Point of VIEW

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# ADAR Editing Wobbles the MicroRNA World

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**ABSTRACT** Recent work reveals that adenosine-to-inosine editing occurs in a number of cellular microRNAs (miRNAs). Such editing is shown to diminish the expression of one miRNA and alter the target specificity of another. This changes our current views significantly by not only increasing the repertoire of miRNAs and their potential targets, but also providing mechanisms for how to regulate them and direct them to alternative targets.

MicroRNAs (miRNAs) are small (~22 nt) noncoding RNAs abundantly produced by metazoans. miRNAs target specific messenger RNAs (mRNAs) in a cell to diminish expression of the encoded proteins and consequently modulate many aspects of development and cell behavior. Since the discovery in 1993 of the first miRNA in *Caenorhabditis elegans*, ingenious molecular methods have been developed to isolate miRNAs from many model organisms, including human (*1*). Several-hundred human miRNAs are known so far, and they are predicted to target thousands of protein-coding genes. A single miRNA may target hundreds of genes because of the imperfect base pairing with target RNAs that is inherently involved in miRNA action. Now, a study by Kazuko Nishikura and her colleagues from the Wistar Institute in Philadelphia reveals a whole new dimension of the miRNA world: RNA editing can diversify miRNAs and redirect them to alternative target loci (*2*).

Gene silencing by small (19–22 nt) RNAs can occur by two mechanisms. The targeted mRNA is degraded when the interfering RNA forms a perfect duplex with its target, as in gene knockdown experiments with short interfering RNAs that target exons. The mRNA is cleaved by an endonuclease residing in the multiprotein complex called RNA-induced silencing complex (RISC) that helps to bring the ~22-nt antisense RNA strand to the complementary mRNA site (*1*). An alternative mode of action, translational repression, has been documented for natural miRNA function in humans. The miRNAs target 3′-untranslated regions (3′-UTRs) and form imperfect duplexes that inhibit translation by an as-yet poorly defined mechanism (*1, 3*). Recent evidence revealed that miRNA-bound mRNAs are eventually targeted to cytoplasmic processing bodies, where they are degraded or retained for future translation triggered by new environmental cues (see ref 3 for review). Thus, the fate of an mRNA targeted by miRNA is governed by the constituents of the RISC complex that binds the mRNA–miRNA duplexes.

So, what does RNA editing have to do with RNA interference (RNAi)? Cells implement RNA editing to recode genetic information. Editing can add or delete nucleotides, or change one nucleotide to another. A classic example of this genetic recoding process is the modification of adenosine (A) to inosine (I) in a transfer RNA (tRNA) anticodon that enables decoding of three codons by the same tRNA *via* wobble base pairs I○C, I○A, and I○U. A-to-I editing also occurs in mRNAs, and this generates protein isoform diversity vital to nervous system function (see ref 4 for review). This mRNA editing is mediated by adenosine deaminase acting on RNA (ADAR), which promotes hydrolytic deamination of adenosines on double-stranded RNA (dsRNA) substrates (*4*). Vertebrates possess two distinct catalytically active ADARs, which are expressed in many cells and tissues. Interestingly, ADAR1 expresses two isoforms: a short isoform ADAR1-S detected in the nucleoplasm and nucleolus, and a large-form ADAR1-L that is induced by interferon and located mostly in the cytoplasm. ADAR2 is enriched in the

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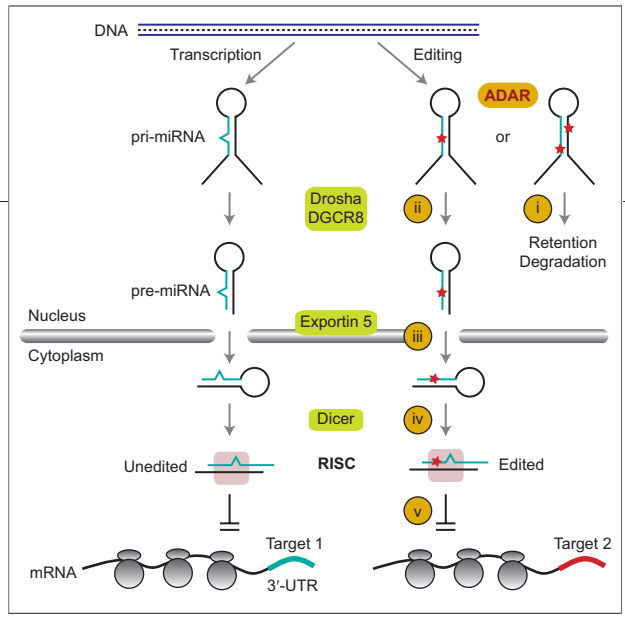


**Figure 1. Steps in miRNA biogenesis and function that might be modulated by ADAR editing. The first step of miRNA biogenesis is transcription, usually carried out by RNA polymerase II, generating pri-miRNAs. Cotranscriptional editing of the pri-mRNA can occur because of the recognition of extended double-stranded regions of the nascent transcript by ADAR1 or ADAR2. i) One possible consequence of editing, documented for miR-142 (see text), is to interfere with nuclear processing of the pri-mRNA by the microprocessor complex, affecting secondary structure, Drosha/DGCR8 binding, or cleavage by Drosha. Consequently, the edited pri-miRNA may be degraded, as was shown for miR-142. ii) In other instances, altered processing by Drosha may generate new pre-miRNA isoforms. iii) The export of some pre-miRNAs to the cytoplasm is mediated by exportin 5 (*23*); editing may directly interfere with this step and inhibit miRNA function this way. iv) Once in the cytoplasm, Dicer cleaves pre-miRNAs to mature miRNAs, which are incorporated into RISC. Some editing events may affect Dicer recognition or cleavage; editing may also hinder RISC assembly. v) Edited miRNAs may recognize new target mRNAs for gene regulation and may not regulate mRNAs targeted by unedited miRNAs, as was documented for miR-376a-5p (Figure 2). Not shown are very similar phenomena of miRNA targeting, mediated by potential editing of the target sites in 3′-UTRs; this may hinder or facilitate a miRNA function, as discussed in the text.**

nucleolus. The differential localization and tissue distribution of various ADARs hint of functional specialization. In fact, ADAR1 and ADAR2 show unique preferences for specific editing sites in mRNAs. ADAR1 inactivation in mice leads to embryonic lethality (*5*), whereas *Adar2*-null mutants are viable, though they are crippled by epileptic seizures and die several weeks after birth (*6*). Important human disorders may result from a dysfunction of A-to-I editing (*7*); however, we still have limited knowledge of how ADAR editing modulates human development, physiology, and behavior. Because miRNA regulatory networks are implicated in many of these processes (*1*), and RNA–RNA interaction constitutes the core of gene silencing by RNAi, cross-talk between RNA editing and RNAi has long been suspected.

Brenda Bass originally put forward the idea that the RNAi and editing machineries may compete for dsRNA substrates, thereby dictating the outcome of RNAi (*8, 9*). Consistent with this, A-to-I editing was shown to dampen RNAi, and ADAR1-L was shown to be a suppressor of RNAi (*10*). More recently, a number of human miRNAs were found to be edited (*11*). But, does ADAR editing modify miRNAs to execute specific physiological aspects of a cell, and can RNA editing modulate specific miRNA function by acting as a switch?

Two recent studies by the Nishikura group address this key issue. Last year, Yang *et al.* (*12*) demonstrated that ADAR editing affects the processing and expression of a miRNA. miRNAs do not originate from nonspecific RNA degradation; rather, they are the products of *bona fide* regulator genes, whose existence was conceptualized by Jacob and Monod 4 decades ago in their operon model. Transcription of miRNA genes produces long primary transcripts, sometimes in polycistronic forms that encode several miRNAs. From these, mature miRNAs are generated *via* two processing reactions, one within the nucleus that produces precursor miRNAs and one in the cytoplasm that converts pre-miRNA to mature miRNA (Figure 1). The precision with which miRNAs are processed is governed by the secondary structure of the precursors. The mature ~22-nt miRNA is embedded within an ~60–70-nt fold-back hairpin structure. The typical structure is characterized by an imperfect bulged duplex flanked by a loop of varying size at one end and two single-stranded tails on the other end. A bulged duplex is a common feature of many natural mRNA substrates edited by ADARs (*13*). Therefore, it is logical to suspect that ADARs target some miRNAs within the nucleus, where they are transcribed and processed to pre-miRNAs, and in the cytoplasm, after they emerge from the nucleus (Figure 1).

To test this possibility, Yang *et al.* (*12*) chose a set of eight miRNAs and showed that several primary miRNAs (pri-miRNAs) from this group were edited *in vitro* quite efficiently and specifically. Notably, A-to-I editing occurred at sites on both strands of the duplex, and ADAR-modified adenosines are located at various distances from the loop and the single-stranded tails, including some that reside within the mature miRNA sequence (Figure 2). Thus, pri-miRNAs can be edited by ADARs, and editing can alter the pairing potential of a mature miRNA. Does this happen *in vivo*?

One miRNA modified differentially by recombinant ADAR1 and ADAR2 belongs to a special class of miRNAs whose precursor generates a pair of complementary miRNAs representing each of the two strands of pre-miRNA hairpin. This RNA, miR-142, is expressed in hematopoietic tissues, which are affected in *Adar1*-null mouse embryos (*5*). To address the *in vivo* function of editing, Yang *et al.* (*12*) transfected a human embryonic kidney 293 (HEK293) cell line with expression plasmids encoding the unedited version of pri-miR-142, as well as a "pre-edited" version of the same that carries four guanosine (G) residues in place of the edited A residue. In the pre-edited version, four A-U/U-A pairs are substituted by G○U/U○G pairs that are thought to mimic the I○U or

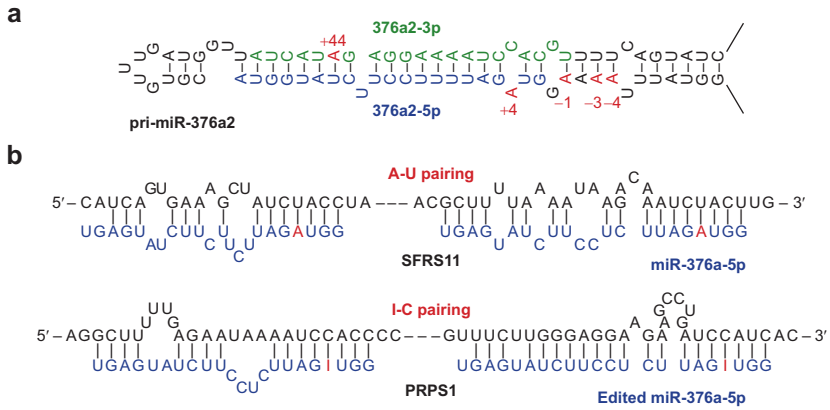


**Figure 2. miRNA editing changes target specificity. a) Sites of editing (shown in red) in the primary transcript containing miR-376a-5p. b) Validated targets of unedited (top) and edited miRNA in mouse cells (*2*). Note the interaction of edited miRNA with a new mRNA target involving an I○C base pair in the seed sequence. Conceivably, I○A or I○U wobble base pairs adjoining the bulged duplex regions may also guide edited miRNAs to different target sites.**

U○I pairs of the edited RNA. A clever set of primer extension experiments monitored the expression of the transgenes and revealed that the pre-edited version of pri-miR-142 is severely defective in generating the pre-miRNA or the mature miRNA forms. This indicates a block in the nuclear processing of the primary transcript carried out by the microprocessor complex (*14*). This inference was borne out by further *in vitro* experiments with a recombinant microprocessor. It is important to note that cotransfection of plasmids producing ADAR1-S- and ADAR2 substantially reduced the expression of mature miRNA-142 from the unedited version of the transgene. Cotransfection experiments showed that the expression of a control miRNA that does not possess editing sites (miR-181b1) was unaffected by the transfected ADARs. Editing clearly affected the processing of pre-miRNA-142, and the RNA was somehow degraded.

The nuclease involved in degrading the edited pri-miRNA is most likely TUDOR-SN, a component of RISC, also known to play a role in degrading I-containing dsRNAs (*15*). Pharmacological inhibition of this enzyme in HEK293 cells for 24 h caused substantial accumulation of edited pri-miR-141. Moreover, ADAR2-deficient mice showed a 3-fold rise in the level of miR-142 in both spleen and thymus. Similar results were obtained with spleen of mice in which ADAR1 is selectively knocked-out in the B-cell lineage. We can thus anticipate that critical new information on the biology of RNA editing and its cross-talk with RNAi will emerge as additional tissue-specific ADAR knockout mice are generated in the future.

Does RNA editing specify alternative targets for a miRNA? To address this, Kawahara and colleagues (*2*) turned to a polycistronic miRNA cluster encoded by syntenic regions in human and mouse genomes. This miR-376 polycistron encodes six miRNAs in humans. Highly homologous to each other and the three counterparts from mouse, these RNAs are expressed in placenta and embryos as well as adult tissues (*16, 17*). Remarkably, the primary transcript encoding the various miR-376 RNAs is extensively edited at specific sites in several human and mouse tissues. Moreover, ADAR1 and ADAR2 modify distinct sites: brain cortices of the ADAR2-deficient mouse show no editing at one site, whereas editing at another site is completely abolished in stage E11.5 embryos that are deficient in ADAR1. ADAR2 deficiency also increases dramatically the efficiency of editing by ADAR1 at its preferred site. Thus, ADAR2, when present, seems to suppress the activity of ADAR1.

Unlike the case of miR-142, however, ADAR editing does not affect miR-376 processing or expression significantly. Moreover, the edited sites in miR-376 happen to lie in the 5′-proximal "seed" sequence believed to be crucial in directing a miRNA to its target. Therefore, the authors were in a position to address whether different mRNAs are targeted by the edited and unedited miRNA. The authors chose as a candidate miR376a-5p (Figure 2), which is conserved in mice and human, and they searched for potential targets by new computational methods that yielded 78 targets for the unedited miRNA and 82 for its edited version. Three members from each group were then chosen randomly, and sensor transgenes were constructed that contain luciferase followed by target 3′-UTRs, bearing complementary sequences for either the edited or the unedited miRNA. HeLa cells were next cotransfected with these expression constructs in combination with either the unedited RNA or the edited (A-to-G-substituted) counterpart. This thoughtfully designed transfection study produced striking results. First, the test transgenes predicted to respond to the unedited RNA did exactly that, whereas the unedited RNA showed no effect in their expression. Reciprocally, the transgenes with targets for the edited RNA responded to the edited RNA but not to unedited RNA. A *C. elegans* miRNA used as a negative control did not affect either set of transgenes. Thus, a compelling case was made for redirecting miRNAs to distinct targets *via* site-specific RNA editing by ADARs. Although A-to-I editing is commonly thought to result in I○C pairing (Figure 2), we suggest that, in the context of miRNA function, I○U and I○A wobble base pairs may also be involved, because they are in tRNA decoding. This would further broaden the poten-

tial impact of editing on miRNA-mediated gene regulation.

One gene targeted by the edited miR-376a-5p RNA encodes phosphoribosyl pyrophosphate synthetase 1 (PRPS1), an essential enzyme involved in purine metabolism and uric acid synthesis. Elevated levels (2–4-fold) of this enzyme are linked to X-linked human disorders associated with gout as well as hyperuricemia, causing neurodevelopmental impairments. Thus, PRPS1 must be tightly regulated, and this may involve the edited form of miR-376a-5p as one regulator. Consistent with this, the PRPS1 gene is expressed at a 2-fold reduced level in the brain cortex of wild-type mice compared with that of the ADAR2-deficient mice. Moreover, the uric acid level parallels the observed gene expression pattern. Finally, in contrast to the picture in the brain, ADAR2 deficiency causes no difference in PRPS1 expression in the liver, which does not edit miR-376a-5p. Thus, the edited miR-376a-5p produced only in specific tissues in mouse represses PRPS1 in a tissue-restricted fashion.

The implications of these findings are far-reaching. The silencing of a miRNA by selective editing of the primary transcript provides a versatile mechanism for the cell to regulate miRNA expression, and in turn, its targets. The site of editing can also guide which mature sequence is ultimately produced and presented by RISC. Consequently, editing of a pre-miRNA may generate more miRNA isoforms than currently predicted. Editing can add yet another dimension in controlling miRNA biogenesis and function: the altered structure of the edited duplexes makes them susceptible to binding by RNA-binding proteins that may not recognize unedited duplexes, and *vice versa*. This impacts all aspects of miRNA life: expression, localization, and targeting (Figure 1).

It is important to note that the 3′-UTR, targeted by typical miRNAs, can itself be a target for modification by ADAR (*18*), especially when it contains inverted repeat sequences (*19*–*21*). A case in point is the discovery by Prasanth *et al.* (*22*) that hyper-editing of 3′-UTR of a mature mRNA retains the mRNA within the nucleus for future export to the cytoplasm in response to stress. We suggest that subtle editing of a 3′-UTR can have just as profound effects on miRNA regulation. First, ADAR editing might recode a 3′-UTR for differential regulation by a miRNA. Second, for mRNAs regulated by multiple miRNAs, 3′-UTR recoding can prohibit one miRNA from functioning and facilitate regulation by another. Combine this with tissue-specific editing of miRNAs and 3′-UTRs and the wobble base pairing that would be afforded by the presence of I in the right structural environment: the result is the emergence of a spectacular new horizon in the miRNA world in which both miRNAs and their targets are edited for a vital cell function, switching gene expression off and on in response to specific physiological and developmental cues.

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