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When microRNAs meet RNA editing in cancer: A nucleotide change can make a difference

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Abstract

RNA editing is a major post-transcriptional mechanism that changes specific nucleotides at the RNA level. The most common RNA editing type in humans is adenosine (A) to inosine (I) editing, which is mediated by ADAR enzymes. RNA editing events can not only change amino acids in proteins, but also affect the functions of noncoding RNAs such as miRNAs. Recent studies have characterized thousands of miRNA RNA editing events across different cancer types. Importantly, individual cases of miRNA editing have been reported to play a role in cancer development. In this review, we summarize the current knowledge of miRNA editing in cancer, and discuss the mechanisms on how miRNA-related editing events modulate tumor initiation and progression. Finally, we discuss the challenges and future directions of studying miRNA editing in cancer.

Keywords

RNA editing; cancer development; A-to-I editing; ADAR; miRNA regulation; cancer treatment; biomarker

1. Introduction

RNA editing is a widespread post-transcriptional mechanism that modifies select RNA transcripts in a site-specific manner. Unlike DNA mutations, RNA editing increases the diversity of transcriptome by changing only a fraction of the RNAs, thus generating a mix of both edited and non-edited (wild-type) RNA molecules [1, 2]. The best characterized and most prevalent RNA editing type in humans is the adenosine (A) to inosine (I) editing, which is subsequently recognized as a guanosine (G) by the translation machinery. A-to-I editing is catalyzed by the adenosine deaminase acting on RNA (ADAR) enzyme family that

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Competing Interests

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includes ADAR1, ADAR2, and ADAR3. Another known type of RNA editing is the cytosine (C) to uracil (U) editing, which is mainly controlled by the APOBEC cytidine deaminases. RNA editing is dynamically regulated in various human cells. Aberrant RNA editing activities skew the balance between edited and wild-type RNAs, resulting in “abnormal protein products” through missense codon change, alternative splicing, and modification of regulatory RNAs [3].

MicroRNAs (miRNAs) are one major class of regulatory non-coding RNAs that undergo extensive post-transcriptional modifications. They participate in nearly all cellular pathways and pathological processes, including cancer initiation and progression [4, 5]. These 21–25nt small RNAs negatively regulate gene expression by base pairing to the 3′UTRs of their target genes. More than 60% of human protein-coding genes show high sequence conservation at miRNA binding sites [6]. MiRNAs recognize their target genes mainly by partial sequence complementarity between the miRNA seed-region (position 2–8 at the 5′ end) and a target site [7]. Upon binding to the 3′UTR of the target gene, mature miRNA can lead to mRNA degradation and translational inhibition [8]. Thus, even a single nucleotide change in the miRNA, especially within the seed region, may redirect a miRNA to a different set of mRNA targets.

Recent next-generation sequencing studies have begun to unravel a full picture of the human editome, and several million high-confidence editing sites have since been identified in the human genome. As both the ADAR and the apolipoprotein B mRNA editing catalytic polypeptide-like (APOBEC) enzymes can edit double-stranded miRNAs, and given the critical roles of miRNAs in cancer development, the patterns and effects of miRNA editing in cancer have attracted considerable interest [10–13]. However, the precise mechanisms underlying miRNA editing events and their downstream functional impacts in human cancers remain largely unknown. In this review, we focus on the evidence of cancer-related miRNA editing events and summarize our current knowledge. We further discuss the potential challenges in utilizing miRNA editing as an investigational paradigm to study cancer development and related clinical applications.

2. Major RNA editing types in humans: A-to-I and C-to-U

A-to-I RNA editing is catalyzed by ADAR enzymes. ADAR1 is the most abundant RNA editing enzyme; it is expressed in almost all tissues and has two isoforms, p150 and p110 (Fig. 1A) [14]. ADAR2 is highly expressed in the brain but is also found in other tissues [15], while ADAR3 expression is restricted to the brain and its catalytic activity remains controversial [16]. ADAR-mediated A-to-I editing of double-stranded RNA (dsRNA) is a catalytic unwinding process. ADARs contain a dsRNA binding domain that makes direct contact with the dsRNA, and a catalytic deaminase domain [2]. Following transcription, the resulting primary miRNA (pri-miRNA) is subjected to nuclear processing initiated by RNase III Drosha [6], wherein the pri-miRNA (<1kb to several kb in length) is cropped into a ~65nt long small hairpin-shaped RNA called the pre-miRNA (Fig. 1A). This pre-miRNA is then exported to the cytoplasm through nuclear pore complex and is further cleaved into a double-stranded miRNA duplex by Dicer. The miRNA duplex is loaded into an Argonaute (AGO) protein to produce a mature RNA-induced silencing complex (RISC). Specifically,

ADAR1 can form a complex with Dicer to promote pre-miRNA cleavage and can assist in the loading of miRNA duplex onto the RISC [17]. A-to-I editing can occur at both pre-miRNA and pre-miRNA levels. Cytoplasmic miRNA editing is mediated by the p150 isoform of ADAR1 as all other ADAR enzymes are primarily nuclear [18]. ADAR editing can either be site-specific, recurrently changing a particular adenosine, or stochastic, modifying all accessible sites [19]. The exact mechanism of ADAR editing site specificity remains unclear; however, several sequence patterns have been observed around frequently edited sites, including depletion of G upstream and enrichment of G downstream of the editing site [20]. A few studies have described a clear UAG motif in the miRNA editing sites [21–23]. It is also believed that the mRNA secondary structure may hold guiding information responsible for the editing site selectivity [24].

C-to-U editing is much less common than A-to-I editing in humans. A C-to-U change is affected by a multi-protein complex referred to as the C-to-U editosome (Fig. 1B) [25]. The minimal functional enzyme complex of this editosome includes the deaminase APOBEC1 [26] and its requisite RNA-binding cofactor ACF (APOBEC1 complementation factor, also known as A1CF) [27]. An *in vivo* APOBEC1 knockout completely abolishes C-to-U editing, indicating an absence of functional overlap between APOBEC1 and other cytidine deaminases [28]. The interaction between mRNA substrates and APOBEC1 alone is not sufficiently strong to conduct editing, and therefore, the RNA-binding protein ACF is required for editing activity. Other RNA-binding cofactors such as RNA Binding Motif Protein 47 (RBM47) have also been reported to substitute ACF, expanding the mRNA substrate of APOBEC1 and the regulatory network of C-to-U editing [29]. Unlike A-to-I editing, C-to-U editing is very site-specific; a high preference for cytidines located in AU-rich regions, and a conserved mooring sequence proximal to the editing site has been demonstrated [30]. A previous study reports that overexpression of APOBEC1 led to a cancer phenotype through promiscuous editing of cytosines [31]. More recently, APOBEC3A and APOBEC3G, both APOBEC1 homologs, have exhibited some editing functions under specific stimulations [32, 33].

3. MiRNA editing level is dysregulated in human cancers

In normal tissues, editing level at a specific miRNA editing site can range from <1% to 100% [11, 21]. In tumor samples, the RNA editing level varies between patients and between cancer types. Through normal-tumor comparisons, Gong *et al.* found that the editing levels of 11 A-to-I editing sites were significantly different in breast, liver, and lung cancers: while 4 of these sites were hyper-edited in tumor tissues, the remaining 7 sites were hypo-edited in tumor tissues [34]. More recently, we performed a comprehensive analysis of miRNA editing hotspots in ~9,000 patient samples across 20 different cancer types using The Cancer Genome Atlas (TCGA) miRNA-seq data [10]. Of the 15 validated A-to-I editing hotspot sites, 9 sites demonstrated significant editing-level differences in at least one cancer type relative to matched normal tissues: 3 sites were hyper-edited and 4 sites were hypo-edited in tumors samples across cancer types, while the remaining 2 editing sites showed inconsistent patterns among different cancer types. Additionally, Tomaselli *et al.* reported a systematic loss of RNA editing in all detected ADAR2-mediated editing sites in glioblastoma cell lines

and tissues compared to healthy brain tissues [35]. Overexpression of ADAR2 could successfully rescue this loss of RNA editing in the glioblastoma cell lines.

In general, the highly diverse patterns of miRNA editing between tumor and normal samples may result from miRNA expression and tissue specificity of ADAR enzymes. ADAR expression often shows a great variation in different tumor contexts, leading to altered A-to-I editing patterns. Overexpression of ADAR1 is frequently observed in cancers. Two major mechanisms have been proposed to contribute to the related overediting in human cancer [18, 36]: inflammation and copy number amplification of chromosome 1q, where ADAR1 gene resides. Unlike ADAR1, ADAR2 activity is often reduced in brain tumors, and the decreased ADAR2-mediated editing level is associated with increased tumor grade in children [35]. Although the underlying mechanisms of how ADARs cooperate with each other and govern the editing patterns remain largely unknown, the distinct functions of ADAR1 and ADAR2 can partially explain the divergent editing patterns observed in human cancers.

C-to-U editing in miRNAs has been under-examined in cancer, and no comprehensive analysis or individual cases of altered C-to-U miRNA editing have been reported thus far. However, an elevated expression level of APOBEC1 homolog, APOBEC3 is frequently noted in tumor samples and various cancer cell lines. The lack of exploration may be due to the fact that APOBEC3-mediated RNA editing only occurs under specific conditions including hypoxia and/or IFN exposure [33].

4. Towards a miRNA “editome” in cancer

Some intrinsic characteristics of miRNAs present considerable challenges in identifying true RNA editing sites from high-throughput sequencing data. The fact that miRNAs are short, contain repeats and undergo extensive post-transcriptional modifications all result in promiscuous cross-mapping and high false positive rates of miRNA editing site calling [37]. These problems are even more critical for miRNA editing calling in tumor samples since the editing signals are further interfered by somatic mutations and tumor purity. In several initial attempts of identifying miRNA editing events from next-generation sequencing data, a large majority of miRNAs were reported to undergo RNA editing modifications including many non-A-to-I editing events. This high false discovery rate may be due to several technical problems such as cross-mapping, DNA mutation contamination, prevalent RNA modifications, and sequencing errors [21, 37]. Dedicated efforts have been made to improve the miRNA editing calling pipelines. Only recently, high-quality miRNA editing pipelines have been applied to large-scale tumor samples [10, 21]. By screening ~9,000 patient samples in 20 cancer types, we identified 193 unique A-to-I editing candidate sites. However, only 19 of these A-to-I editing sites occurred in a highly recurrent manner with significant editing levels, suggesting that only a relatively small proportion of miRNA editing events in tumors have the potential to affect cellular functions [10, 21, 34, 38].

Through a decade of efforts, ~130 miRNA A-to-I editing sites have been identified [2, 6, 10–12, 21, 22, 34, 35, 39, 40]. Some of these editing sites are only found in specific tissues, while others show a universal editing pattern. Over the years, our knowledge of miRNA

editome has expanded from individual cases in a single disease to comprehensive catalogs across many cancer types.

As for the location of editing sites in miRNAs, Alon *et al.* identified 24 miRNA editing sites in human brain tissues and glioma cell lines, of which 22 (92%) were in the miRNA seed region [21]. Gong *et al.* discovered 25 widespread miRNA editing sites in 4 different tissues, 18 (72%) of which were in the seed region [34]. In our pan-cancer miRNA editing analysis, 18 (95%) of the 19 miRNA editing hotspots were also located in the seed region [10]. Similarly, Paul *et al.* analyzed RNA editing events in mature miRNAs across 13 different human tissues and found 73.33% of them to be in seed regions [41]. In addition, more than half of the edited miRNAs showed increased stability compared to the unedited miRNAs. Thus, RNA editing sites appear to be enriched in the miRNA seed regions, highlighting the potential importance of edited miRNA in regulating gene expression.

5. Key miRNA editing events inhibit tumor progression

Given the critical roles of miRNAs in cancer, the effect of nucleotide changes introduced in a miRNA through RNA editing on tumor development and progression has attracted great attention. Intriguingly, individual miRNA editing events have been reported to function as both oncogenes and tumor suppressors (Table 1). Here we first review cases where miRNA editing makes a negative contribution to tumor development.

The first example that links miRNA editing to cancer is the A-to-I editing in miR-376a-5p [11]. The miR-376 family, including pri-mir-376a-1, pri-mir-376a-2, pri-mir-376b, and pri-mir-376c, undergoes extensive A-to-I editing that redirects their targets to a new set of genes [39]. Choudhury *et al.* reported a dramatic decrease in the editing level of miR-376a-5p, from 95% in normal brain tissues to a negligible level in glioma cell lines. The editing level of miR-376a-5p correlated negatively with tumor volume in glioma patients. Overexpression of mature unedited miR-376a-5p promoted migration and invasion of glioma cells *in vitro*, and this can be reversed by a stable expression of edited miR-376a-5p. Furthermore, mice injected with cells expressing unedited miR-376a-5p developed more aggressive tumors of irregular shapes and with a higher tendency to displace from the primary inoculation site. The authors identified Ras-related protein Rap-2A (RAP2A) as a unique target of the unedited miR-376a-5p, harboring six binding motifs in its 3'UTR, whereas Autocrine Motility Factor Receptor (AMFR) was a unique target of the edited miR-376a-5p, with 2 binding sites in the 3'UTR. Only unedited miR-376a-5p significantly reduced the mRNA level and protein expression of RAP2A in glioma cell lines. Knockdown of RAP2A, and overexpression of AMFR, both led to phenotypes similar to the overexpression of unedited miR-376a-5p, which further validated the functional role of unedited miR-376a-5p in promoting cell migration and invasion. Thus, the decreased editing level of miR-376a-5p leads to more aggressive glioblastoma both *in vitro* and *in vivo*, and poor patient prognosis, through the inhibition of RAP2A, the target gene of unedited miR-376a-5p.

Another such example is miR-455-5p editing in metastatic melanoma [12]. Loss of ADAR1 has been reported to result in more aggressive melanoma phenotype through an RNA editing-independent mechanism [42]. Subsequent to this finding, a miRNA-editing mediated

pathway was reported to promote melanoma progression. Using miRNA expression data from ADAR1 silenced and activated melanoma cell lines, Shoshan *et al.* identified A-to-I editing sites in miR-378-3p, miR-324-5p, and miR-455-5p catalyzed by ADAR1. Focusing on miR-455-5p, they showed that ADAR1 expression reduced the binding ability of edited miR-455-5p to Drosha and Dicer, thereby decreasing mature miR-455-5p expression. Next, combining gene expression profiling data and 3'UTR sequence complementarity, the authors identified tumor suppressor Cytoplasmic Polyadenylation Element Binding Protein 1 (CPEB1) as a potential target of the wild-type miR-455-5p. ADAR1 overexpression, which led to a decreased miR-455-5p expression, effectively increased CPEB1 expression by 2-fold at the mRNA level and 1.5-fold at the protein level. *In vivo*, significant tumor growth and lung metastasis were observed in mice overexpressing wild-type miR-455-5p compared to those overexpressing the edited miRNA. Using a nano-liposome delivery system, the authors demonstrated that mice treated with edited miR-455-5p exhibited a dramatically reduced number of lung metastases, while treatment with wild-type miR-455-5p promoted metastasis. Taken together, ADAR1 inhibits melanoma growth and metastasis *in vitro* and *in vivo* through editing activities of miR-455-5p.

In a third example, Tomaselli *et al.* investigated the role of ADAR2 in shaping the miRNA transcriptome of glioblastoma [35]. Based on miRNA-seq profiling, ADAR2 was found to be responsible for 19 miRNA editing events and ~90 miRNA expression changes in glioma cells. Loss of ADAR2 is common in glioma cell lines, while its rescue impedes tumor development. Specifically, ADAR2 edited onco-miR-222/221 and miR-21, and inhibited their maturation both *in vitro* and *in vivo*, leading to suppressed cell proliferation and migration. The authors concluded that ADAR2 maintains a balance between onco-miRNAs and tumor suppressor miRNAs in brain tissue.

6. Key miRNA editing events promote tumor growth

In contrast to the above examples where A-to-I RNA editing could suppress tumor growth and metastasis, several examples of miRNA editing have been shown to drive tumor development (Table 1).

Zipeto *et al.* investigated how ADAR1 amplification promoted leukemia stem cell self-renewal through an editing-dependent pathway [43]. A previous study had noted that dysregulated ADAR1 expression promoted the transformation of chronic myeloid leukemia from chronic phase to a more aggressive therapy-resistant blast crisis phase, in part through elevated inflammatory cytokine signaling that activates JAK-STAT binding to the ADAR1 promoter [44]. Zipeto *et al.* further found that JAK2 signaling promoted ADAR1 expression and related A-to-I editing events. Following JAK2 transfection, the expressions of LIN28B and miRNAs of the let-7 family were strongly inhibited. When co-transfected with BCR-ABL1, a known driver of blast crisis transformation, miRNA-maturation-related genes were reduced, profoundly impairing biosynthesis of let-7 family miRNAs, enhancing self-renewal capacity, and worsening survival times *in vivo*. Utilizing RNA-seq profiling data, they demonstrated that ADAR1 overexpression significantly promoted the expression of genes related to stem cell pluripotency and that most of the up-regulated genes were let-7 targets. In transfection studies, a +3 site edited miRNA led to a significant loss in mature let-7d

miRNA expression, while a +59 site edited and unedited pre-let-7d had little impact on the level of mature let-7d. Thus, both *in vitro* and *in vivo* data support the role of ADAR1 in promoting leukemia stem cell self-renewal through enhanced pri-let-7 family editing.

In another example, ADAR1 activation corresponded with miR-381 over-editing in non-small cell lung carcinoma (NSCLC) [45]. Here, the authors observed that shRNA-mediated knockdown of ADAR1 in lung cancer cell lines significantly reduced cell growth and colony-formation. *In vivo*, ADAR1 depleted cells showed significantly slower tumor formation rate and lung metastases in nude mice. To validate that ADAR1 increased tumorigenesis through RNA editing, the authors performed whole-transcriptome sequencing and subsequently focused on two editing sites in NEIL1, a well-studied DNA repair enzyme. Upon transfection of ADAR1, editing levels of the two sites increased from 28% and 45% to 92% and 82%, respectively. Cells transfected with edited NEIL1 and miR-381, an ADAR1 editing target reported in glioma, exhibited dramatically enhanced growth and migration compared with cells transfected with wild-type NEIL1 and miR-381. Using *in vitro* analyses, the authors showed that ADAR1 enhanced tumor growth and migration in NSCLC by editing NEIL1 and miR-381. ADAR1 amplification is seen in 10% of lung cancer patients, and the presence of ADAR1 gene amplification is associated with worse relapse-free survival time, especially among patients in early stage of the disease.

Liu *et al.* studied the impact of miRNA editing on mature miRNA expression in hepatocellular carcinoma (HCC) [46]. Among 16 previously reported deregulated miRNAs, miR-214 and miR-122 undergo ADAR2-catalyzed editing. HCC patients with increased ADAR2 expression show similar editing patterns to those observed in ADAR2 overexpressing cell lines. Interestingly, the authors observed not only A-to-I editing but also unusual U-to-C editing that may have been a result of A-to-I editing of the reverse strands of pri-miRNAs. *In vitro*, pri-mir-214 editing disrupted the maturation of miR-214-3p, resulting in an increased expression of a RAS family oncogene RAB15.

While most functionally characterized miRNA editing events have been noted in specific cancer types, in a recent pan-cancer analysis, we identified an editing event at the fifth position of miR-200b-3p across all surveyed cancer types [10]. This editing event was consistently increased in tumor samples, and patient survival analysis further suggested an oncogenic role of the edited miRNA as patients with high editing levels of miR-200b-3p tended to have worse survival times. Our data suggested that this widely observed editing event switched the function of miR-200b-3p, a well-established tumor suppressor, to that of an oncogenic miRNA. We performed cell viability, migration, and invasion assays in breast, ovarian, and lung cancer cell lines, and obtained consistent results supporting the oncogenic role of edited miR-200b-3p in promoting tumor migration and invasion. By analyzing the transcriptome data of cell lines transfected with edited and unedited miR-200b-3p, we identified target genes of the wild-type and edited miR-200b-3p. Interestingly, only 3 genes were shared between the two target gene sets, suggesting a strong redirection effect caused by the single nucleotide change. The top candidate target of edited miR-200b-3p was Leukemia Inhibitory Factor Receptor (LIFR), a well-studied tumor suppressor gene that functions through Yes-Associated Protein (YAP)-Hippo regulation pathway [47]. Knockdown of LIFR resulted in a phenotype akin to the overexpression of edited miR-200b-3p.

Collectively, over-editing of miR-200b-3p promotes tumor migration and invasion by inhibiting the tumor suppressor LIFR *in vitro*. Besides miR-200b, we identified a total of 19 miRNA editing sites that are recurrently modified in tumor samples, among which the editing in miR-376a and miR-381 have been functionally characterized.

7. 3'UTR editing changes miRNA target sites in cancer

RNA editing occurs mostly in non-coding RNAs [48]. As we note earlier, the detailed mechanism of ADAR editing preference remains unknown; however, the non-coding-region-enriched RNA editing pattern may result from enriched *Alu* elements since the resultant double-stranded RNA structures can serve as substrates for ADAR enzymes [49]. Previous computational analyses suggest that A-to-I editing in 3'UTRs have the potential to block miRNA-mRNA interactions, but that RNA editing tends to avoid miRNA target binding sites [50]. Besides RNA editing in mature miRNAs, 3'UTR editing may redirect miRNA regulation. Interestingly, as much as 20% of the RNA editing sites reside in 3'UTRs, potentially preventing canonical miRNA inhibition or gaining new miRNA targets [51]. A large-scale analysis by Paz-Yaacov *et al.* investigated 222,778 *Alu* repeats in 3'UTRs and reported 3,689 sites in 503 genes with significantly altered miRNA binding sequences [52]. One such example is the RNA-editing-mediated gene dysregulation of Rho GTPase Activating Protein 26 (ARHGAP26) whose 3'UTR undergoes extensive modifications catalyzed by ADAR1. The ARHGAP26 expression was positively associated with the ADAR1 expression as this 3'UTR editing effectively blocked binding of its original inhibitors miR-30b-3p and miR-573 [53]. Recently, Zhang *et al.* discovered a similar mechanism for the Mouse Double Minute 2 (MDM2) gene, a well-known oncogene in the p53 pathway that is frequently amplified across cancer types. Increased editing level of MDM2 3'UTR is commonly observed in TCGA RNA-seq data from 14 tumor types, and the editing level shows a strong positive correlation with MDM2 expression in patients. In particular, the 3'UTR binding sites of miR-200b/c in MDM2 are among the most over-edited regions in cancer. The authors experimentally demonstrated that overexpression of miR-200b could effectively reduce MDM2 expression by 60% in cell lines with a low editing level, but had no effect in cell lines with a high editing level. This result suggests that MDM2 escapes the inhibitory regulation of miR-200b/c by over-editing their 3'UTR binding motifs. Another intriguing example is the dihydrofolate reductase (DHFR) gene in breast cancer with 26 reported editing sites in its 3'UTR. In MCF-7 cells, ADAR1 positively regulated DHFR gene and protein expression by disrupting the binding of miR-25-3p and miR-125a-3p to its 3'UTR through RNA editing [54]. Importantly, methotrexate is a competitive inhibitor of DHFR, a key enzyme in folate metabolism. Knockdown of ADAR1 increased methotrexate sensitivity in MCF-7 cells and overexpression of ADAR1 led to enhanced tumor growth and methotrexate resistance [54].

In addition to disrupting existing miRNA binding sites, 3'UTR editing can create novel miRNA regulatory networks. Nakano *et al.* observed a negative correlation between ADAR1 expression and aryl hydrocarbon receptor (AhR) protein level in HCC cell lines due to the creation of a miR-378 binding motif in the AhR 3'UTR by RNA editing [55]. In 32 human liver samples, the editing level of AhR 3'UTR was similar, while the ADAR1 expression varied by up to 220-fold, and there existed a significant negative correlation between

miR-378 level and AhR protein abundance. This may explain individual differences in liver metabolism potentials. Furthermore, down-regulated AhR impaired downstream xenobiotic-metabolizing transcripts and decreased the resistance to xenobiotics [55].

8. MiRNA regulates ADAR expression

Finally, miRNAs may regulate ADAR enzymes in human cancer, which, in turn, can alter miRNA editing, thereby affecting their function, and abundance. Nemlich *et al.* found that in metastatic melanoma cells, miR-17 and miR-432 were frequently amplified to silence ADAR1 by directly binding to its 3' UTR [42]. Notably, downregulation of ADAR1 is a marker of metastatic transition in melanoma, resulting in a more aggressive tumor. There is also evidence that knockdown of ADAR1 in stomach cancer cell lines increased miR-17-5p level by 47.9%, suggesting a feedback loop between down-regulated ADAR1 and up-regulated miR-17 in melanoma [1].

9. Conclusions and future directions

Unlike DNA mutations, RNA editing generates tunable “mutations” only at the RNA level that can be adjusted at different stages or contexts of cancer development. Since mRNA-miRNA interactions are transient, RNA editing enables time- and location-specific regulation that changes cellular properties. This may explain the varied patterns (increased or decreased) of miRNA editing in different cancer types or different samples within the same disease. This dynamic regulation allows cancer cells to alter specific pathways to better adapt to the tumor microenvironment, e.g., the dual role of chronic inflammation and the immune system in tumor initiation and development [56]. Therefore, samples at different stages or different conditions (such as drug-sensitive vs. drug resistant) are worthy of careful analysis. Another interesting but unanswered question is how this dynamic regulation is communicated among cancer cells, and passed from generation to generation. Understanding the dynamics of miRNA editing could help elucidate the evolutionary process of tumors, and potentially serve as biomarkers. To this end, analyzing miRNA editing in single-cell sequencing data is of particular interest. Finally, lncRNAs, which usually undergo extensive editing, can interact with miRNAs by base-pairing and thus serve as miRNA target competitors. A recent study reports that about 200,000 editing sites in lncRNAs have the potential to change the secondary RNA structures and affect lncRNA-miRNA binding [57]. However, a detailed functional characterization of lncRNA editing in cancers has not been attempted probably because of our limited knowledge of the lncRNAs themselves.

In summary, extensive studies over the last decade have indicated that miRNA editing is dysregulated in human cancers, and some miRNA editing events can make notable contributions to cancer development and tumor response through impaired biosynthesis or altered interactions with miRNA targets. Except for a few well-characterized cases, the role of miRNA editing events detected in tumors is largely unexamined. While many of these events (especially those with low editing level, occurring sporadically) are “passengers”, some miRNA editing may represent exciting targets for scientific discovery and clinical applications. Given the key impacts of miRNA regulation and the diversified roles of

miRNA editing in different tumor contexts, edited miRNAs have the potential to serve as biomarkers, therapeutics, and therapeutic targets in cancer management and treatment.

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Fig. 1. Schematic representation of biological process and functional impact of miRNA editing
 A) In the nucleus, a miRNA is transcribed from DNA to pri-miRNA. The pri-miRNA is then processed by Drosha to become a smaller pre-miRNA. Then, the pre-miRNA is exported into the cytoplasm, where it will be further truncated into the mature miRNA. RNA editing mediated by ADAR enzymes can take place at both pri-miRNA and pre-miRNA stages when the miRNA forms a double-stranded structure. Functionally, miRNA editing can lead to disrupted miRNA maturation or miRNA redirection. B) C-to-U editing mainly happens in the nucleus. The minimal editing machine consists of APOBEC1 and a dsRNA binding cofactor such as ACF and RBM47.

Table 1

Functional effects of edited miRNAs in human cancers

miRNA	Enzyme	Editing pos(s)	Editing type	Cancer type(s)	Role in cancer	Function of edited miRNA	miRNA target(s)
miR-376a-5p	ADAR1	3	A-to-I	Glioblastoma	Tumor suppressor	Inhibit tumor progression	Edited: MAFK Unedited: RAP2A
miR-455-5p	ADAR1	2, 17	A-to-I	Melanoma	Tumor suppressor	Inhibit tumor progression	Unedited: CPEB1
pri-miR-222/221/21	ADAR2	-1, +1, +34, +64, +187/-4, +53/+16, +46, +51	A-to-I	Glioblastoma	Tumor suppressor	Disrupt miRNA maturation	NA
let-7d-5p (let-7 family)	ADAR1	3	A-to-I	Leukemia	Onco-miRNA	Disrupt miRNA maturation	NA
miR-381-3p	ADAR1	4, 7	A-to-I	Non-small cell lung cancer	Onco-miRNA	Promote tumor growth and migration	NA
miR-214-3p	ADAR2	6, 16	A-to-I	Hepatocellular Carcinoma	Onco-miRNA	Disrupt miRNA maturation	Unedited: RAB15
miR-200b-3p	ADAR1 ADAR2	5	A-to-I	Pan-cancer	Onco-miRNA	Promote tumor migration and invasion	Edited: LIFR Unedited: ZEB1/ZEB2