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Structural and functional effects of inosine modification in mRNA

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ABSTRACT

Inosine (I), resulting from the deamination of adenosine (A), is a prominent modification in the human transcriptome. The enzymes responsible for the conversion of adenosine to inosine in human mRNAs are the ADARs (adenosine deaminases acting on RNA). Inosine modification introduces a layer of complexity to mRNA processing and function, as it can impact various aspects of RNA biology, including mRNA stability, splicing, translation, and protein binding. The relevance of this process is emphasized in the growing number of human disorders associated with dysregulated A-to-I editing pathways. Here, we describe the impact of the A-to-I conversion on the structure and stability of duplex RNA and on the consequences of this modification at different locations in mRNAs. Furthermore, we highlight specific open questions regarding the interplay between inosine formation in duplex RNA and the innate immune response.

Keywords: inosine; RNA modification; RNA structure; RNA function; A-to-I editing; ADARs

INTRODUCTION

The term epitranscriptome refers to all the post-transcriptional biochemical transformations (e.g., methylation, acetylation, and deamination) in constituent nucleosides of RNA molecules that do not involve changes in the RNA sequence (Wang et al. 2017; Zhao et al. 2017; Rosselló-Tortella et al. 2020). Inosine (I) is one of the most widespread forms of RNA modification and is a product of the deamination of adenosine (A) (Wang et al. 2017; Dutta et al. 2022; Sun et al. 2023). The enzymes responsible for inosine production in mRNAs are the double-stranded RNA (dsRNA)-specific adenosine deaminases (ADARs) and duplex structure is required at sites of inosine modification in mRNA (Wang et al. 2017; Mendoza and Beal 2023). ADARs catalyze the hydrolytic deamination of adenosine (Fig. 1A). This reaction proceeds by activation of a water molecule by binding to a zinc ion in the ADAR active site for direct nucleophilic attack on the purine, forming a tetrahedral intermediate. This is followed by ejection of ammonia from the intermediate and formation of inosine (Haudenschild et al. 2004). Conversion of A-to-I by this mechanism makes the formation of inosine in RNA essentially irreversible. The direct reverse reaction is highly unlikely given the vanishingly low concentration of ammonia (in comparison to water) in biological systems. Indeed, there are no known natural processes that directly convert inosine back to adenosine in RNA (i.e., no known inosine "erasers"). However, once generated in an RNA in living cells, inosine at that specific nucleotide position is not permanent since RNAs are subject to constant turnover by nuclease degradation and regeneration by transcription. Thus, the amount and persistence of inosine at a specific position in a cellular RNA is a function not only of the activity of the adenosine deaminase responsible but also the half-life of that RNA. While not the focus of this Perspective, inosine is also found in the anticodon loops of several mammalian tRNAs where it can modulate decoding specificity. The enzymes responsible for the incorporation of inosine in tRNAs are referred to as ADATs (adenosine deaminases acting on tRNA) (Torres et al. 2014).

Critically, the A-to-I conversion changes the hydrogen bonding potential of the edited nucleobase from a *donor–acceptor* Watson–Crick face in adenosine to an *acceptor–donor* face in inosine (Fig. 1A). Hence, it follows that the conversion of adenosine to inosine in an RNA duplex is accompanied by alteration in duplex structure and stability, which can have far-reaching implications for RNA processing and functionality. Two catalytically active forms of ADARs, ADAR1 and ADAR2, are the key players in facilitating dsRNA-specific adenosine deamination in humans (Melcher et al. 1996). ADAR1 also exists as two abundant

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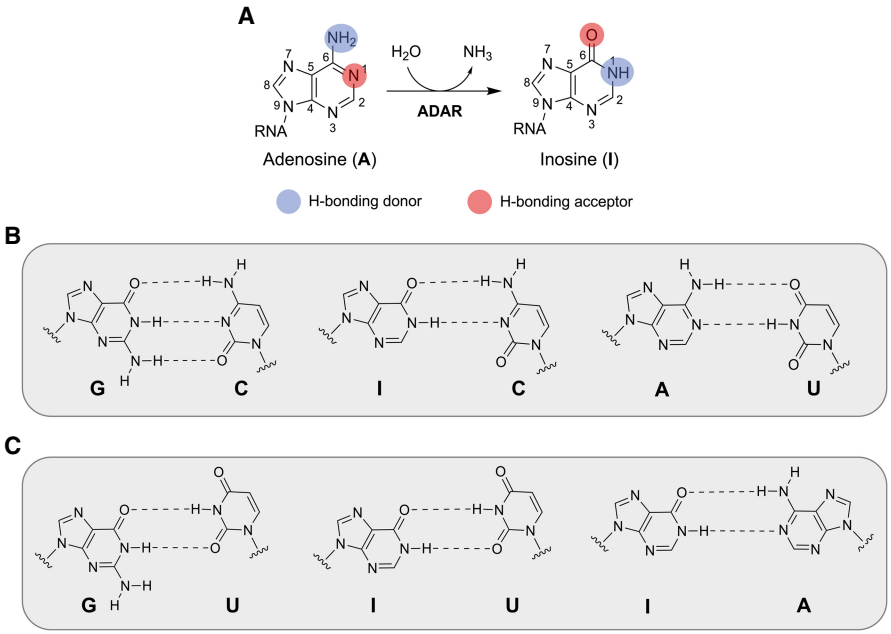

FIGURE 1. (*A*) ADAR-catalyzed deamination of adenosine to inosine in RNA changes the H-bonding potential of the modified nucleobase. (*B*) Hydrogen bonding patterns for G–C, I–C, and A–U Watson–Crick base pairs. (*C*) Hydrogen bonding patterns for G–U, I–U, and I–A wobble base pairs. H-bonds are shown as dashed lines.

isoforms: the constitutively expressed ADAR1 p110 and the interferon (IFN)-inducible ADAR1 p150 (Patterson and Samuel 1995; George and Samuel 1999). ADARs are primarily localized in the nucleus except for ADAR1 p150, which shuttles between the nucleus and cytoplasm (Poulsen et al. 2001). ADAR-catalyzed deamination reactions occur at millions of sites in the human transcriptome, predominantly in noncoding regions (Eisenberg and Levanon 2018). However, inosine modifications can also be found in coding regions, often leading to recoding events (Picardi et al. 2017). Hence, ADAR-mediated adenosine deamination is also considered a form of RNA editing.

The study of inosine generation in mRNA by ADARs not only expands our understanding of RNA biology but also offers a promising avenue for therapeutic interventions, as dysregulation in these processes has been implicated in various diseases, including cancers and neurological and immune disorders (Gallo et al. 2017; Baker and Slack 2022). In this Perspective, we focus on the impact of the A-to-I conversion on the structure and stability of duplex RNA and on the consequences of this modification at different locations in mRNAs.

EFFECT OF INOSINE ON RNA DUPLEX STRUCTURE AND STABILITY

The incorporation of inosine into RNA can have profound effects on RNA duplex structure and stability due to its unique base-pairing properties. Among the four standard ribonucleotides, inosine is closest in structure to guanosine (G); hence, like guanosine, inosine also preferentially base-pairs with cytidine (C) (Fig. 1B). However, the absence of the N2 amino to O2 carbonyl H-bonding pair in I–C renders an RNA duplex bearing this base pair at an internal position to be ~2.0 kcal/mol less stable than the same duplex with a G–C pair (Wright et al. 2018). With uridine (U), inosine can also form a base pair that is isosteric to a G–U wobble pair (Fig. 1C). However, although both base pairs can form two H-bonds, an RNA duplex containing an internal I–U pair is substantially less stable than the same duplex with a G–U pair (difference of ~1.9 kcal/mol) (Wright et al. 2007). Thus, the exocyclic amine group in guanosine can substantially contribute to stability, possibly due to enhanced base-stacking interactions and/or improved hydration (Serra et al. 2004; Wright et al. 2007).

Both ADAR1 and ADAR2 have a general preference for deaminating adenosines in an A–C mismatch (Wong et al. 2001), which leads to the formation of the more stable I–C base pair. Indeed, replacing an A–C with an I–C pair in the center of a Watson–Crick RNA helix results in helix stabilization by an average of 4.1 kcal/mol (Wright et al. 2018). Deamination of adenosines in an A–U Watson–Crick base pair (Fig. 1B) can also take place (Wong et al. 2001), which leads to the production of the noncanonical I–U pair. Although both pairs can assume two H-bonds, the shift in conformation from an A–U to an I–U pair gives rise to a substantial decrease in the thermodynamic stability of the RNA duplex. Indeed, duplexes with an internal I–U pair have been shown to be ~2.3 kcal/mol less stable compared to their A–U pair counterparts (Wright et al. 2007). The

significant reduction in stability may be attributed to a number of factors, including the loss of favorable base-stacking interactions, induction of unfavorable helix distortion, and weaker H-bonding interactions in an I–U pair compared to an A–U pair (Wright et al. 2007).

Consistently, a decrease in duplex RNA stability has also been noted upon an increase in the number of I–U pairs within the duplex (Serra et al. 2004; Wright et al. 2007; Špačková and Réblová 2018). Furthermore, Westhof and colleagues have shown that tandem I–U pairs are remarkably destabilizing compared to tandem A–U (or G–U) pairs (Serra et al. 2004). They found that a model 8 bp RNA duplex with two adjacent I–U pairs in the center had a melting temperature 21°C lower than the corresponding duplex with tandem A–U pairs and 24°C lower than the duplex with tandem G–U pairs. Thus, the conversion of the adenosine in an A–U pair to inosine generating an I–U pair is highly duplex destabilizing, particularly when multiple A–U to I–U modifications are localized at a specific site on the duplex. Importantly, the vast majority (>99%) of ADAR-mediated adenosine deamination events occurs at multiple clustered sites in long, complementary (A–U pair rich) Alu repeat sequences (Athanasiadis et al. 2004; Levanon et al. 2004; Bazak et al. 2014). Deamination of these targets results in substantial destabilization due to the creation of closely spaced I–U pairs. These observations are consistent with the original proposed function of ADARs in unwinding double-helical RNA structures (Bass and Weintraub 1988; Wagner et al. 1989). Interestingly, parallel analysis of RNA secondary structures sequencing (PARS-seq) revealed a notable decrease in the global dsRNA to single-stranded RNA (ssRNA) ratio in ADAR1 knockdown cells, signifying the overall impact of ADAR1-mediated adenosine deamination in the stabilization of a large subset of imperfect (likely A–C pair containing) RNA duplexes (Solomon et al. 2017). This observation may be unexpected at first glance, given the large fraction of A-to-I modification sites found in A–U rich Alu repeat sequences which are mostly attributed to ADAR1 editing activity (Levanon et al. 2004). However, since editing at these sites is very low (<1%) (Bazak et al. 2014), duplex destabilization due to ADAR1-mediated editing of these targets does not appear to significantly impact the global RNA secondary structure content (Solomon et al. 2017). These authors did note, however, that Alu repeat sequences were enriched in cellular RNAs that showed higher levels of duplex structure in ADAR1 knockdown cells (Solomon et al. 2017).

BIOLOGICAL CONSEQUENCES OF A-TO-I MODIFICATION IN mRNA

Since inosine modification clearly influences the structure and stability of RNA duplexes, it also has the potential to influence any biological process that relies on sequence-specific base-pairing interactions as well as duplex structure-specific interactions with the RNA. A-to-I editing can have distinct functional consequences depending on where the modification occurs within an mRNA molecule (Fig. 2A). As stated earlier, the Alu repeat sequences are the major sites for most of the A-to-I events in the human transcriptome (Athanasiadis et al. 2004; Levanon et al. 2004; Bazak et al. 2014). These transcripts arise from *Alu* transposable elements that are often inserted in the genome as inverted copies of itself (i.e., inverted repeats) and hence can form nearly perfect intramolecular duplexes that are ideal substrates for ADARs. Alu repeat sequences are prevalent in the noncoding regions of mRNAs, particularly in the intronic and 3′ untranslated regions (UTRs), making these sites the primary hotspots for inosine modification (Athanasiadis et al. 2004).

Among the different mRNA components, introns have one of the highest inosine contents (Peng et al. 2012) where it can have an impact in splicing regulation (Reuter et al. 1999; Lev-Maor et al. 2007; Parada et al. 2014; Tang et al. 2020). ADAR-mediated A-to-I editing can modulate mRNA splicing by creating new or destroying existing splice sites—an important mechanism for generating novel protein isoforms. For instance, ADAR2 regulates its own expression by editing its own pre-mRNA, thereby creating an alternative splice acceptor site (Reuter et al. 1999). The role of inosine modification in alternative splicing, particularly in exon inclusion, is also exemplified in A-to-I editing-dependent conversion of a noncanonical GU–AA splice site to a canonical GU–AI splice site in the NRK (Nik-related protein kinase) transcript (Parada et al. 2014). This editing event results in the inclusion of a 42 nt exon in the mature mRNA. Moreover, exon 8 of the human NARF (nuclear prelamin A recognition factor) transcript is exonized upon A-to-I editing of the Alu-containing intronic region between exon 7 and exon 9 (Lev-Maor et al. 2007). Finally, ADAR1-mediated editing at an intronic site in the CCDC15 (coiled-coil domain containing 15) transcript promotes binding of SRSF7 (serine/arginine-rich splicing factor) which then blocks the inclusion of CCDC15 exon 9 (Tang et al. 2020).

Inosine modification in the untranslated regions of mRNA transcripts rarely takes place in the 5′ UTRs but is very common in the 3′ UTRs, which harbor more self-complementary regions likely due to high Alu repeat sequence content. In general, the structural effects brought about by inosine modification in these mRNA elements affect recognition or accessibility by *trans*-acting factors, including RNA-binding proteins and other regulatory noncoding RNAs (Nakano et al. 2016; Stellos et al. 2016; Brümmer et al. 2017; Solomon et al. 2017). In particular, the introduction of inosine in mRNA, especially in the 3′ UTR, can result in the modulation of miRNA target sites. Analysis of the A-to-I editome across human populations revealed that ADAR-mediated editing at 3′ UTR stabilizes RNA secondary structures which in turn reduces the accessibility of miRNAs associated with AGO2 (Argonaute 2) in these regions

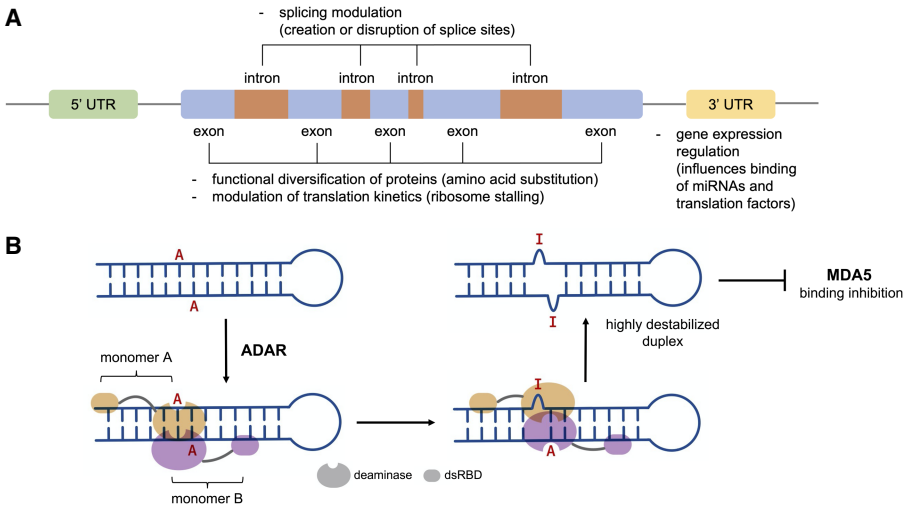

FIGURE 2. (*A*) Biological consequences of inosine modification at specific locations in the mRNA. (*B*) Proposed editing of an ADAR dimer at tandem A–U sites. Editing leads to the formation of a highly destabilized duplex which can no longer bind and activate the dsRNA sensor, MDA5. (dsRBD) dsRNA binding domain.

(Brümmer et al. 2017). ADAR1 also facilitates the editing of a site in the 3′ UTR of AhR (aryl hydrocarbon acceptor) transcript, creating a miR-378 target site (Nakano et al. 2016). In addition, ADAR1-mediated A-to-I editing destabilizes an Alu-containing stem–loop in the 3′ UTR of CTSS (cathepsin S) transcript, enabling the recruitment of the HuR (human antigen R) protein. HuR binding at the 3′ UTR of CTSS consequently regulates mRNA stability and expression (Stellos et al. 2016). As described above, analysis of translation profiles and PARS-seq data showed that genes whose transcripts are destabilized by A-to-I editing, mainly at the 3′ UTR, are frequently up-regulated in ADAR1 knockdown samples (Solomon et al. 2017). These results further establish the importance of inosine modification in gene expression regulation by defining 3′-UTR mRNA structures that modulate the binding of translation factors in these regions.

While A-to-I editing events are primarily observed in the noncoding regions of mRNAs, thousands of sites have also been identified in coding regions (Picardi et al. 2017). Due to similarities in structure and base-pairing properties, inosine modifications in coding sequences are often read as guanosine during translation. Hence, A-to-I editing in exons can result in amino acid substitutions (i.e., recoding) that may alter protein function. However, in the human transcriptome, there are <100 sites known where efficient recoding (>20%) is observed (Nishikura 2016; Gabay et al. 2022; Keegan et al. 2023). Furthermore, for most of these sites, no information is available on the impact the recoding event has on the function of the encoded protein. This has been established for a handful of sites, however. For instance, inosine is common in transcripts coding neurotransmitter receptors and ion channels where recoding occurs and is known to be functionally significant (Sommer et al. 1991; Higuchi et al. 1993, 2000; Burns et al. 1997; Fitzgerald et al. 1999; Niswender et al. 1999). A-to-I editing of the GluR-B (glutamate receptor B) transcript at the Q/R site catalyzed by ADAR2 results in a glutamine (Q) to arginine (R) substitution at the protein level, leading to a reduction of permeability of this ion channel to calcium ions (Sommer et al. 1991; Higuchi et al. 1993, 2000). Another prominent example of inosine-dependent recoding is the 5-HT_{2C}R (5-hydroxytryptamine receptor 2C) transcript, where A-to-I conversion at five sites in exon 5 (sites A–E) regulates the receptor's G-protein coupling activity (Burns et al. 1997; Fitzgerald et al. 1999; Niswender et al. 1999). A-to-I recoding has also been characterized for the DNA repair enzyme NEIL1 (Nei-like DNA glycosylase 1) (Yeo et al. 2010), transcription factor GLI1 (glioma-associated oncogene 1) (Shimokawa et al. 2013), actin-binding protein FLNB (filamin B) (Chan et al. 2014), and cell proliferation regulator AZIN1 (antizyme inhibitor 1) (Chen et al. 2013). More effort is needed to fully define the impact of recoding in other transcripts and the role this type of regulation plays in the function of the target proteins.

While inosine is primarily decoded as guanosine during translation, it is important to note that this decoding process is not entirely strict. As described earlier, both inosine and guanosine can form a Watson–Crick or wobble base pair with cytidine or uridine, respectively (Fig. 1B). However, inosine can additionally form a wobble base pair with adenosine (Fig. 1C). Indeed, a recent study using an in vitro translation reporter system coupled with mass spectrometry revealed that while inosine is generally interpreted as guanosine, it can also be decoded as adenosine, and more rarely as uridine (Licht et al. 2019). This lack of absolute fidelity in inosine decoding allows for additional flexibility in the genetic code and contributes to the potential diversity in the translated protein sequence. Interestingly,

inosine has also been found to affect translation efficiency by causing ribosome stalling, especially in the presence of multiple inosines in the codon (Licht et al. 2019). This finding is consistent with the significant reduction in translation rates observed upon introduction of inosines within two distinct, proximal codons of the 5-HT_{2C}R transcript (C- and D-sites) and the complete translation inhibition when two inosines were incorporated in one codon of the 5-HT_{2C}R transcript (A- and B-sites) (Hoernes et al. 2018). Hence, while A-to-I editing of mRNAs enhances genetic flexibility, this could potentially be offset by a reduction in translation efficiency.

INOSINE AND THE INNATE IMMUNE RESPONSE

Inosine also plays a significant role in the innate immune response by influencing the stability of RNA duplex structures, thereby modulating the binding and activity of innate immune sensors. dsRNAs are a common feature of viral infection which are recognized by cytoplasmic dsRNA sensors such as MDA5 (melanoma differentiation-associated protein 5) (Wu et al. 2013). This subsequently triggers a series of events leading to the activation of an IFN-mediated immune response. However, as mentioned earlier, the human transcriptome is abundant in Alu repeat sequences which form long, nearly complementary (i.e., A–U rich) dsRNA structures that are potential ligands for MDA5 (Ahmad et al. 2018; Mehdipour et al. 2020; Levanon et al. 2023). To prevent unintended immune activation, endogenous dsRNAs (self) are distinguished from viral dsRNAs (nonself) by ADAR-mediated A-to-I editing (Mannion et al. 2014; Liddicoat et al. 2015). In other words, the incorporation of inosine alters the structure and stability of endogenous dsRNAs to the point where they are no longer recognizable by MDA5 (Fig. 2B). Indeed, several studies provided strong evidence of the role of A-to-I editing activity of the cytoplasmic ADAR1 p150 isoform in preventing MDA5 sensing of endogenous dsRNAs as nonself (Mannion et al. 2014; Liddicoat et al. 2015; Pestal et al. 2015).

MODULATING A-TO-I EDITING FOR DISEASE INTERVENTION

The importance of inosine in influencing dsRNA immunogenicity and maintaining immune homeostasis is further demonstrated in the involvement of aberrant A-to-I editing in a substantial number of autoimmune and inflammatory disorders as well as cancers (Gallo et al. 2017; Baker and Slack 2022). Following our discussion above, autoimmune and inflammatory disorders are believed to arise from under-editing of endogenous immunogenic dsRNAs, leading to accumulation of these RNAs and activation of MDA5-mediated immune response. Indeed, mutations that reduce ADAR1 editing activity were found to cause Aicardi–Goutières syndrome (AGS), an autoimmune disorder characterized by elevated IFN levels and encephalopathies (Rice et al. 2012). On the other hand, cancer cells have been found to utilize ADAR1 hyper-editing of endogenous dsRNAs to suppress the immune system (Paz-Yaacov et al. 2015). This essential role of ADAR1 in cancer cell survival has recently been demonstrated in the increased sensitivity of tumor cells to immunotherapy upon loss of function of ADAR1 (Ishizuka et al. 2019). Thus, A-to-I editing inhibition is emerging as a promising approach for the treatment of cancers.

However, critical open questions remain regarding the interplay between inosine formation in dsRNA and the innate immune response. First, what are the identity and structural features of the endogenous dsRNA ligands that trigger the immune response via MDA5? Second, what is the cytoplasmic load or threshold of these immunogenic dsRNA targets that is required for immune activation? Third, for endogenously expressed RNAs that are particularly effective MDA5 activators unless modified by ADAR1, how, precisely, does the ADAR reaction block MDA5 activation by those RNAs (i.e., what are the sites of A-to-I editing on those RNAs and how does inosine incorporation at those specific sites prevent activation of MDA5 by that RNA?). Answers to these questions will aid in the deeper understanding of disorders associated with immune signaling and will facilitate the development of therapeutic strategies targeting MDA5 interaction with immunogenic dsRNAs. On another note, Alu repeat sequences, which are potential MDA5 ligands, contain multiple A-to-I editing sites that are in proximity to each other as previously described (Athanasiadis et al. 2004; Levanon et al. 2004; Bazak et al. 2014). Recent crystal structures of ADAR2 bound to dsRNA revealed the formation of a dimer with the deaminase domain of one monomer engaged in catalysis and the other deaminase domain involved in protein–protein interactions at the dimer interface. Interestingly, the active site of the "noncatalytic" monomer is within 20 Å of the nucleotide in the base pair adjacent to the editing site and on the opposite strand (Thuy-Boun et al. 2020; Doherty et al. 2022). Thus, one can easily imagine the deaminase domains in an ADAR dimer swapping roles in the complex to react with closely spaced adenosines on opposing strands (Fig. 2B). Therefore, it will be interesting to see whether ADAR dimerization plays a role in facilitating editing at clustered sites and whether disrupting dimerization can serve as a strategy for regulating dsRNA immunogenicity.

Dysregulated A-to-I editing is also implicated in cancer and neurological disorders through mechanisms that are different from those discussed above. In line with its effect on protein recoding, the NEIL1 K242R mutation arising from a recoding inosine modification in the NEIL1 transcript has been found to reduce the enzyme's DNA repair activity, leading to increased cell proliferation and colony formation in multiple myeloma (Teoh et al. 2018). Since A-to-I editing at 3′ UTRs can influence miRNA target sites, multiple

studies have demonstrated the importance of this mechanism in regulating the stability and expression of critical cancer genes (Nakano et al. 2016, 2017; Jiang et al. 2019). Meanwhile, reduced ADAR2 editing activity has been associated with brain disorders such as Alzheimer's disease, amyotrophic lateral sclerosis (ALS), schizophrenia, and bipolar disorder (Hideyama et al. 2012; Gaisler-Salomon et al. 2014; Kubota-Sakashita et al. 2014). Given the context-dependent role of inosine modification in the pathology of these diseases, inhibition, activation, or enhancement of A-to-I editing can be utilized as strategies for disease treatment in a case-to-case basis.

To date, no FDA-approved ADAR editing modulatory drugs are available in the market. However, some published reports suggest the use of small molecule nucleoside analogs of adenosine as ADAR editing inhibitors (Hang et al. 2010; Zipeto et al. 2016; Ding et al. 2020; Ramírez-Moya et al. 2020). For example, 8-azaadenosine (8-aza-A) has been used as an inhibitory tool compound to evaluate ADAR function in chronic myeloid leukemia and thyroid cancer (Zipeto et al. 2016; Ramírez-Moya et al. 2020). However, a follow-up study using an extensive set of control experiments has shown that this nucleoside analog does not specifically inhibit ADAR (Cottrell et al. 2021). Recently, in vitro deamination studies using a dsRNA substrate mimic of ADAR bearing the adenosine analog 8-azanebularine (8-azaN) showed selective inhibition of ADAR1 but not ADAR2. The nucleoside monomer by itself was established to be noninhibitory; however, incorporating it in a dsRNA structure effectively increased inhibitory activity (Mendoza et al. 2023). Precise inhibition of specific A-to-I editing events has also been observed with the use of antisense oligonucleotides (ASOs) that specifically bind to ADAR transcript targets, effecting a change in RNA structure that prevents ADAR recognition and subsequent editing (Mizrahi et al. 2013; Tay et al. 2021). ADARs are also currently being utilized to implement specific A-to-I editing events at specific locations in the transcript via the process called site-directed RNA editing (SDRE) (Khosravi and Jantsch 2021; Diaz Quiroz et al. 2023; Pfeiffer and Stafforst 2023). SDRE uses antisense oligonucleotides complementary to therapeutically relevant target sites to form the duplex structure required for ADAR recruitment and editing. Apart from reversing disease-causing G-to-A mutations in the transcript, SDRE also has the potential to suppress nonsense mutations by targeted inosine modification at premature termination codons (PTCs), as recently applied in PTCs causing the neurological disorder Rett syndrome (Doherty et al. 2022; Brinkman et al. 2023; Jacobsen et al. 2023).

CONCLUDING REMARKS

As we learn more about the various modified nucleotides present in mRNA, common questions arise regarding the modification sites and frequency, the identity, structures, and mechanisms of the enzymes responsible, the functional impact of the modification, and the extent to which human health is affected by perturbation of the modification pathway. In many ways, inosine stands as a useful exemplar for the epitranscriptomics field since a rich body of literature has developed around answering each of these questions for inosine. Indeed, we are at a point now in the study of inosine in mRNA where clear causal links between changes in ADAR activity and human disease have been established, new tools to direct inosine formation for therapeutic purposes are being perfected, and drugs that bind to and modulate the activity of the ADAR enzymes are being developed. Nevertheless, knowledge gaps exist in our understanding of the biological impact of inosine in mRNA; for instance, in precisely how A-to-I changes at specific locations in immunostimulatory duplex RNAs block MDA5 activation, and how recoding regulates the activity of many of the affected proteins. Future studies will bring answers to these questions and tell us whether ADAR-based therapeutics can be made safe and effective.

COMPETING INTEREST STATEMENT

P.A.B. is a consultant and equity holder for the RNA editing therapeutics company ProQR Therapeutics.

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