The Other Face of an Editor: ADAR1 Functions in Editing-Independent Ways

Konstantin Licht and Michael F. Jantsch*

The RNA editing enzyme ADAR1 seemingly has more functions besides RNA editing. Mouse models lacking ADAR1 and sensors of foreign RNA show that RNA editing by ADAR1 plays a crucial role in the innate immune response. Still, RNA editing alone cannot explain all observed phenotypes. Thus, additional roles for ADAR1 must exist. Binding of ADAR1 to RNA is independent of its RNA editing function. Thus, ADAR1 may compete with other RNA-binding proteins. A very recent manuscript elaborates on this and reports competition of ADAR1 with STAUFIN1, thereby modulating RNA-degradation. ADAR1 is also known to recruit proteins such as DROSHA to nascent transcripts. Still, many open questions remain. For instance, the biological role of the Z-DNA binding domains in ADAR1 is not defined. Moreover, the impact of ADAR1 on the RNA-folding landscape is unclear. In sum, moonlighting functions of ADAR1 may be manifold and have a great impact on the transcriptome.

1. Introduction

Adenosine to inosine RNA editing (A-to-I editing) is a major modification of protein-coding mRNA and non-coding RNAs.[1,2] A-to-I editing is catalyzed by the adenosine deaminase acting on RNA (ADAR) class of enzymes. The bulk of editing is thought to occur co-transcriptionally.[3] Editing deaminates adenosines and results in the generation of inosines. Inosines are interpreted as guanosine by cellular machines.[4] Therefore, editing can change the information encoded in the genome, leading to amino acid changes in the respective protein.[3]

Two catalytically active enzymes, ADAR1 and ADAR2, have been identified.[6] Both editases are double-stranded RNA-binding (dsRNA-binding) proteins. Consequently, they exclusively act on dsRNA substrates. The functions of ADAR1 and ADAR2 have been defined using knockout mice. ADAR2 null mice do not live longer than 3 weeks past birth.[7] Lethality of ADAR2 knockout mice can be rescued by expression of a constitutively edited version of the glutamate receptor subunit 2 (Gria2), showing that Gria2 editing by ADAR2 is a prerequisite for life.[7] Several other protein-coding transcripts are also targets of ADAR2, but the consequences of these editing events are currently largely unknown.[8]

ADAR1 knockout mice on the other hand die around embryonic day e11.5 to e12.5 in utero associated with a number of hematopoietic defects.[9–11] Interestingly, loss of ADAR1 in hematopoietic stem cells leads to an upregulation of interferon-stimulated genes (ISGs).[10] Moreover, double-stranded RNA containing I-U basepairs can dampen the induction of ISGs.[11] These phenotypes all hinted toward an involvement of ADAR1 in the immune response.

Two isoforms of ADAR1 exist (Figure 1), a shorter version (ADAR1α110) that is ubiquitously expressed across tissues and a longer isoform (ADAR1α150) that is interferon (IFN)-inducible.[12] Interestingly, mutations in the human Adar1 gene have been linked to the Aicardi–Goutières syndrome (AGS), an inflammatory disorder associated with aberrant expression of type I interferon.[13] In mice, deletion of ADAR1 causes similar aberrant anti-viral responses associated with elevated expression of ISGs.[15,16] ISGs are also upregulated in a mouse expressing a constitutively edited version of ADAR1, suggesting that the phenotype is tightly linked to editing.[17] These mice can be rescued when either MDA5 or MAVS – proteins sensing viral dsRNA – are deleted in ADAR1 null or ADAR1 editing-dead mice.[15–17] Taken together, this suggests that the main function of ADAR1 is to edit endogenous double-stranded RNA which may help to distinguish it from exogenous (viral) dsRNA. Unedited endogenous RNA apparently triggers the interferon response via the MDA5–MAVS pathway. In ADAR1 null mouse embryonic fibroblasts (MEFs) the interferon response can be rescued by transfecting inosine-containing dsRNA oligonucleotides into these cells, again demonstrating that editing suppresses inflammatory responses.[15]

Interestingly, mice lacking both, ADAR1 and the proteins sensing dsRNA (MAVS or MDA5) show strong phenotypic differences, depending on the ADAR1 allele investigated. While a point mutation in the catalytic domain of ADAR1 (E861A) leading to editing-dead ADAR1 can be fully rescued, mice with larger deletions in the ADAR1 protein are still severely impaired.[10,15–17] The critical determinants of the severity of
the different phenotypes seem to be the nucleo-cytoplasmic localization and the RNA-binding domains of ADAR1. However, as different Ifih1 (the gene coding for MDA5) and Mavs alleles have been used in these studies, differences in the alleles of MDA5 or MAVS may also contribute to the different phenotypes observed.\textsuperscript{15–17} Still, binding of dsRNA by ADAR1 may also affect the transcriptome, independent of RNA editing (Figure 2).

Here, we will discuss published evidence supporting this idea and future directions to challenge this view.

2. ADAR1 Functions in Different Editing-Independent Ways

2.1. Protection of Transcripts Against Degradation

Binding of STAUFN1 or STAUFN2 to structured regions in mRNAs can recruit UPF1 and consequently trigger STAUFN-mediated RNA-decay (SMD).\textsuperscript{18,19} STAUFN proteins recognize dsRNA and consequently can compete with other dsRNA-binding proteins such as ADARs.

Recently, Nishikura and colleagues have proposed an anti-apoptotic role of ADAR1 under stress conditions involving STAUFN-mediated RNA-decay.\textsuperscript{20} They show that ADAR1\textsuperscript{p110} translocates to the cytoplasm when cells are stressed by UV-irradiation or heat shock (Figure 2A). The translocation is induced by phosphorylation of ADAR1\textsuperscript{p110} via the MAP kinase pathway and depends on p38 and MSK1/2.\textsuperscript{20} ADAR1\textsuperscript{p110} is a nucleo-cytoplasmic shuttling protein that is only transiently found in the cytoplasm and primarily localizes to the nucleus. Nuclear import is mediated by a region surrounding the third dsRBD found in this protein that is recognized by the nuclear import factor Transportin1 (TRN1).\textsuperscript{21,22} The import factor competes with the export factor Exportin5 (XPO5).\textsuperscript{21} XPO5 binds double-stranded RNAs and hence proteins are believed to be co-exported with bound RNAs.\textsuperscript{23,24} The phosphorylation of ADAR1\textsuperscript{p110} was shown to map to a region immediately downstream of the TRN1 binding site. The Nishikura lab shows that this phosphorylation facilitates XPO5 binding. Whether this interaction is RNA-dependent, (as generally believed) or whether also TRN1 binding is affected by phosphorylation remains to be determined.

In any case, stress-induced phosphorylation suggested that ADAR1\textsuperscript{p110} might be involved in regulating apoptosis.\textsuperscript{20} Indeed, knockdown of ADAR1\textsuperscript{p110} increased apoptosis about fivefold. Most interestingly, this effect could be rescued by overexpressing ADAR1\textsuperscript{p110} using transient transfection, but also by overexpressing an editing-dead version of ADAR1\textsuperscript{p110}. The anti-apoptotic effect of ADAR1\textsuperscript{p110} is also independent of the MDA5-MAVS pathway as these two factors could be knocked down without rescuing the apoptotic phenotype. Thus, the authors concluded that an editing-independent function of ADAR1 plays a role here. In search for interacting factors they found that ADAR1\textsuperscript{p110} competes with the dsRNA-binding protein STAUFN1. Knockdown of ADAR1 under UV-stress conditions leads to a decrease in mRNA-levels for about 500 genes, but most of the genes were unaffected when ADAR1 and STAUFN1-knockdown was combined. This suggests that ADAR1 may protect a subset of RNAs containing long double-stranded RNA-repeats in their 3'UTRs from binding to STAUFN1 and subsequent degradation, although another study found knockdown of STAUFN1 protein has only a very modest effect on RNA levels, thereby questioning the general existence of SMD.\textsuperscript{18,20,25} However, it is plausible that stress-induced cytoplasmic ADAR1\textsuperscript{p110} can interfere with binding of STAUFNs and other dsRBD proteins thereby affecting the stability of selected transcripts like Rad51 or Atm. RAD51 or ATM proteins have been implicated in protection against DNA-damage or oxidative stress, and may help cells to survive under (UV)-stress conditions.\textsuperscript{209}

2.2. Competition or Cooperation With RNA-Binding or Processing Factors

Knockdown of ADAR1 in a human cell line leads to differences in the length of several 3'UTRs.\textsuperscript{206} For instance, the UTR of Lamc1 and Aph1b gets longer upon knockdown of ADAR1.\textsuperscript{206} Conversely, overexpression of ADAR1 leads to a shortening of both UTRs. Interestingly, also overexpression of an editing-dead ADAR1 mutant can cause shortening. Only about 25% of the lengthened or shortened UTRs contain at least one editing site overlapping or in the vicinity.\textsuperscript{206} Thus, the regulation of the UTR length upon ADAR1 knockdown seems editing-independent in most cases. As ADAR1 is found largely associated with chromatin, it may also bind nascent RNA and preclude binding of other proteins such as canonical 3'UTR processing factors (Figure 2A).\textsuperscript{206} This can explain the differences in 3'UTR length that the authors observe upon knockdown of ADAR1. The observed chromatin association also fits well with an earlier report showing that A-to-I editing primarily takes place on nascent RNA.\textsuperscript{3}

Interestingly, ADAR1 and HuR (ELAVL1) were found to cooperatively bind to RNA.\textsuperscript{27} As HuR is known to stabilize transcripts the interaction promotes transcript abundance of HuR/ADAR1 targets.\textsuperscript{27} However, in this case it is unclear if the cooperative effect depends on editing, although HuR-binding motifs are enriched around A-to-I editing sites.\textsuperscript{27}
2.3. Recruitment of Proteins to dsRNA

The Nishikura group has shown that ADAR1 can recruit and promote DICER activity on pre-miRNAs and siRNA precursors.\cite{28} Interestingly, this stimulatory effect was found to be independent of editing or RNA-binding. In contrast, ADAR1 can promote primary miRNA processing.\cite{26} However, here, ADAR1 mutants that lost either RNA-binding or editing activity also were impaired in their stimulatory activity on pri-miRNA cleavage.\cite{26} Interestingly, a previous study reported that lack of ADAR1 and ADAR2 affects miRNA abundance.\cite{29} It was shown that this effect is largely editing-independent. However, most of the miRNAs were upregulated in mouse embryos lacking both ADAR1 and ADAR2. Thus, it is possible that ADAR2 and ADAR1 affect miRNA levels in opposing directions. Taken together, these observations show that besides editing also RNA-binding is important for pri-miRNA processing (Figure 2B). In contrast, pre-miRNA processing does not require RNA interaction of ADAR1 itself but seemingly only relies on ADAR1-DICER interactions.

2.4. Camouflaging “Self”

As mentioned above, the embryonic lethality observed in all ADAR1 alleles can be rescued to different extents by removal of sensors of viral RNAs, depending on the ADAR1 allele investigated. MDA5 a cytoplasmic sensor of viral RNA and its downstream signaling mediator MAVS are critical components of antiviral responses (Figure 3A).\cite{30,31} A complete deletion of the ADAR1 protein can be rescued until birth and shortly beyond by MAVS.\cite{15} ADAR1 has two isoforms, a cytoplasmic p150 version and the above mentioned, predominantly nuclear ADARp110 version.\cite{32} Deletion of only the p150 version also leads to embryonic lethality but can be rescued till weaning when MAVS is simultaneously deleted.\cite{16,33} Deletion of exons 7–9 encoding the nuclear localization signal, parts of one RNA-binding domain and the catalytic domain in both ADAR1 isoforms led to a more severe phenotype. Here, removal of MAVS could only rescue until birth with only a minor fraction of mice surviving until day 10 past birth.\cite{16} Most interestingly, an editing-dead point mutation in ADAR1 (AdarE861A) also
displays embryonic lethality at day e13.5. However, the Adar1 E861A allele can be fully rescued by an MDA5 deletion.\[17\] Thus, a major function of ADAR1 seems to "mark" endogenous RNA by modification to allow its distinction from exogenous (viral) RNAs. However, the failure to fully rescue complete ADAR1 deletions by the disruption of the MDA5-MAVS pathway suggests additional functions of ADAR1 beyond editing, possibly also by camouflaging unedited endogenous RNAs by sequestration (Figure 3B).

However, as editing by ADAR1 also requires RNA-binding, the contribution of RNA-binding cannot be studied without disrupting editing at the same time. Nonetheless, the fact that ADAR1 can prevent ADAR2 from accessing some editing sites suggests that ADAR1 can bind RNAs without editing them.\[34\] Thus, ADAR1 may sequester some RNAs and prevent activation of other pathways and enzymes that sense or bind double-stranded RNAs.

3. Beyond Editing: In Search of “The Other Face of ADAR1”

3.1. Does ADAR1 Shape RNA-Structures?

As ADAR1 binds and edits transcripts very early in their life cycle when they are still nascent it would be very interesting to see how the structure of RNA changes in the absence of ADAR1 (Figure 4A).\[3,26\] Ideally, this would be done with knockout/knockin systems that lack ADAR1 completely or only contain an editing-dead knockin in order to discriminate between effects of ADAR1 editing loss or complete loss of ADAR1.\[15,17\] In any case, doing so might give insight into the role ADAR1 plays in shaping the dsRNAome, a possible trigger of antiviral responses.\[35\]

3.2. Does ADAR1 in General Modulate Binding of Proteins to Nascent RNA?

The above mentioned studies demonstrated modulation of interactions with STAUFEN1, DROSHA, or DICER by ADAR1.\[20,26,28\] It may be revealing to test if ADAR1 generally prevents other proteins from binding dsRNA or recruits proteins to dsRNA and to determine potential biological consequences (Figure 4B). This could be tested using a recently published method where RNA binding proteins are crosslinked to their transcripts followed by enrichment of poly(A) containing mRNAs and mass spectrometry in order to "define the mRNA interactome of proliferating human HeLa cells."\[36\] Comparing wild type and ADAR1 deficient cells should allow identification of proteins that exhibit increased binding in the absence of ADAR1.

3.3. What is the Biological Role of the Z-DNA Binding Domains in ADAR1?

ADAR1 harbors a peculiar Z-DNA binding domain (Figure 4C).\[2,37\] Z-DNA is left-handed DNA exhibiting a particular zigzag backbone confirmation that is stabilized by negative supercoiling occurring during transcription.\[38\] The Z-DNA binding domain Z-alpha was shown to bind Z-DNA very efficiently, suggesting that it may help to direct ADAR1 to nascent RNA and to actively transcribed genes.\[37,39\] The second Z-DNA domain – Z-beta – in turn was suggested to promote dimerization of ADAR1.\[37\] While these findings rely on in vitro observations, no in vivo data is available for these domains. Clearly, transgenic mice with an in-frame deletion of the Z-DNA binding domain Z-alpha or Z-beta may help to uncover the biological role of Z-DNA binding domains in ADAR1.

3.4. Protein Interactors of ADAR1?

The above mentioned interactions with DICER or DROSHA were all based on individual observations while systematic studies searching for direct protein interactors of ADAR1 are still lacking (Figure 4D). Protein-interaction databases like BioGRID (https://thebiogrid.org) may fill the gap.\[40\] In case of ADAR1 BioGRID lists 67 protein-interactors for human. Many of these interactions come from high-throughput studies. Here, we want to exemplify two of the studies as they alone provide evidence for 40% of the listed ADAR1 interactions.\[41,42\] Li et al identify high confidence interactions of ADAR1 with the
transcription factors FOXA2 and FOXA3 in the chromatin fraction again suggesting that ADAR1 is enriched in chromatin and therefore may preferentially act on nascent RNA. They use different transcription factors as baits and perform tandem affinity purifications followed by mass spectrometry. Their data also suggest high-confidence interactions with other members of the FOX proteins. The laboratory of Matthias Mann on the other hand identified 17 different interactors for ADAR1. As they did not focus on one specific class of proteins and took 1125 bait proteins from different classes, the reported interactions are presumably less biased. This list includes transcription or splicing factors, which is not surprising as ADAR1 acts co-transcriptionally. However, the list also includes one ribosomal protein, namely the ribosomal protein L35 (RPL35) and the ribosome binding protein 1 (RRBP1), which is associated with the ER. This may point to an unknown role of ADAR1 for translation. Moreover, as the second Z-DNA (Z-beta) binding domain in ADAR1 has been linked to protein–protein interactions it can also be of interest to study the ADAR1-interactome in the presence and absence of Z-Beta using tagged ADAR1 protein.

4. Conclusion

Although the major function of RNA editing by ADAR1 seems to mark endogenous structured RNA as self by RNA editing many questions that go beyond editing alone remain unanswered. Nishikura and colleagues have explored one important editing-independent function of ADAR1 by showing that binding of the protein alone can protect transcripts from degradation. Moreover, binding of ADAR1 very early during transcription to nascent RNA may play a very important role. ADAR1 – in contrast to ADAR2 the editase acting primarily on protein-coding transcripts – does contain a Z-DNA-binding domain, which has been suggested to direct ADAR1 to actively transcribing genes immediately to nascent RNA. Why is it needed only for ADAR1? What is its biological function? This is only one of the many open links for ADAR1. Here, we have given ideas how to tackle gaps in our knowledge of ADAR1 and shed light on open questions.

Abbreviations

ADAR, adenosine deaminase acting on RNA; ADAR1p110, short ADAR1 isoform, ubiquitously expressed, primarily nuclear; ADAR1p150, long ADAR1 isoform, interferon-inducible, nuclear-cytoplasmic shuttling; dsRNA, double-stranded RNA; IFN, interferon; ISG, interferon-stimulated gene; MAVS, signaling protein/acts downstream of MDA5; MDA5, sensor/receptor for dsRNA; UTR, untranslated region; Z-DNA, left-handed DNA with a zigzag backbone conformation.

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