The RNA-Specific Adenosine Deaminase ADAR1 Inhibits Human Protein Kinase R Activation

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To the Editor:

ADAR1, AN RNA EDITING ENZYME, catalyzes the conversion of adenosine (A) to inosine (I) in double-stranded RNA (dsRNA) by hydrolytic deamination of purine C-6. A-to-I editing is a form of nucleotide substitution editing, because I is decoded as guanosine (G) instead of A by ribosomes during translation and by polymerases during RNA-dependent RNA replication. In addition, A-to-I editing can alter RNA structure stability as I:U mismatches are less stable than A:U base pairs. ADAR1 contains three dsRNA binding motifs, followed by a highly conserved C-terminal catalytic domain and a zinc finger binding domain. Two major variants of human ADAR1 exist due to different promoter usage and are found in distinct intracellular locations. The full-length protein, ADAR1 p150, is expressed from an interferon (IFN) inducible promoter and localizes to the cytoplasm, whereas the N-terminal truncation variant, p110, localizes to the nucleus. The RNA editing function of ADAR1 is dependent on the activities of its RNA binding domain and the deamination catalytic domain. Most RNA transcripts subjected to ADAR1 editing were found in noncoding regions of transcribed RNAs, especially repeated sequences, such as *Alu* elements that compose 10% of the human genome, whereas ADAR1 also modifies the protein codon and splicing sites on messenger RNAs (mRNAs) and microRNA “seed” sequences. Global knockout (KO) of ADAR1 is embryonic lethal in mice, with fetal demise at 11.5–12.0 days after coitus due to massive production of IFN, loss of embryonic liver hematopoietic cells, and widespread apoptosis, although the molecular cause of cell death remains unknown.

We have previously reported that ADAR1 blocks endogenous dsRNA from activating the retinoic acid-inducible gene I-like receptor-dependent innate immune pathway (6). A genome-wide transcriptome analysis of the microarray data from ADAR1^{-/-} embryonic liver hematopoietic stem cells revealed that ADAR1 gene deletion was strongly associated with a gene expression signature of types I and II interferon-stimulated genes (ISGs). Interestingly, among the dsRNA-induced gene expression of RNA binding proteins, only IFN-inducible genes were upregulated in ADAR1^{-/-} cells. Protein levels of IFN- α and IFN- β were also dramatically increased in the embryonic tissue, although type II

IFN (i.e., IFN- γ) was not detectable. These findings implicated ADAR1 in the regulation of IFN and ISGs in hematopoietic cells. Indeed, subsequent studies demonstrated that ADAR1 plays an essential role in suppressing “endogenous” RNA from activating the MDA5-MAVS-mediated innate immune pathway; MDA5 KO rescues ADAR1 KO-induced type I IFN production and MDA5-ADAR1 double knockout (DKO) also rescued the embryonic lethality (2). Surprisingly, IFNAR1 KO failed to rescue ADAR1 KO animals from dying, implying that ADAR1 KO-induced cell death is not solely attributed to overproduction of IFN (3).

Among those dsRNA binding proteins, protein kinase R (PKR) is activated during viral infections and then phosphorylates the α subunit of eukaryotic translation initiation factor 2 (eIF2), leading to inhibition of translation and viral replication. A recent report showed that human ADAR1 prevents translational shutdown during the type I IFN response by blocking PKR activation (1). The authors observed that ADAR1 KO neuronal progenitor cells exhibit MDA5-dependent IFN production, PKR activation, and cell death, raising an interesting possibility that perhaps without ADAR1, endogenous RNA engages and activates PKR, resulting in cell death. If true, this could provide a mechanistic explanation of how ADAR1 KO induced embryonic lethality. Contrary to such a hypothesis, we previously genotyped 140 pups (F2) after interbreeding ADAR1^{+/-} with PKR^{+/-} mice (5). The distribution of genotypes clearly followed Mendelian pattern. Notwithstanding, none of the viable offspring carries PKR^{-/-} ADAR1^{-/-} DKO genotype, indicating that PKR KO failed to rescue the lethality conferred by ADAR1 KO. Therefore, at least in mice the main cause of the observed cell death in the absence of ADAR1 is unlikely attributed to PKR activation. In reconciling the discrepancy, the authors pointed out that ADAR1 may play a more critical role in preventing PKR activation in humans but not in mice. In support, it has been previously noticed that substitution of positively selected residues in human PKR with residues found in related species altered sensitivity to PKR inhibitors from different poxviruses. Species-specific differences in sensitivity to poxviral pseudosubstrate inhibitors were identified between human and mouse PKR, which were traced to positively selected residues near the eIF2 α -binding site (4). Lastly, PKR is typically activated by virally derived

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dsRNA. Working with systems without viral infection, Chung *et al.* (1) strikingly reported that ADAR1 prevents endogenous RNA from activating PKR, which then allows efficient translation during the type I IFN response. It is puzzling, however, that PKR activation by endogenous dsRNAs does not occur in mock-treated, as opposed to IFN-treated, ADAR1-deficient cells given the high abundance of unedited Alu elements present in the transcriptome. Only future work will be able to solve the mystery.

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