018).
9. Gary., J. D. et al. *Prog. Nucleic Acid Res Mol. Biol.* **61**, 65–131 (1998).
10. Gordon., H. et al. *RNA Biol.* **10**, 1714–1725 (2013).