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miR-EdiTar: A database of predicted A-to-I edited miRNA target sites

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ABSTRACT

Motivation: A-to-I RNA editing is an important mechanism which consists of the conversion of specific adenosines into inosines in RNA molecules. Its dysregulation has been associated to several human diseases including cancer. Recent work has demonstrated a role for A-to-I editing in microRNA (miRNA) mediated gene expression regulation. In fact, edited forms of mature miRNAs can target sets of genes that differ from the targets of their unedited forms. The specific deamination of mRNAs can generate novel binding sites in addition to potentially altering existing ones.

Results: This work presents miR-EdiTar, a database of predicted A-to-I edited miRNA binding sites. The database contains predicted miRNA binding sites that could be affected by A-to-I editing and sites that could become miRNA binding sites as a result of A-to-I editing.

Availability: miR-EdiTar is freely available online at http://microrna.osumc.edu/mireditar.

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1 INTRODUCTION

A-to-I editing is an essential post-transcriptional mechanism common to all eukaryotes. This form of editing is catalyzed by enzymes of the Adenosine Deaminase Acting on RNA (ADAR) family and results in the conversion of single adenosines into inosines which are recognized as guanosines by various cellular machineries (Bass 2002). This can affect splicing and alter coding and non-coding sequences in RNA molecules, thus contributing to the diversity of the transcriptome (Rueter et al 1999; Yang et al. 2008). Alterations of A-to-I editing have been associated to several human diseases, such as infections, neurological diseases and cancer (Maas et al. 2006; Dominissini et al. 2001; Gallo and Locatelli 2012).

A-to-I editing can also influence miRNA-mediated gene regulation (Nishikura 2010). Several cases of A-to-I editing of miRNA precursors have been reported (Kawahara et al. 2007; Alon et al. 2012). This phenomenon can suppress processing by Drosha and Dicer and the presence of inosines in the mature sequences can alter the recognition of their target sites (Yang et al. 2006).

A-to-I editing is most abundant in the 3' UTR regions of the human transcriptome (Athanasiadis et al. 2004; Levanon et al. 2004). This could affect existing miRNA binding sites as well as generate novel binding sites (Liang and Landweber 2007).

The importance of RNA editing in miRNA activity suggests the need for computational tools to predict and analyze the effects of RNA editing on miRNA-mediated regulation. This work presents miR-EdiTar, a database of predicted A-to-I edited miRNA binding sites. In this paper we describe the database and suggest some plausible scenarios of the involvement of editing in miRNA activity.

2 RESULTS

2.1 Prediction of A-to-I edited miRNA binding sites

We collected 1139 human 3' UTR sequences with 10,571 total A-to-I editing sites from the DARNED database (Kiran and Baranov 2010) and used the computational method miRiam (Laganà et al. 2010) to predict miRNA-target interactions that involve the edited sites. miRiam makes use of empirical binding rules and thermodynamics features, such as the structural accessibility of the target site and the energy of the miRNA/target duplex. We performed the predictions on the complete set of 1922 human miRNA sequences

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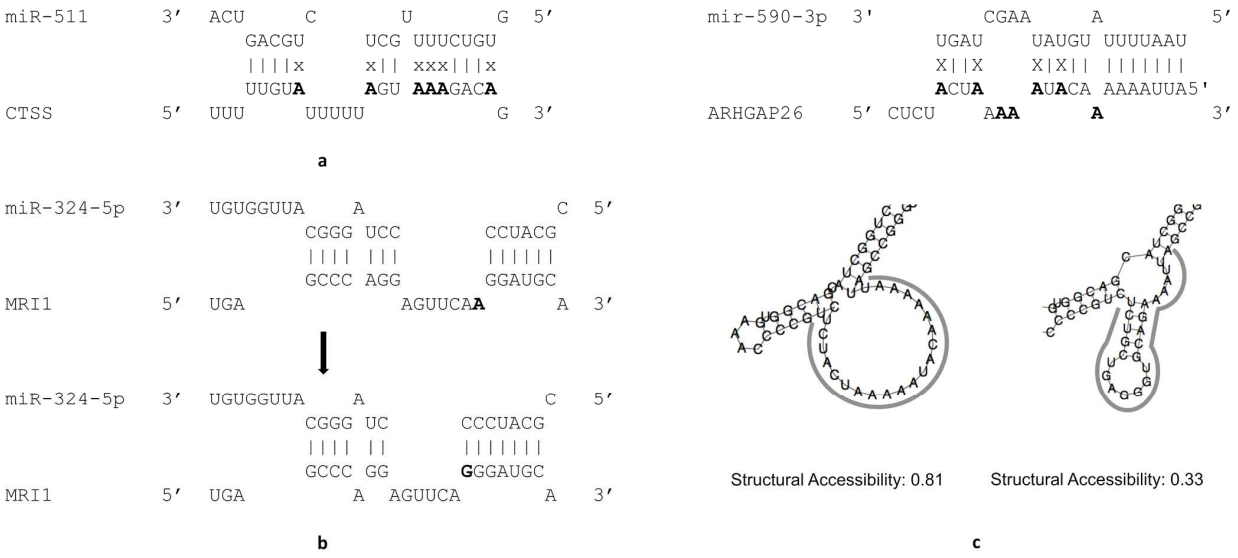

Fig. 1. Examples of predicted miRNA binding sites potentially affected by A-to-I editing. (a) Predicted binding site for miR-511 in the 3' UTR of CTSS. (b) An edited adenosine in a potential binding site for miR-324-5p on the 3' UTR of MRI1 may improve the seed match by adding an extra CG bond and changing the type from 7mer-A1 to 8mer. (c) Example of variation of structural accessibility of predicted miRNA binding sites affected by A-to-I editing. The estimated structural accessibility of a predicted binding site for miR-590-3p in the 3' UTR of the gene ARHGAP26 decreases by 40% due to editing events. The predicted interactions are shown along with the secondary structures of the un-edited and edited versions of the binding sites.

retrieved from miRBase Release 18 (Kozomara and Griffiths-Jones 2011). The duplexes were then classified into two categories, depending on whether the edited adenosines were located on a miRNA seed binding region or not. Seed matches were classified as 6mer, 7mer-A1, 7mer-m8 and 8mer, as in Bartel (2009).

The descriptive statistics are summarized in the supplementary material.

2.2 miR-EdiTar contents and web interface

miR-EdiTar contains a collection of predicted human miRNA binding sites in A-to-I edited 3' UTR sequences. The database contains two kinds of sites: (i) "current" sites are sites that are predicted to be miRNA binding sites but could be affected by A-to-I editing, and (ii) "novel" sites are sites that are not predicted to be miRNA binding sites but could become miRNA binding sites as a result of A-to-I editing.

The Website can be searched by miRNA and/or by target. Given a miRNA, the list of its predicted targets is shown in a box. When a target is selected, the corresponding interaction details are displayed on a table and available for download in csv format. The binding sites are grouped into two categories based on their type (current sites or novel sites). Several data elements are provided, such as the position of the binding site on the UTR, the seed type, the free energy of the duplex, the structural accessibility degree, the interaction score and the duplex structure. The edited bases are highlighted in bold characters and the corresponding alignment pipes are replaced with an X, indicating the potential disruption of the corresponding bond. In the case of current sites, an entry indicates if the edited bases are located in the seed region. Moreover, the values of seed type, free energy, accessibility, interaction score and duplex structure are provided for both the edited and the unedited forms of the site. Similar results can be obtained by choosing a target from the list and then selecting one of its predicted miRNAs.

Check boxes can be used to filter the results visualized. In particular, users can choose to filter the interactions based on the type of predicted site (current or novel), the fact that the seed region is edited or not, the type of seed match (6mer, 7mer-A1, 7mer-m8 and 8mer) and the energy of the duplex.

Finally, miR-EdiTar is connected to miRo', a web environment which provides users with miRNA functional annotations inferred through their validated and predicted targets (Laganà et al. 2009).

More details on the data and the methods used can be found in the supplementary material.

3 DISCUSSION

The modifications of predicted miRNA binding sites are classified into two categories, based on whether the editing events occur in the seed region or in another part of the duplex. The replacement of adenosines with inosines in the seed region can change A-U matches into G-U wobbles which are sometimes tolerated, especially in the presence of compensatory matches elsewhere in the duplex, but which have been reported to weaken the interaction or even abrogate the binding (Brennecke et al. 2005) (Fig. 1a, Fig. S1a). Editing events which occur outside of the seed binding region could also influence the targeting. They might either reduce

the stability of the duplex, through the introduction of G-U wobbles and mismatches, or increase it by improving the seed match or by creating new matches outside the seed area (Fig. 1b, Fig. S1b, c).

The presence of inosines in miRNA binding sites could also alter their secondary structure and, as a consequence, increase or reduce the chance of binding. It has been demonstrated that SNPs can significantly change mRNA secondary structure (Shen et al. 1999; Halvorsen et al. 2010) and that changes in secondary structure can significantly affect the binding of miRNAs (Kertesz et al. 2007; Haas et al. 2012). Therefore, it is plausible that editing events may yield similar effects (Fig. 1c, Fig. S2).

Other than affecting existing miRNA binding sites, A-to-I editing can generate novel miRNA/target interactions by either changing mature miRNA sequences or creating new sites on UTRs, as already reported by a few studies (Kawahara et al. 2007, Borchert et al. 2009). In supplementary material we show that deamination of the 3' UTR of the gene MDM4 could generate a novel binding site for miR-500a-3p.

All these hypotheses and preliminary experiments suggest a new layer of dynamic regulation in miRNA-mediated gene expression control and encourage further investigations. We plan to update the database with new editing sites and new predictions as soon as new data is available, as well as consider other types of RNA editing, like C-to-U editing. Moreover, since editing sites in DARNED do not necessarily reflect editing events as they occur in mRNAs, future work will also include the implementation of an EST-based filter in order to refine the miRNA binding sites predictions. Finally, we plan to include data about the editing of miRNA sequences along with their affected and newly created predicted targets, in addition to data on tissue specificity of both miRNAs and editing events.

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