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RESEARCH HIGHLIGHT

RNA editing by ADAR1 marks dsRNA as "self"

A paper recently published in *Science* reports that adenosine deaminase acting on RNA 1-dependent adenosine-to-inosine editing marks endogenous double strand RNA (dsRNA) as self and prevents their immune recognition by cytosolic RNA sensor MDA5.

To distinguish non-self from self is the core mission of immune system. The innate immune system is the first line of defense that defends host from invasion by pathogenic microorganism. In response to exogenous RNAs derived from invading pathogens, host immune system can detect the pathogenic RNAs via cytosolic RNA sensors, such as retinoic acid inducible gene-I (RIG-I), melanoma differentiation associated gene 5 (MDA5) and laboratory of genetics and physiology 2 (LGP2), and initiate the downstream signaling pathway [1]. However, recognition of endogenous RNAs in the cytoplasm by these sensors should be avoided. How to mark endogenous dsRNA as "self" while recognize exogenous dsRNA as "non-self" is a dilemma for immune system. Now Liddicoat *et al*. [2] showed in a recent *Science* paper that ADAR1 can mediate the adenosine-to-inosine (A-to-I) editing of self dsRNA and thus avoid the recognition of endogenous dsRNA by MDA5.

RNA editing is a molecular process through which cells can make discrete changes to specific nucleotide sequences within a RNA molecule. In humans, the most common type of RNA editing is adenosine to inosine [3], which is catalyzed by the ADAR proteins [4]. The study by Liddicoat *et al*. [2] links RNA editing to RNA recognition, providing an explanation for unresponsiveness of RNA sensors like MDA5 to endogenous dsRNA and highlighting a crucial physiological role of ADAR1.

In 2008, the Walkley group reported that ADAR1 is essential for maintenance of hematopoietic stem cells (HSCs) and suppression of interferon (IFN) signaling [5]. Loss of ADAR1 in HSCs led to global upregulation of IFN-stimulated genes (ISGs) and rapid apoptosis. However, the exact mechanism is unclear. To address this, the Walkley lab generated a constitutive knock-in of an editing-deficient $ADAR1^{E861A}$ allele in mice. Compared to wild-type mice, the $Adar1^{E861A/E861A}$ mice died at ~E13.5, and the size of fetal liver was smaller and contained massive apoptotic cells. Three hundred eighty-three transcripts were upregulated in $Adar1^{E861A/E861A}$ fetal liver, and most of them (258 of 383) were ISGs. The phenotypes of $Adar1^{E861A/E861A}$ mice were analogous to those with ADAR1 deficiency. By comparing the gene set associated with the response to IU-dsRNA (inosine uracil-paired dsRNA) in human cells, they found that the gene signatures (most of them were ISGs) of both $Adar1^{E861A/E861A}$ fetal liver and $Adar1^{-/-}$ HSCs were highly enriched for the IU-dsRNA response. Thus the authors proposed that the A-to-I RNA editing catalyzed by ADAR1 was a key step for suppression of the IFN response.

To determine the ADAR1-specific RNA editing events *in vivo*, Liddicoat *et al*. analyzed A-to-I mismatches from RNA-seq data. Among the edited RNA transcripts, there were three hyper-edited transcripts (*klf1*, *optn*, *oip5*) and their ADAR1-specific A-to-I hyper-edited sites were located within long 3′ untranslated regions (3′UTRs). They excluded the possibility that editing in 3′UTRs could affect the alternative splicing or the gene expression of targeted substrates. The authors then modeled secondary structures for potential hyper-edited substrates by predicting secondary structures for 3′UTRs of *klf1*, *optn*, and *oip5* after replacing adenosine with inosine or guanosine. For both cases, the mismatches were predicted to destabilize perfect dsRNA stem loops within hyper-edited 3′UTRs so that the RNA could not form long matched dsRNA. Meanwhile, they analyzed the transcriptional profile of the $Adar1^{E861A/E861A}$ fetal liver and found that it was similar to those with RIG-I and MDA5 activation. Therefore, the authors hypothesized that endogenous RNA without A-to-I editing could be recognized by MDA5 and activate immune response against dsRNA (Figure 1). Moreover, they observed that MDA5 deficiency could rescue the phenotype of $Adar1^{E861A/E861A}$ mice. These data convincingly support that ADAR1-mediated A-to-I editing of endogenous RNAs is essential for avoiding their recognition by MDA5.

Findings presented in this paper illustrated an essential role of ADAR1-mediated RNA editing in prevention of immune response to endogenous dsRNA, thus providing a mechanism of how organisms distinguish self and non-self dsRNA. A recent paper published in *Nature Immunology* reported that the RNA helicase SKIV2L, as a component of the RNA exosome, was required for the degradation of the modified endogenous RIG-I like receptor ligands generated
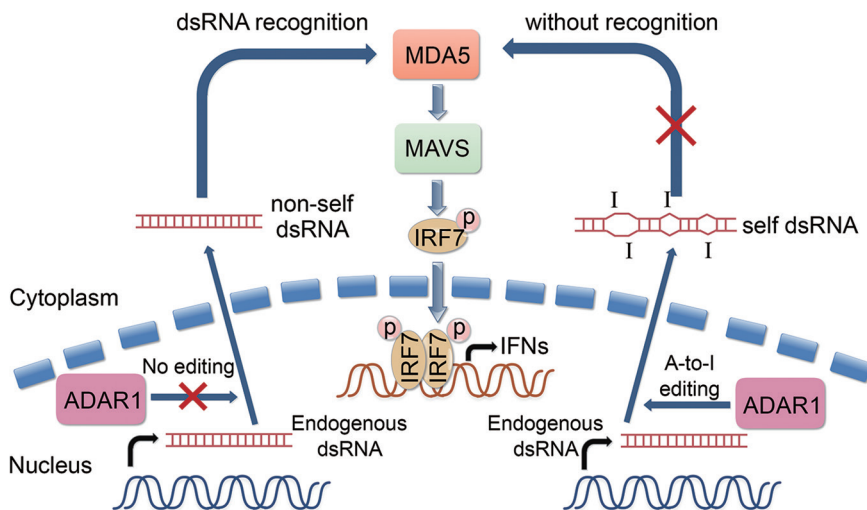
Figure 1 ADAR1-mediated A-to-I editing of endogenous dsRNA. In the presence of ADAR1, endogenous dsRNA is edited and regarded as self dsRNA that does not initiate immunogenic MDA5 recognition. In case of ADAR1 deficiency or mutation, endogenous dsRNA is recognized by MDA5 as non-self, and then activates MAVS-dependent phosphorylation of IFN-regulatory factor 7 (IRF7) and induction of IFNs, probably leading to the pathogenesis of autoimmune diseases.

during the unfolded protein response via IRE1-dependent RNA decay [6]. The 3′-to-5′ exonuclease TREX1 and the triphosphohydrolase SAMHD1 have been implicated in the control of endogenous *de novo* generation of DNA to avoid its recognition by DNA sensors [7, 8]. Despite these advances in understanding the prevention of innate recognition of endogenous RNA and DNA, investigations are required for the further understanding of mechanisms used by host to discriminate endogenous versus exogenous nucleic acids.

Both RIG-I and MDA5 were identified as the major sensors of cytosolic RNA and played crucial roles in dsRNA-induced innate antiviral responses [9]. However, they preferred different RNA chains. Long fragments analog poly (I:C) (> 4 kb) were preferred by MDA5, whereas short fragments generated by enzyme digestion (~300 bp) were recognized by RIG-I [10]. Therefore, MDA5 was suggested to sense the long dsRNA chain. However, Liddicoat *et al*. [2] proposed that the unedited endogenous dsRNA in the 3′UTR of different transcripts (200-300 bp) may

be recognized as non-self by MDA5, which is not well-fitted into the knowledge of dsRNA preference by RIG-I and MDA5. This disagreement suggests that the recognition pattern of dsRNA by RIG-I and MDA5 needs further investigations. It is also possible that the recognition pattern of endogenous dsRNA by MDA5 is different from the features characterized in exogenous dsRNA.

The role of MDA5 as a dsRNA sensor in innate immune response to self RNA is contradictory to a previous study. Mannion *et al*. [11] observed a similar phenotype of *Adar1*^{−/−} mice; however, they found that the newborn *Adar1*^{−/−}*Mavs*^{−/−} double mutant mice were able to feed, but they die within a day after birth. Their preliminary data suggested that the newborn *Adar1*^{−/−} *Mavs*^{−/−} mice still showed elevated type I IFN levels in blood. Given that MAVS is an essential adaptor utilized by both RIG-I and MDA5 to trigger type I IFN production [12], apart from the MDA5-MAVS axis, there may exist MDA5-dependent but MAVS-independent mechanisms that mediate the

innate immune responses in *Adar1*^{−/−} or *Adar1*[E861A/E861A] mice. Moreover, since aberrant recognition of endogenous RNA and DNA usually leads to autoimmune diseases, such as the linkage between loss-of-function mutations of *Trex1* gene and the Aicardi-Goutières syndrome, it may be meaningful to examine the existence of *Adar1* mutation and its relation to autoimmune diseases.

In conclusion, the work by the Walkley group uncovers a new mechanism of distinguishing the self and non-self dsRNA by the immune system and opens up a new field in immune recognition research.

Zhou Yu[1], Taoyong Chen[2], Xuetao Cao[1, 2]

[1]*National Key Laboratory of Medical Molecular Biology & Department of Immunology, Institute of Basic Medical Sciences, Peking Union Medical College, Chinese Academy of Medical Sciences, Beijing 100005, China;* [2]*National Key Laboratory of Medical Immunology & Institute of Immunology, Second Military Medical University, Shanghai 200433, China*
Correspondence: Xuetao Cao
E-mail: caoxt@immunol.org

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