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miRNA Editing—We Should Have Inosine This Coming

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Abstract

In a recent issue of *Science*, Nishikura and colleagues provide the first evidence that editing of a microRNA (miRNA) precursor by ADARs can modulate the target specificity of the mature miRNA (Kawahara et al., 2007).

MicroRNAs (miRNAs) direct the translational repression and/or degradation of their target messenger RNAs (mRNAs) by base pairing with specific sites in the 3′ untranslated region of the message. Also, a small region near the 5′ end of the miRNA, termed the seed element, is important in defining target specificity (Bartel, 2004). Therefore, modifications to the seed element are expected to alter miRNA target specificity.

One mechanism by which a miRNA sequence could be altered is through adenosine-to-inosine (A-to-I) editing, a reaction catalyzed by adenosine deaminases that act on RNA (ADARs). As double-stranded RNA binding proteins (dsRBPs), ADARs bind their substrates based upon structure rather than sequence and compete with other dsRBPs for dsRNAs in vivo (Knight and Bass, 2002; reviewed in Nishikura [2006]). Since inosine acts like guanosine and preferentially base pairs with cytidine, editing can alter base pairing specificity. Consequently, several labs have investigated if ADARs edit miRNA precursors and whether this influences miRNA processing or alters target selection of mature miRNAs.

Editing of a miRNA precursor was first shown by Maas and colleagues (Luciano et al., 2004), who found evidence for A-to-I editing events in the primary transcript of miRNA-22 (pri-miR-22). Although low levels (~5%) of pri-miR-22 editing were found in several different tissues, no physiological role was elucidated. Next, 12 of 14 viral KSHV-miR-K12-10 clones sequenced by Tuschl and colleagues (Pfeffer et al., 2005) revealed an editing event in the miRNA seed element. They also detected the first edited mature miRNA (versus just a precursor), indicating that editing does not necessarily impede miRNA biogenesis. Subsequently, however, Nishikura and colleagues (Yang et al., 2006) demonstrated that editing of pri-miR-142 inhibits Drosha cleavage in vitro, raising the possibility that editing can alter processing of some miRNAs in vivo. Consistent with this idea, expression of an artificially edited (G substituted for I) pri-miR-142 in HEK293 cells led to an accumulation of the primary transcript and loss of mature miR-142. These authors also showed in vitro that edited pri-miR-142 can be degraded by Tudor-SN, an inosine-specific ribonuclease (Scadden, 2005) and member of the staphylococcal nuclease family. Since edited pri-miR-142 accumulated in vivo when HEK293 cells were grown in the presence of a staphylococcal nuclease inhibitor, the authors suggested that Tudor-SN is involved in a degradation pathway specific to at least some edited miRNA precursors. Lastly, Stratton and colleagues determined that 6 of 99 surveyed

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pri-miRNAs were found to be edited in at least one of ten human tissues (Blow et al., 2006). Furthermore, editing at specific adenosines was as high as 70% in some cases, providing the first evidence for high levels of editing at specific sites in mammalian pri-miRNAs.

The above papers clearly established that ADARs edit endogenous miRNAs but did not show that editing serves a biologically relevant function. In fact, if highly edited pri-miRNAs are degraded by Tudor-SN, one might suspect that they are unwanted cellular byproducts, which are "cleaned up" by this ribonuclease. Although results from the above experiments are largely suggestive of functional edited miRNAs, substantial support for this idea was lacking.

In a recent issue of *Science*, Nishikura and colleagues provide the first evidence that edited miRNAs have biological significance in vivo (Kawahara et al., 2007). Similar to previous studies, they identified mammalian miRNA precursors containing A-to-I editing. Unique to this study was the detection of editing within a mature mammalian miRNA (miR-376a). Intriguingly, the editing occurred within the seed element, suggesting that the editing event could redirect the miRNA to a new set of targets. A subset of these predicted targets was tested using a reporter assay, but, most importantly, the authors validated in vivo that the endogenous expression of PRPS1, a mouse protein involved in purine metabolism and uric acid synthesis and a predicted target of the edited miR-376a, was dependent upon ADAR2 and (by inference) edited miR-376a. Specifically, they showed that PRPS1 protein and uric acid levels were ~2-fold higher in the cortex of ADAR2$^{-/-}$ mice, a tissue in which they detected high levels of miR-376a editing. Their findings suggest that editing of miRNAs can modulate target specificity.

While Nishikura and colleagues provide the best evidence to date that edited miRNAs have a biological function, the story would benefit from experiments directly demonstrating that regulation of PRPS1 in ADAR2$^{-/-}$ mice is dependent on the edited miRNA. This is of particular importance, since the only endogenous edited miR-376a target evaluated in vivo was PRPS1. Future experiments might include demonstrating that PRPS1 is restored to wild-type levels in ADAR2$^{-/-}$ mice following expression of a miR-376a precursor that mimics editing using A-to-G substitutions. Also, since their model predicts that the regulation of both edited and unedited targets is dependent upon the components of the miRNA pathway, it is pertinent to show that PRPS1 regulation depends on these constituents. Given the uncertainty regarding the fate(s) of dsRNA containing inosine, and the little-understood role of Tudor-SN, these latter experiments seem particularly relevant.

One question emerging from these studies is whether the observed tissue and editing site specificities are related solely to ADARs or if other factors contribute. Furthermore, is editing within a given cell constitutive or regulated in response to stimuli? The notion of redirecting miRNAs implies that they are limiting in the cell. Thus, editing should not only decrease expression of the edited target but also increase expression of the unedited target, as editing of miRNAs would be expected to deplete the population of unedited miRNAs. Although this was not observed for the proposed target of unedited miR-376a (TTK) (Kawahara et al., 2007), the idea cannot be excluded without analysis of more potential targets. Alternatively, the role of editing may be simply to generate two pools of miRNAs with distinct sequences and targets in the cell.

How prevalent is editing within miRNAs? A number of groups are using deep sequencing methodologies to clone and sequence large populations of small RNAs. While the results are not yet in, the disproportionate number of edited miRNA precursors relative to edited mature miRNAs identified to date may suggest that edited mature miRNAs are rare. Perhaps the more common fate of edited miRNAs is either defective processing or degradation. Future studies

are needed to determine whether such fates constitute another level of miRNA regulation or highlight a control mechanism to protect against unwanted ADAR activity.

In summary, these studies suggest a new level of genetic diversity provided by ADAR editing. In addition to producing alternate protein isoforms through editing mRNA, ADARs also expand miRNA populations. These findings also suggest that ADARs may have helped shape the landscape of mammalian miRNAs during evolution by introducing a selective force on miRNA sequence and structure.

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