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REVIEWS

Review

The Editor's I on Disease Development

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Adenosine-to-inosine (A-to-I) editing of RNA leads to deamination of adenosine to inosine. Inosine is interpreted as guanosine by the cellular machinery, thus altering the coding, folding, splicing, or transport of transcripts. A-to-I editing is tightly regulated. Altered editing has severe consequences for human health and can cause interferonopathies, neurological disorders, and cardiovascular disease, as well as impacting on cancer progression. ADAR1-mediated RNA editing plays an important role in antiviral immunity and is essential for distinguishing between endogenous and viral RNA, thereby preventing autoimmune disorders. Interestingly, A-to-I editing can be used not only to correct genomic mutations at the RNA level but also to modulate tumor antigenicity with large therapeutic potential. We highlight recent developments in the field, focusing on cancer and other human diseases.

Highlights

Adenosine deaminase acting on RNA (ADAR)1 expression is largely correlated with tumor progression and has been identified as a new promising target for cancer immunotherapy.

It has recently emerged that RNA editing plays a pivotal role in coronary heart disease.

Double-stranded RNA sensors such as MDA5 provide innate immunity against several viral infections; however, dysregulation of RNA sensing can lead to autoimmune disorders.

A-to-I RNA editing is tightly controlled and regulated at multiple levels.

Site-directed RNA editing may develop into RNA therapeutics against human disease.

A-to-I Editing and ADAR Enzymes

High-throughput sequencing and mass spectrometry have identified massive and diverse RNA modifications which have bolstered the idea that gene expression can be regulated at the transcript level [1,2]. **A-to-I editing** (see Glossary) is a prevalent type of RNA modification during which adenosines in double-stranded (ds) or structured RNAs are deaminated, leading to nucleotide differences between RNA and DNA. Inosine is primarily interpreted as guanosine by the cellular machinery but can also be decoded as adenosine or uridine [3,4]. Hence, adenosine deamination can affect coding potential and translation efficiency, change splice sites,or mask endogenous RNAs from the innate immune system [1,4]. In humans, editing occurs at millions of sites, mostly in noncoding parts of the transcriptome such as introns and untranslated regions (UTRs) [5–7]. By contrast, editing in protein-coding regions of transcripts is relatively rare, and only about 40 such sites are conserved in mammals [8].

A-to-I editing is catalyzed by the **adenosine deaminase acting on RNA (ADAR)** family of enzymes which require dsRNA for binding and editing. Two catalytically active ADARs (ADAR1 and **ADAR2**) are expressed in mammals. ADAR3 is a third member of the family but is catalytically inactive [9]. ADAR1 is expressed in two isoforms. A shorter isoform (**ADAR1-p110**) is expressed from a constitutively active promoter, whereas a longer isoform (**ADAR1-p150**) is interferon (IFN)-inducible [10]. Mice lacking ADAR1 die at embryonic (E) day 12.5 accompanied by an elevated IFN response [11–13]. Lethality can be rescued when cytoplasmic sensors of (viral) dsRNAs are also deleted, suggesting that inosines help to discriminate 'self' from 'non-self' dsRNA [14–16]. *Adar2* knockout mice die within 3 weeks after birth [17]. This phenotype can be rescued when a pre-edited allele of the glutamate receptor *Gria2* gene is coexpressed, demonstrating that ADAR2-mediated editing of *Gria2* is essential [18].

We review the role of A-to-I editing in disease, including its impact on cancer progression and innate immunity. We also discuss recent advances in site-directed editing which can be used to repair genomic mutations at the RNA level, and how this technology may pave the way towards the establishment of new RNA-based drugs.

Keeping ADARs under Control: Regulation of A-to-I Editing

A-to-I editing is tightly regulated, and deregulation of editing is associated with neurological disorders and some cancers [19–22]. Conceptually, A-to-I editing can be regulated by (i) altering the expression and activity of ADARs, (ii) changing the subcellular localization of ADARs, (iii) competition between RNA-binding proteins and noncoding RNAs that alter target accessibility, and (iv) by competing RNA modifications.

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(i) Analysis of **genotype tissue expression (GTEX)** RNA-seq data from hundreds of human tissue-derived datasets shows a moderate correlation (R = 0.55) of overall editing levels with the expression of ADAR1 and ADAR2 when the negative impact of ADAR3 on editing is subtracted [23]. However, the moderate correlation suggests additional regulatory mechanisms. AIMP2 may be the strongest negative regulator affecting ADAR protein stability [23]. Similarly, the E3 ubiquitin ligase WWP2 targets ADAR2 for degradation [24]. Conversely TDP-43 enhances ADAR1 expression (Figure 1A) [22].

(ii) ADAR1-p110 and ADAR2 preferentially localize to the nucleus. However, ADAR1-p110 shuttles between the nucleus and the cytoplasm [25]. Different stimuli lead to increased cytoplasmic localization; these include binding to dsRNA and stress-induced phosphorylation of ADAR1 which may help cells to cope with stress (Figure 1B) [26,27]. Within the nucleus, ADAR activity is regulated by nucleolar sequestration [28,29]. The importance of correct localization is exemplified by a group of patients with amyotrophic lateral sclerosis (ALS) and frontotemporal dementia where ADAR2 partially localizes to the cytoplasm, causing alterations in editing patterns [30].

(iii) Numerous RNA-binding proteins alter editing in a site-specific manner, likely by altering the accessibility of ADAR to its target or by changing the secondary structure of the target (e.g., helicases) [22,31,32]. The fragile X proteins FMRP and FXR1P are interesting examples. FMRP promotes site-specific editing whereas FXR1P inhibits editing, which may in part explain the global hypoediting observed in individuals with autism spectrum disorder (ASD) (Figure 1C) [21,22,33]. Similarly, small noncoding RNAs can alter A-to-I editing. The small nucleolar (sno)RNA HBII-52 seemingly interferes with editing of the serotonin receptor *HTR2C* transcript

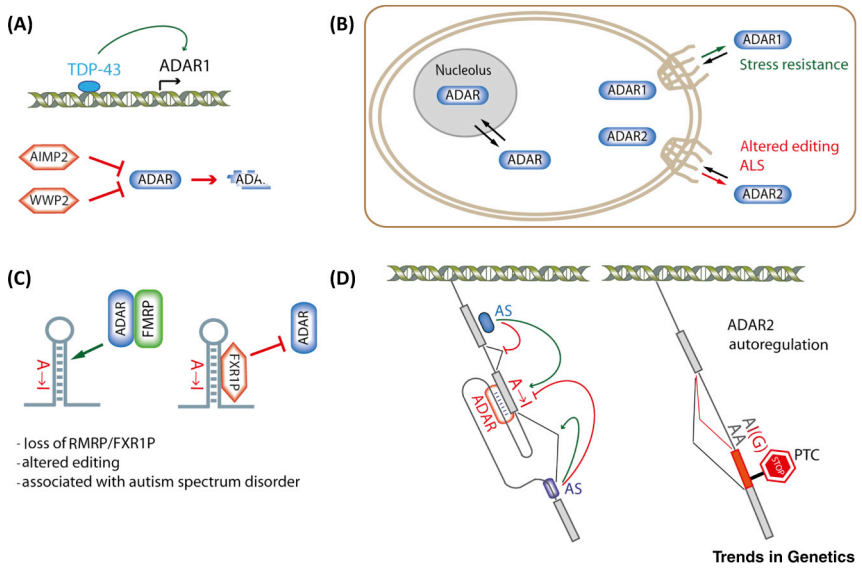

Figure 1. A-to-I Editing Is Regulated at Multiple Levels.
(A) TDP-43 enhances adenosine deaminase acting on RNA (ADAR)1 transcription, whereas AIMP2 and WWP2 promote protein degradation. (B) ADAR1 shuttles between the nucleus and the cytoplasm. Enhanced cytoplasmic localization helps the cell to cope with stress. ADAR2 localizes to the nucleus. Aberrant cytoplasmic localization has been linked to amyotrophic lateral sclerosis (ALS). (C) Several factors either promote (e.g., FMRP) or reduce (e.g., FXR1P) site-specific A-to-I editing. (D) Both A-to-I editing and mRNA splicing occur cotranscriptionally. Thus, alternative splicing (AS) factors can either increase A-to-I editing by reducing the splicing efficiency or decrease editing by increasing the efficiency of splicing. Furthermore, A-to-I editing affects splice sites. For instance, ADAR2 levels are autoregulated via alternative splicing: efficient editing promotes the use of an alternative 3′ splice site, leading to inclusion of a premature stop codon (PTC) and thereby decreasing the levels of functional ADAR2 protein.

Glossary

Adenosine deaminase acting on RNA (ADAR): three proteins (ADAR1, ADAR2, and ADAR3) are known in humans.
ADAR1-p110: a constitutively expressed isoform of ADAR1; localizes mostly to the nucleus.
ADAR1-p150: a longer isoform of ADAR1 expressed from an interferon-inducible promoter upstream of the promoter driving ADAR1-p110 expression; preferentially localizes to the cytoplasm.
ADAR2: the second active ADAR, edits a variety of targets in the coding region leading to amino acid exchanges.
Aicardi–Goutières syndrome (AGS): a severe autoimmune disorder mostly affecting newborns and infants; several mutations in ADAR1 are linked to AGS.
A-to-I editing: adenosine-to-inosine editing catalyzed by ADAR deaminases.
Filamin A (*FLNA*) mRNA: a protein-coding editing substrate that is edited in exon 42 of the pre-mRNA leading to a Gln to Arg amino acid exchange.
Genotype tissue expression (GTEX): a collection of RNA-seq datasets from over 700 human donors and samples for ~50 different tissues.
Guide RNA: the RNA used to direct ADAR towards a specific target for site-directed editing; not to be confused with CRISPR guide RNAs.
Mitochondrial antiviral-signaling protein (MAVS): the receptor for MDA5; amplifies the antiviral signal recognized by MDA5 or similar pattern recognition proteins.
Melanoma differentiation antigen 5 (MDA5): encoded by gene *IFIH1*, MDA5 senses viral dsRNA and unedited endogenous dsRNA. Loss of MDA5 rescues the ADAR1 lethality phenotype.
Off-target editing: editing events outside the intended targeted editing site.
RIG1-like receptors (RLRs): a group of cytoplasmic pattern recognition receptors that are similar to retinoic acid-inducible gene I (RIG1) and sense intracellular viral RNA.
Site-directed RNA editing (SDRE): A-to-I editing approaches

in the nucleolus [34]. Conversely, expression of HBII-52 is decreased in patients with Prader–Willi syndrome, while editing levels of the *HTR2C* transcript are increased [35]. Interestingly, mice solely expressing the fully edited HTR2C protein isoform exhibit characteristics of Prader–Willi syndrome [36]. However, the mechanism underlying how snoRNA HBII-52 affects A-to-I editing is unclear. It is possible that HBII-52-guided methylation may affect RNA structure and thereby editing. Alternatively, the snoRNA could affect editing by changing the alternative splicing of *HTR2C* (see below and [35]).

(iv) The regulation of editing via modifications in the target RNA is less well explored. *In vitro* data demonstrate that both adenine methylation at the 6 position (m6A) and 2′O-methylation prevent editing by ADARs [37,38]. Moreover, depletion of m6A leads to increased A-to-I editing, suggesting a negative correlation between the two types of modification [39]. However, there is currently no indication that methylation *in vivo* directly prevents editing. Likely, structural changes introduced by the m6A modification affect the binding of ADAR [39]. Nevertheless, it might be interesting to test how other abundant RNA modifications affect the editing landscape.

that aim at repairing RNAs downstream of genomic mutations.
The Cancer Genome Atlas (TCGA): a database of SNPs and expression data from human cancer samples.

Interplay of A-to-I Editing and mRNA Processing

The majority of editing takes place cotranscriptionally, presumably on nascent RNA [40,41]. Consistently, A-to-I editing is particularly prevalent in intron regions of transcripts [5]. In addition, exonic editing sites frequently require intronic elements to form editing-competent RNA duplexes [42–44] (Figure 1D). In these cases A-to-I editing needs to take place before mRNA splicing [45]. In line with this, A-to-I editing modulates alternative splicing for a large set of transcripts [41,46–48]. A particularly interesting example is the autoregulation of ADAR2 [46]: ADAR2 edits an AA dinucleotide to an AI dinucleotide within its own pre-mRNA. This leads to the creation of a novel 3′ spice site (AI = AG) leading to inclusion of an additional 47 nt in the open reading frame, and ultimately to the creation of a truncated and probably unstable ADAR2 protein [46] (Figure 1D). Similarly, an A-to-I editing event in the tyrosine phosphatase PTPN6 pre-mRNA converts a branch-point adenosine into inosine and prevents splicing. This leads to intron retention and ultimately to the generation of a nonfunctional protein, which may play a role in acute myeloid leukemia [49].

Similarly, only edited *Gria2* pre-mRNA is spliced efficiently, and unedited pre-mRNA is retained in the nucleus [18,47,48] (Figure 1D). Moreover, ADAR proteins can interfere with pre-mRNA processing independently of A-to-I editing [50]. For instance, ADAR1 can compete with 3′-UTR processing factors and thereby influence transcript length [51]. ADAR proteins can also reduce circular RNA expression [52,53]. It is assumed that ADARs increase the rate of 'backsplicing' required for circular (circ)RNA biogenesis by binding to these reverse complementary sequences, editing these sequences, and thereby decreasing their stability, which in turn reduces circRNA biogenesis [53].

Most interestingly, the efficiency of splicing can determine the extent of editing. Transcripts that are less efficiently spliced exhibit higher editing levels [54,55]. Consistently, poorly spliced transcripts and alternatively spliced pre-mRNAs are edited more frequently [40]. Excitingly, splicing efficiency can contribute to the regulation of tissue-specific editing, as revealed by analysis of GTEx data [55]. Collectively, editing levels are controlled cotranscriptionally. Consequently, proteins binding to pre-mRNA or influencing splicing are potent regulators of editing [22,31,32].

ADARs and Cancer

A-to-I editing affects the progression and metastasis of some cancers. Systematic analysis of RNA-seq data from **The Cancer Genome Atlas (TCGA)** showed that A-to-I RNA editing is dysregulated in 17 different cancer types relative to normal tissues [56]. Interestingly, the majority of these tumor samples showed tumor-specific hyperediting, and only a small fraction of tumors showed hypoediting [56] (Figure 2 and Table 1, Key Table). Overall, editing levels correlated with the expression levels of ADAR1 but not of ADAR2. Moreover, ADAR1 overexpression was found to be a common phenomenon in many tumors [56–59]. By comparing tumor subtype, stage, and survival rate, a series of

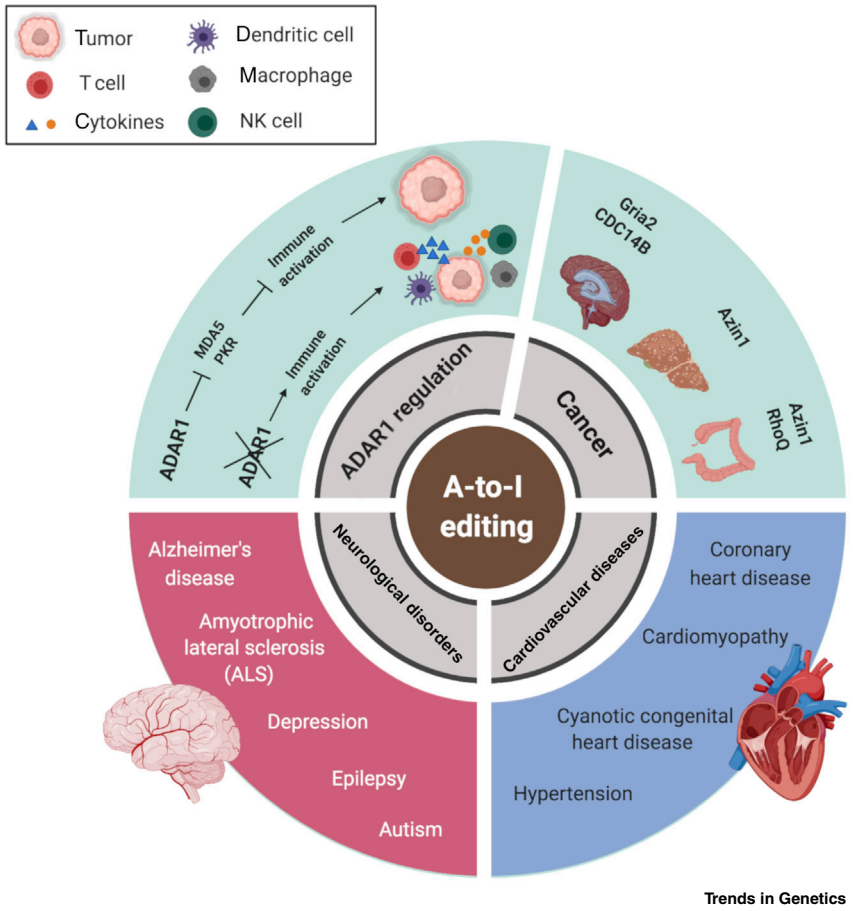

Figure 2. Impact of Adenosine Deaminases Acting on RNA (ADARs) on Disease Development.
Misregulated ADARs can promote tumorigenesis. For instance, *AZIN1* editing is increased in hepatocellular carcinoma. Cardiovascular diseases such as cardiomyopathy and hypertension are crucially affected by ADAR1 and ADAR2. Editing levels are altered in neurological diseases such as Alzheimer's, amyotrophic lateral sclerosis (ALS), depression, epilepsy, and autism. In tumors, ADAR1-mediated RNA editing leads to suppression of RNA sensors (MDA5 and PKR), thereby inhibiting the immune response and thus favoring tumor growth. Conversely, an absence of ADAR1-mediated RNA editing activates the immune response and tumor infiltration by cytotoxic T cells, natural killer (NK) cells, and other immune cells, thus impeding tumor growth and survival.

clinically relevant editing sites could be identified that were mostly located in nonrepetitive noncoding RNAs, or within conserved mRNAs, leading to nonsynonymous codon changes. Most interestingly, several of these editing events can promote cell growth and survival [56].

RNA editing levels can show a positive or negative correlation with cancer progression, but the functional consequences of these editing events for tumor progression are poorly understood (Table 1). Nevertheless, for some substrates such as transcripts encoding antizyme inhibitor 1 (Azin1), RhoQ, the actin-crosslinking protein podcalyxin-like (PODXL), the cell-cycle regulator CDC14B, and the DNA glycosylase NEIL1, their direct involvement in cellular migration, cell division, or DNA repair is consistent with a uniform alteration in editing patterns across different tumors [56,60–64] (Figure 2 and Table 1). Other editing events, such as in bladder cancer-associated protein (BLCAP) transcripts, can increase or decrease in different cancers, possibly because they are a target for both ADAR1 and ADAR2 [65]. Similar differences in editing patterns were found for GABRA3 mRNA that shows editing in nonmetastatic primary human breast cancer samples but not in metastatic counterparts [66].

Key Table

Table 1. List of Coding Editing Targets Implicated in Cancer Development

Gene	Editing site	Full name	Enzyme	Type of regulator	Type of cancer	Refs
Azin1	S367G	Antizyme inhibitor 1	ADAR1	Positive	Hepatocellular carcinoma Esophageal squamous cell carcinoma Colorectal cancer	[45,64]
Gabra3	I342M	GABA receptor α3	ADAR1	Negative	Breast cancer	[66]
Gria2	Q607R	Glutamate ionotropic receptor AMPA-type subunit 2	ADAR2	Negative	Glioblastoma	[82]
PODXL	H241R	Podocalyxin-like	ADAR2	Negative	Gastric cancer	[61]
CDC14B		Cell division cycle 14B	ADAR2	Negative	Astrocytoma Gliobastoma multiforme	[60]
RhoQ	N136S	Ras homolog family member Q		Positive	Colorectal cancer	[63]

Until recently, RNA editing in cancer has been monitored at the RNA level. For the first time, Peng *et al*. studied the effects of A-to-I RNA editing at the proteomic level by using mass spectrometry, integrating TCGA and Clinical Proteomic Tumor Analysis Consortium (CPTAC) data, and reported highly significant effects in breast cancer [67].

Role of ADAR1 in Immunity

Loss-of-function mutations in human ADAR1 are associated with the severe autoimmune disorder **Aicardi–Goutières syndrome (AGS)** that is accompanied by elevated expression of IFN-stimulated genes (ISGs) [68]. Deletion of mouse *Adar1* causes early embryonic lethality as a result of elevated levels of IFN and ISG expression [11,12]. Simultaneous deletion of genes encoding the cytoplasmic sensor of viral dsRNA, **melanoma differentiation antigen 5 (MDA5)**, or its downstream interaction partner **mitochondrial antiviral signaling protein (MAVS)** can rescue the embryonic lethality of ADAR1 null mice until birth [14–16]. These studies show that ADAR1 activity antagonizes the MDA5–MAVS signaling pathway and helps to distinguish self from non-self. Consistently, I:U mismatched dsRNA has been shown to prevent the type I IFN response through their ability to bind to and inhibit **RIG1-like receptor (RLR)** proteins such as MDA5 and RIG1 [69] (Figure 3).

MDA5 forms a filament-like structure on dsRNA and induces MAVS oligomerization via their caspase activation and recruitment domain (CARD) regions [70]. Recent studies suggest that MDA5 multimerizes along base-paired human *Alu* and viral sequences, and that this could be prevented by A-to-I editing [71–73] (Figure 3). Interestingly, selective deletion of the IFN-inducible p150 isoform of ADAR1 is crucial for antagonizing MDA5 signaling, and cannot be rescued by the constitutive p110 version of ADAR1 [15,74]. Creation of a catalytically dead version of ADAR1 has also shown that catalysis is necessary to antagonize MDA5 signaling [16]. Whether ADAR1-p150 has a different substrate repertoire from its p110 counterpart and how editing prevents activation of innate immune signaling remains to be established [75,76].

ADAR-induced inosines in dsRNA not only suppress MDA5–MAVS signaling but also antagonize activation of the antiviral sensor protein kinase R (PKR) [75,76]. This activity was recently shown to control the immune checkpoint in IFN-positive tumors. Such tumors were found to be crucially dependent on ADAR1 [76,77]. In the absence of ADAR1, tumors were infiltrated by cytotoxic T cells and natural killer cells, and infiltration was dependent on type I and type II IFN secretion. Lack of ADAR1 and the concomitant elevation of IFN signaling stimulate the expression of MDA5 and PKR (Figure 3). In a CRISPR/Cas9 knockout screen, PKR was crucially involved in the attraction of immune cells towards

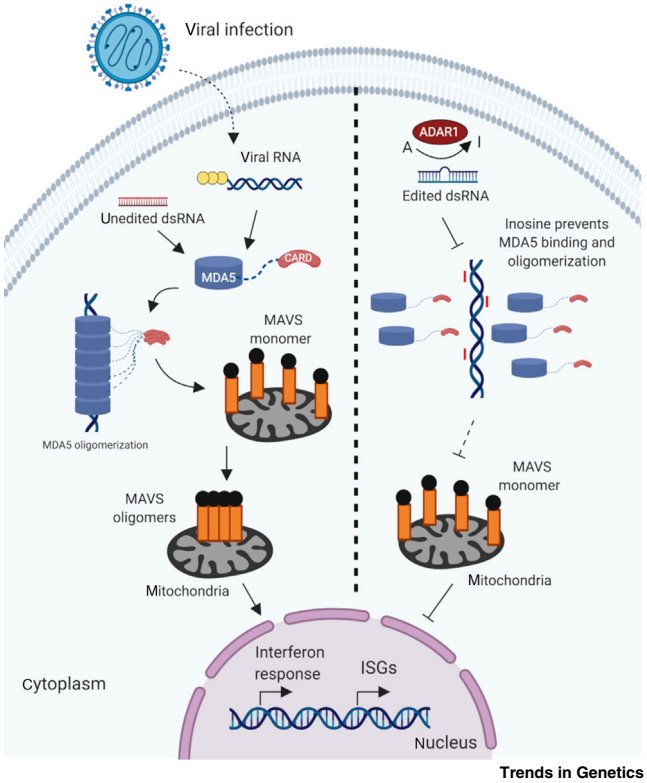

Figure 3. Sensing of Viral or Endogenous Double-Stranded (ds) RNA in the Cytoplasm.
(Left) Unedited viral or endogenous dsRNAs activate RNA sensors such as melanoma differentiation antigen 5 (MDA5) and stimulate the binding, orchestration, and oligomerization of MDA5 on dsRNAs. CARD domains shown in red oligomerize and activate mitochondrial antiviral signaling protein (MAVS) via oligomerization. MAVS oligomerization stimulates the expression of interferon-stimulated genes (ISGs) in the nucleus, causing an inflammatory response. (Right) In the presence of edited dsRNA, inosine prevents the binding of MDA5 to dsRNA, thus blocking MDA5 oligomerization. This further keeps MAVS in an inactivate monomeric state (shown in orange), preventing inflammatory signaling.

tumors, possibly via cytokine signaling [76,77]. Thus, targeting ADAR1 in IFN-positive tumors may be a way to enhance tumor immune therapies or to overcome resistance against immune checkpoint blockade in cancer. For this to work, ideally drugs targeting ADAR1-p150 will be necessary because only ADAR1-p150 expression depends on an IFN-inducible promoter [10].

RNA Editing in Neurological Disorders and Cardiovascular Disease

A-to-I RNA editing regulates important functions of the brain by modifying mRNAs encoding proteins involved in neurotransmission (Figure 3). Prominent neuronal targets of ADARs include the RNAs encoding serotonin receptor HTR2C, several glutamate receptors, and the GABRA3 receptor, to name only a few [78–80]. Overall, reduced A-to-I RNA editing is not only associated with Alzheimer's disease but also with spinal cord injury and individuals with ASD [20,81]. Similarly, glutamate receptor *GRIA2* editing is significantly reduced in the motor neurons of ALS patients as well as in patients with schizophrenia and bipolar disorder [82,83]. Reduced editing is accompanied by reduced ADAR2 expression in these patients (Figure 2).

Although the impact of RNA editing on neuronal function is widely appreciated, several recent papers have also shown that RNA editing has important functions in the cardiovascular system. For instance, cathepsin S mRNA (*CTSS*), which encodes a cysteine protease associated with angiogenesis

and atherosclerosis, has been associated with human vascular diseases [84]. ADAR1-mediated editing in the 3′-UTR of *CTSS* mRNA favors the binding of ELAVL1, thus regulating *CTSS* mRNA expression and stability. Both *CTSS* 3′-UTR editing and mRNA expression are significantly increased in RNA from peripheral blood mononuclear cells of patients with coronary artery disease relative to healthy controls [84]. This study shows the potential of ADAR1-mediated A-to-I RNA editing in vascular inflammatory disorders.

Recently, ADAR2-mediated **filamin A (*FLNA*) mRNA** editing was shown to regulate blood pressure and possibly the development of cardiovascular disease [85]. Surprisingly, *FLNA* mRNA editing and ADAR2 expression was found to be highest in the arterial system [23,85,86]. In patients suffering from cardiac myopathies, *FLNA* editing was found reduced by 50% compared with normal hearts. Consistently, mice with FLNA editing deficiency developed diastolic hypertension and showed cardiac remodeling [85]. Thus, FLNA editing is the first example of an editing-induced recoding event that is associated with vascular function and cardiac diseases. Interestingly, the editing-induced amino acid exchange in FLNA alters protein interactions [85]. These studies highlight that both ADAR1- and ADAR2-mediated editing events have important functions in the cardiovascular system (Figure 2).

Site-Directed A-to-I Editing: New Drugs on the Horizon?

In recent years the use of CRISPR/Cas9 to correct genetic disorders has been widely explored but has several risks [87]. Nucleic acid-based therapeutics transiently targeting the transcript rather than stably altering the genetic information may be an alternative. In fact, A-to-I editing can be used to correct a genetic mutation at transcript level by an approach called **site-directed RNA editing (SDRE)** (Figure 4). SDRE offers several advantages over CRISPR gene editing: (i) **off-target editing** is less severe because the genome remains unaffected, (ii) SDRE can be applied in a dose-dependent manner rendering it more drug-like, and (iii) humans may have a pre-existing adaptive immunity against popular Cas9 proteins [88].

SDRE requires ADAR deaminase activity and a **guide RNA** that forms a ds complex with the target RNA, ideally with a cytosine directly opposing the targeted adenosine. Thereby a bulge is created which fosters high levels of editing [89,90]. Guide RNAs come in different flavors (Figure 4): (i) the simplest comprises a chemically modified guide that forms a short dsRNA [91]. (ii) Guide RNAs

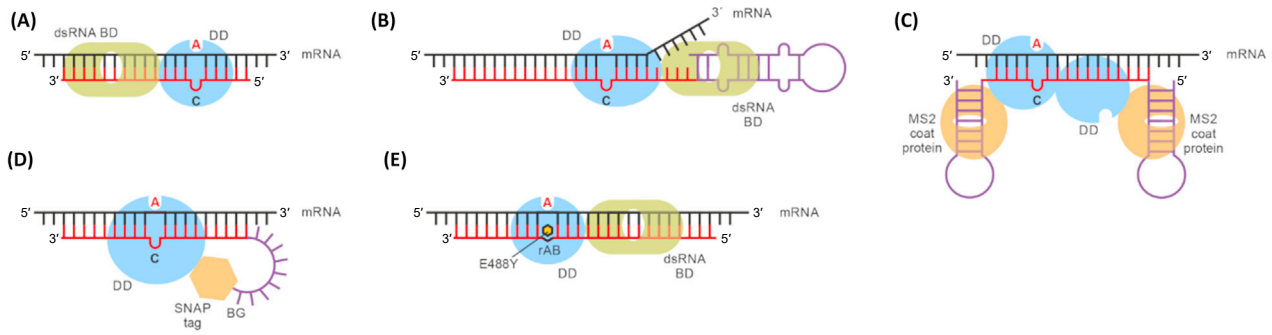

Figure 4. Strategies for Site-Directed RNA Editing.
(A) Site-directed editing by recruiting endogenous adenosine deaminase acting on RNA (ADAR) to antisense-induced double-stranded (ds) regions. (B) A dsRNA resembling a well-characterized editing substrate (*Gria2*) is attached to an antisense guide RNA (in purple). (C) Protein-tethering motifs (e.g., MS2) are attached to the guide RNA to attract engineered editases (deaminase domain fused to MS2 coat protein). (D) The deaminase domain expressed as a fusion protein with a SNAP-tag. The SNAP-tag covalently links an *O*-benzylguanine (BG) moiety in the guide RNA to the protein. (E) A 'bulky' tyrosine is introduced into the catalytic center of the deaminase domain (E488Y), thereby only targeting regions in which a guide RNA carrying a reduced abasic site (rAB) is opposite the editing site. Abbreviations: dsRNA BD, dsRNA binding domain (green); DD, deaminase domain (blue); target mRNA (black); complementary guide RNA (red); motifs attached to the guide for protein recruitment (purple); A, edited adenosine (red); C, cytosine opposing the editing site in the guide RNA (black).

may contain an attached stem loop which serves as binding platform for ADAR [92,93]. (iii) Alternatively, guide RNAs with a protein-tethering motif (e.g., MS2 repeats or lambda N) that require engineered deaminases containing the complementary binding domain can be used [94–96]. (iv) Finally, the guide RNA itself can be covalently attached via a SNAP-TAG to the deaminase domain, allowing low concentrations of both guide and deaminase to be used. However, this system cannot be genetically encoded. Because it requires delivery of a modified oligonucleotide, the SNAP-TAG approach may be less suitable as a drug but more useful as a research tool [97,98]. To improve such research tools and reduce off-target editing, another chemical approach using a mutated deaminase domain with a 'bulky' tyrosine in the active site may be a useful tool. The tyrosine interferes with natural editing, but allows editing with an adapted and chemically modified guide RNA that can accommodate the bulky tyrosine [91].

Where do we stand regarding therapeutics? Using SDRE in primary neurons of a mouse model of Rett syndrome with a point mutation in the *Mecp2* transcript and adenoviral delivery, 72% of the mRNA was 'corrected' [99]. The first step to apply SDRE *in situ* has been taken in mouse models of Duchenne muscular dystrophy (DMD) and ornithine transcarbamylase (OTC) deficiency using adenoviral delivery [96]. However, the editing yields achieved remain low: 2–3% for the DMD model and 3–4% for the OCT model. As an alternative to these genetically encoded systems, guide RNAs can be delivered via injection methods. In this case, chemically stabilized guide RNAs designed to attract endogenous ADAR enzymes can be used [92]. It will be exciting to see how such guide RNAs perform in a mouse model.

Concluding Remarks and Future Perspectives

A-to-I editing is deregulated in human diseases including cancer, Alzheimer's, depression, ASD, and cardiovascular disease (Figure 2). Additional information can be found in the Editome Disease Knowledgebase [100]. The regulation of A-to-I editing is complex and includes multifaceted management of ADAR expression and localization, specific features of RNA targets, and other intermediate regulators such as RNA-binding proteins. Interplay of editing and splicing also plays a central role in ADAR regulation. However, the regulation (or 'misregulation') of A-to-I editing is far from being understood in human disease (see Outstanding Questions). Even more importantly, it is mostly unknown whether misregulation of A-to-I editing in a particular disease is a cause or a consequence. However, the development of drugs against a selected editing event or ADAR enzyme will only contribute to curing the disease if misregulation is the cause. Taken together, A-to-I RNA modification has a great deal to offer in providing new therapeutic targets in neurodegenerative diseases and aggressive tumors. Further studies aiming to improve specific RNA targeting would be instrumental in designing new RNA drugs in the future.

Outstanding Questions

How do dsRNA sensors distinguish between self and non-self RNA? This would aid in designing distinct RNAs that activate MDA5 in antiviral therapies.

What are the distinct functions of ADAR1-p150 and ADAR1-p110? Isoform-specific regulation may help understand the complex function of the ADAR enzymes.

Is A-to-I editing misregulation a cause or a consequence of a particular disease?

Is it possible to target and correct A-to-I misediting for selected protein-coding substrates?

What are the key regulators of ADAR-mediated editing in disease? Do ADARs contribute to human disease independently of A-to-I editing ADARs?

ADAR1 has been identified as a potential target for cancer immunotherapy. Can ADAR1 be targeted to treat cancer?

The efficiency of site-directed editing in a mouse model is currently poor. How can site-directed RNA editing be improved and developed for therapeutic applications?

Acknowledgments

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