RNA Editing

Unexplored Opportunities in the Cardiovascular System

Shizuka Uchida, Steven P. Jones

Increasing usage of next-generation sequencing technology will illuminate the extent of RNA modifications, which may affect various pathophysiological conditions. Here, we will highlight one such category of RNA modification called RNA editing, which can be detected from RNA sequencing data by simply modifying the data analysis pipeline using bioinformatics methods.

Life on our planet depends on RNA. Despite our early understanding of RNA as an enabling messenger, it is not simply a static reflection of genetic activation. Indeed, the life span of RNA is more complex than previously thought. On transcription from the DNA template, RNA can be modified by various enzymes, which results in over 100 RNA modifications. Because of the potential involvement of RNA modifications in a variety of pathophysiologies, epitranscriptomics is quickly gaining momentum, and our gaps in understanding epitranscriptomics require further study.[1]

RNA editing is a post-transcriptional modification to alter the sequence of RNA molecules.[2] RNA editing of exons of protein-coding genes may lead to the production of an amino acid sequence that differs from the original DNA sequence. In addition, editing of 3′-untranslated regions may affect binding of RBPs (RNA-binding proteins) or microRNAs (miRNAs), thereby modulating RNA stability or translation. There are 2 types of RNA editing: adenosine to inosine (A-to-I) and cytidine to uridine (C-to-U); A-to-I is the more common form. Furthermore, RNA editing can be identified in RNA sequencing (RNA-seq) data without any special treatment before the sequencing library construction. A-to-I RNA editing occurs through RNA-editing enzymes called ADARs (adenosine deaminases acting on RNA), which convert adenosine in double-stranded RNA into inosine. In humans, there are 3 ADARs (*ADAR1*, *ADAR2*, and *ADAR3*). In mice, the deletion of *Adar1* and *Adar2* (also known as *Adarb1* [adenosine deaminase, RNA-specific, B1]) is embryonically and postnatally lethal, respectively,[3,4] highlighting the importance of RNA editing in normal physiology. Human *ADAR3* (also known as *Adarb2* [adenosine deaminase, RNA-specific, B2]) is considered to be catalytically inactive and less important in RNA editing.[5] In humans, mutations in *ADAR1* have been associated with dyschromatosis symmetrica hereditaria and Aicardi–Goutières syndrome[6]; however, whether the expression of ADARs changes in various cardiovascular conditions remains unknown.

RNA Editing Sites Are Found Mostly in Nonprotein-Coding Regions

Although A-to-I editing occurs at the level of RNA, when reverse transcribed to cDNA, an inosine is converted to guanine (G). This A-to-G conversion can be identified by comparing to the reference genome as an evidence for RNA-editing sites. Many studies detected RNA-editing events from RNA-seq data, including recent study in endothelial cells and atherosclerosis.[7] Because of the detection in RNA-seq data, several databases for RNA-editing events have been constructed to provide evidence for the frequency of RNA editing in various conditions. When the content of one of such database called RADAR[8] is examined for humans, ≈89% of RNA-editing sites are found in introns of both protein-coding genes and noncoding RNAs (ncRNAs) followed by ≈5% in intergenic regions that include long intergenic ncRNAs, ≈4% in 3′-untranslated region, ≈1% in exons of ncRNAs, and <1% in 5′-untranslated region. Surprisingly, only 0.17% of RNA-editing sites are found in exons of both protein-coding genes and ncRNAs, which do not code for amino acids. This distribution of RNA-editing sites agrees with previous studies that examined RNA-editing sites in humans, where they occur mostly in *Alu* repetitive elements as they form double-stranded RNA, which ADARs bind to.[9] Thus, RNA-editing sites are found mostly in nonprotein-coding regions of the human transcriptome.

RNA-Editing Events in the Cardiovascular System Are Underexplored

There are only 3 publications that examined RNA editing in diseased hearts. The first study reported increased RNA-editing events in *MED13* (gene encoding a component of the mediator complex) in cyanotic congenital heart disease patients compared with acyanotic patients.[10] Another recent study reported that cathepsin S (*CTSS*), which encodes a cysteine protease associated with angiogenesis and atherosclerosis, is highly edited.[7] Such RNA editing enables the recruitment of stabilizing RBP HuR (human antigen R; encoded by *ELAVL1*) to the 3′-untranslated region of *CTSS* transcript, thereby controlling *CTSS* mRNA stability and expression. ADAR1 levels and the extent of CTSS RNA editing are associated with changes in CTSS levels in patients with atherosclerotic vascular diseases, including subclinical atherosclerosis, coronary artery disease, aortic aneurysms, and

From the Cardiovascular Innovation Institute (S.U.) and Institute of Molecular Cardiology, Diabetes and Obesity Center, Division of Cardiovascular Medicine, Department of Medicine (S.P.J.), University of Louisville, KY.

Correspondence to Shizuka Uchida, PhD, Cardiovascular Innovation Institute, University of Louisville, 302 E Muhammad Ali Blvd, Louisville, KY 40202. E-mail heart.lncrna@gmail.com

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advanced carotid atherosclerotic disease. Above 2 studies are of mammalian hearts, whose adult cardiomyocytes have a very limited capability of regeneration. Interestingly, in the newt hearts, whose cardiomyocytes can be regenerated on injury, the increased expression of *adar1* mRNA was observed and nucleocytoplasmic shuttling of the adar1 protein in the regeneration zone of regenerating newt heart, suggesting that RNA editing might be necessary for cardiac regeneration in newts.[11] However, there is no study yet that comprehensively compared the expression of ADARs and RNA-editing events in the cardiovascular system under various conditions, including pathological conditions.

Detecting RNA-Editing Events From RNA-Seq Data

RNA editing can be detected by comparing the sequencing reads from RNA-seq data to the reference genome by examining A-to-G nucleotide changes present only in the read sequence but not in the genomic DNA. Currently, many bioinformatics tools can identify RNA-editing sites from RNA-seq data (reviewed here[12]), including our RNAEditor bioinformatics tool.[13] Although it sounds rather straightforward to identify A-to-G mismatches to the reference genome, a set of filters must be applied to remove biases arising from the sequencing technique (eg, sequencing errors, misalignment of reads, PCR duplicates during library preparation leading to misinterpretation of RNA-editing sites) and genomic variations (eg, single-nucleotide polymorphisms). In addition, the Short Genetic Variations database (dbSNP), a public domain archive for sequence variations and the primary source for the filter separating RNA-editing sites from known SNPs, contains the sequence variations from both genomic DNAs and cDNAs (eg, generated during RNA-seq).[12] Thus, the computational pipeline for the detection of RNA-editing sites is complicated by the propensity for errors and extensive hardware time. Furthermore, how to normalize the identified RNA-editing sites have not been well established, which makes it difficult to compare the number of RNA-editing sites observed in one sample to another.

Most recent comprehensive analysis of various human tissues for RNA-editing sites indicates that transcripts in human hearts are edited.[14] Given that many cardiac RNA-seq data have been published and several bioinformatics tools are available, it is straightforward to conduct a large-scale bioinformatics analysis of published RNA-seq data to identify condition-dependent RNA-editing events. In this context, our RNAEditor tool will be of great help as this tool takes an FASTQ file as input and reports the frequency of RNA-editing sites and islands, which is the term we coined to describe the highly edited sites in the given loci. Our previous study indicates that RNA-editing islands overlap to ADAR1-bound peaks from RNA immunoprecipitation assay followed by next-generation sequencing (RIP-seq) data using anti-ADAR1 antibody.[15] Because these bioinformatics tools are readily available, there is an urgent need to profile RNA-editing events in the cardiovascular system under various conditions.

Concluding Remarks

Because of the unprecedented availability of genetic tools, it is now possible to edit an individual's genome. For example, using CRISPR (clustered regularly interspersed short palindromic repeats)/Cas9 (clustered regularly interspaced short palindromic repeat–associated 9), permanent change to the human genome is possible; however, there are many concerns with, and limitations to, CRISPR/Cas9 technology, which leaves open the prospects for improvement. Given the potential for greater flexibility, improved specificity, and non-genomic DNA sequence editing, application of RNA-editing (particularly ADAR-mediated editing with newer Cas varieties)[16] technology has the potential to extend further our armamentarium of genetic tools, and such possibilities warrant further study for potential eventual applications in correcting heritable disease traits.

Compared with other fields (eg, cancer, immunology), cardiovascular research has lagged considerably in its examination of RNA-editing events. The first step in understanding the cardiovascular editome is to perform a large-scale reanalysis of published RNA-seq data to identify the expression patterns of ADARs and frequencies of RNA-editing events in diseased hearts. Because the source of RNA-editing events is known (ie, the enzymatic activity of ADARs to convert adenosine into inosine), the effects of RNA-editing events may be investigated by conducting studies in mice deficient in *Adar1* and *Adar2* (Figure).[3,4] Furthermore, there are many overexpression constructs readily available for ADARs that have been tested in other fields. As reported, the effect of RNA-editing

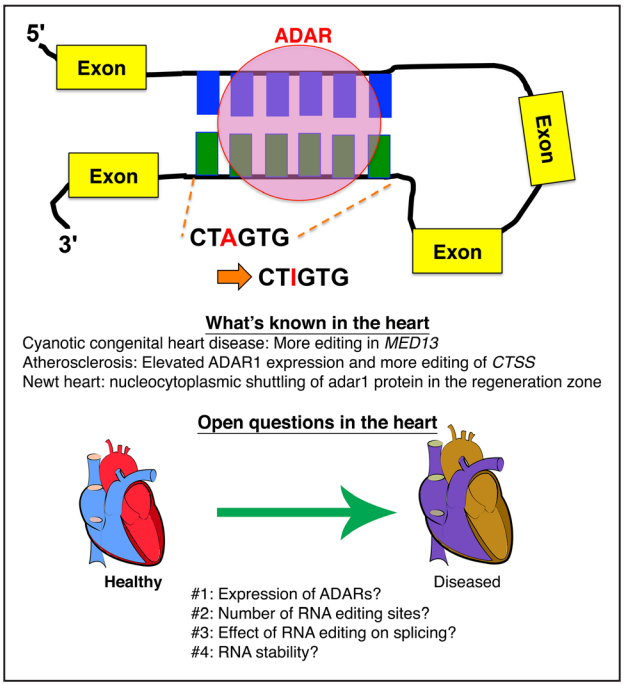

Figure. The effects of RNA editing. ADARs (adenosine deaminases acting on RNA) preferentially bind double-stranded RNA and convert adenosine (A) into inosine (I). On transcription to cDNA, inosine is converted to guanine (G) in RNA, which is not present in the corresponding genomic DNA. In diseased human hearts, more RNA-editing sites have been recorded for some genes, whereas in the regenerating newt heart, the adar1 protein is localized more in the nucleus of regenerating cardiomyocytes than in the cytoplasm. Because of lack of comprehensive studies of RNA editing in the heart, more systematic research is needed to uncover the roles of ADARs and the effects of RNA editing in cardiovascular disease.. *CTSS* indicates cathepsin S.

events in the vasculature is prominent[7]; however, it is not yet clear whether RNA editing plays a role in cardiomyocytes. Thus, more functional studies are needed to elucidate the pathophysiological roles of ADARs in cardiomyocytes to understand the impact of RNA-editing events in the heart and associated disease.

Given that a majority of RNA-editing sites are found in nonprotein-coding regions, especially in introns, an open question remains (Figure): what is the function of RNA-editing sites in nonprotein-coding regions? One possible answer is that RNA editing may alter alternative splicing patterns via modification of splicing acceptor and donor sites. By modulating splicing, RNA editing may result in diversifying protein isoforms from a single gene. Given that alternative splicing is known to be altered in diseased hearts compared with the healthy ones, by studying RNA-editing patterns, it might be possible to dissect splicing patterns further related to cardiovascular disease.

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