



THE UNIVERSITY *of* EDINBURGH

Edinburgh Research Explorer

ADARs have effects beyond RNA editing

Citation for published version:

Heale, BSE, Keegan, LP & O'Connell, MA 2009, 'ADARs have effects beyond RNA editing' *Cell Cycle*, vol 8, no. 24, pp. 4011-2. DOI: 10.4161/cc.8.24.10214

Digital Object Identifier (DOI):

[10.4161/cc.8.24.10214](https://doi.org/10.4161/cc.8.24.10214)

Link:

[Link to publication record in Edinburgh Research Explorer](#)

Document Version:

Publisher's PDF, also known as Version of record

Published In:

Cell Cycle

Publisher Rights Statement:

© 2009 Landes Bioscience

General rights

Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.



ADARs have effects beyond RNA editing

Bret S.E. Heale, Liam P. Keegan and Mary A. O'Connell*

MRC Human Genetics Unit; Institute of Genetics and Molecular Medicine; Western General Hospital; Edinburgh, UK

Adenosine in RNA is converted into inosine by members of a family of enzymes; ADARs (adenosine deaminases that act on RNA) (reviewed in ref. 1). Inosine base pairs like guanosine during translation; editing can result in a different amino acid being inserted at the editing site. Short, imperfectly paired stretches of double-stranded (ds)RNA formed within transcripts mediate site-specific RNA editing. However ADARs can also bind to and edit any long dsRNA as it is primarily the A-form dsRNA duplex that is recognized. When the proteins involved in RNA interference were identified many were found to contain dsRNA-binding domains similar to those found in ADARs. Since both ADAR-mediated RNA editing and RNA interference converge on dsRNA, do they act synergistically or antagonize each other?

One of the original studies proposed that 10% of mature miRNAs were edited.² From deep sequencing data obtained since then this number appears to be accurate. Editing of miRNAs was found to have diverse consequences; it can affect the processing, turnover and abundance of a miRNA or it can redirect a miRNA to target another transcript (reviewed in ref. 3).

To elucidate how ADARs could affect the microRNA pathway Heale⁴ and colleagues chose to study it in a well defined system; the human mir-376 cluster previously shown to be edited.⁵ Many of the miRNAs within this cluster have similar sequences so attention was focused on mir-376a2 as this is preferentially edited by ADAR2 at the +4 position and ADAR1 at the +44 position, both sites being within the seed sequences of mature miRNAs. The surprising finding was that ADAR2 interfered with the processing of pre-miRNA and that this is independent of editing activity. As depicted in Figure

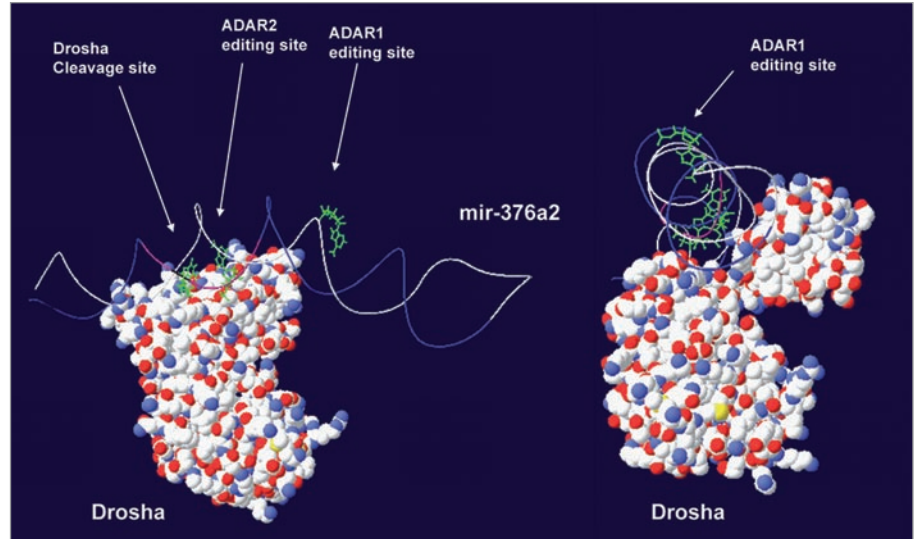


Figure 1. Model of DROSHA binding to mir-376a2, based on the crystal structure of Irc7.pdb and a Mfold prediction of mir-376a2.¹³ Green bases indicate nucleotides. Blue and purple ribbon is 5' half of mir-376a2 backbone. White Ribbon is 3' half of mir-376a2.

Do not distribute.

1, binding of ADAR2 to the +4 position hinders cleavage of the pre-miRNA by DROSHA as the editing and cleavage sites are very close. Since this occurs in the nucleus it is cleavage by DROSHA that is affected and not cleavage by DICER, a cytoplasmic protein. This result suggests that ADARs could affect the processing of more miRNAs than the 10% that were found to be edited as all pri- and pre-miRNAs have highly duplex structures that could potentially bind ADARs.

RNA editing has been reported in 17% of endogenous siRNAs in *Drosophila*.⁶ To determine if ADARs could also influence the siRNA pathway, transgenic flies were generated that expressed different *Drosophila* or human ADARs under the control of the GAL4/UAS system. These were crossed with flies expressing under GAL4/UAS control a long hairpin to the *white* gene that is exported from the nucleus and silences the pigmentation

in the fly eye so that the eyes are white. Coexpression experiments showed that only the cytoplasmic isoform of human ADAR1 p150 antagonizes the *white* hairpin-mediated silencing and partially restores the red eye pigment. Inactive ADAR1p150 restored the pigmentation to 40% of ADAR1p150 levels suggesting that binding alone can influence processing of long duplexes into siRNAs. Cleavage by DICER is thought to be affected as this antagonism occurs in the cytoplasm and DICER can partially suppress the effect of ADAR1 when coexpressed with it.

These results suggest that ADAR proteins have a wider influence than previously appreciated as most of the work on these proteins has focused on their editing activity rather than on potential roles as dsRNA-binding proteins. The likely significance of dsRNA-binding becomes more evident when one considers that two out of the four ADAR proteins present

*Correspondence to: Mary A. O'Connell; Email: M.OConnell@hgu.mrc.ac.uk

Submitted: 09/30/09; Accepted: 09/30/09

Previously published online: www.landesbioscience.com/journals/cc/article/10214

Comment on: Heale BS, et al. EMBO J 2009; In press.

in mammals are enzymatically inactive. Neither ADAR3 that is expressed in the brain nor TENR that is testis-specific have editing activity however both of these proteins contain dsRNA binding domains and deaminase domains and are highly conserved. Is the biological function of these inactive ADAR proteins to bind to and regulate specific transcripts or dsRNAs? In *Drosophila* there is also an ADAR homolog *DIP1*, that lacks the deaminase domain and, intriguingly, it is located near the beginning of transcripts through the X-centromere *flamenco* locus⁷ that is a major generator of piRNAs in *Drosophila*.

An interesting catalytically inactive ADAR1 is associated with a disorder found in two Japanese families. Loss of function mutations in human *ADAR1* are associated with autosomal dominant Dyschromatosis Symmetrica Hereditaria (*DSH1*) in Chinese and Japanese families. *DSH1* manifests itself as freckle-like, hyper- and hypo-pigmented regions on the hands and feet without any other obvious mental or physical effects. More than fifty mutations including many protein-truncation and frameshift mutations in *ADAR1* are associated with the pigmentation symptoms and the genetic dominance appears to be due to haploinsufficiency.⁸ One missense mutation however, *ADAR1*

G1007R in two families, is associated with more severe effects.⁹ Individuals with this mutation suffer, in addition to the skin pigmentation symptoms, progressive mental deterioration with dystonia, motor defects and calcium accumulation in the brain. Heale and colleagues demonstrate that this mutant protein binds to but does not edit dsRNA. The data are consistent with the idea that the mutant protein is a dominant negative ADAR1 that interferes with the function of the normal ADAR1, or the function of another protein that binds dsRNA, making the effects in these patients more severe.

The symptoms seen in the *ADAR1 G1007R* patients are consistent with a new finding in mice that ADAR1 is an important modulator of interferon signaling. Again, the effect may involve antagonism between ADAR1 and other proteins that recognize intracellular dsRNAs and trigger the interferon response. *ADAR1*^{-/-} mice die at embryonic day 11.5–12.5 with failure of liver haematopoiesis. Recently it has been reported that *ADAR1*^{-/-} mice fail to maintain haematopoietic stem cells (HSCs) in embryonic liver. Microarray analyses show increased activation of interferon-inducible genes in the *ADAR1*^{-/-} HSCs¹⁰ and interferon α and β levels are substantially elevated in mutant embryos. Mice genetically engineered to overproduce

α -interferon in astrocytes develop neurodegeneration and calcium accumulations in the brain.¹¹ Elevated interferon could also underlie the symptoms in the *ADAR1 G1007R* patients. Mental deterioration and brain calcifications sometimes arise in patients with lupus erythematosus, virus infections or other conditions involving Type I interferon expression in the brain.¹²

To conclude, binding of ADAR family proteins to duplex RNA per se may have a range of biological roles that are still to be discovered. So far it has been difficult to define the full range of RNAs that are edited by ADARs. It will indeed be a quest to identify all the RNAs that are bound but not edited by ADARs.

References

1. Valente L, et al. *Prog Nucleic Acid Res Mol Biol* 2005; 79:299-338.
2. Blow MJ, et al. *Genome Biol* 2006; 7:27.
3. Ohman M. *Biochimie* 2007; 89:1171-6.
4. Heale BS, et al. *EMBO J* 2009; In press.
5. Kawahara Y, et al. *Science* 2007; 315:1137-40.
6. Kawamura Y, et al. *Nature* 2008; 453:793-7.
7. Malone CD, et al. *Cell* 2009; 137:522-35.
8. Liu Q, et al. *Br J Dermatol* 2006; 154:636-42.
9. Tojo K, et al. *Mov Disord* 2006; 21:1510-3.
10. Hartner JC, et al. *Nat Immunol* 2009; 10:109-15.
11. Akwa Y, et al. *J Immunol* 1998; 161:5016-26.
12. Rigby RE, et al. *Bioessays* 2008; 30:833-42.
13. Błaszczak J, et al. *Structure* 2004; 12:457-66.