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ORIGINAL RESEARCH

The Integrative Studies on the Functional A-to-I RNA Editing Events in Human Cancers

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Abstract Adenosine-to-inosine (A-to-I) RNA editing, constituting nearly 90% of all RNA editing events in humans, has been reported to contribute to the tumorigenesis in diverse **cancers**. However, the comprehensive map for functional **A-to-I RNA editing** events in cancers is still insufficient. To fill this gap, we systematically and intensively analyzed multiple tumorigenic mechanisms of A-to-I RNA editing events in samples across 33 cancer types from The Cancer Genome Atlas. For individual candidate among ~ 1,500,000 quantified RNA editing events, we performed diverse types of downstream functional annotations. Finally, we identified 24,236 potentially functional A-to-I RNA editing events, including the cases in *APOL1*, *IGFBP3*, *GRIA2*, *BLCAP*, and miR-589-3p. These events might play crucial roles in the scenarios of tumorigenesis, due to their tumor-related editing frequencies or probable effects on altered expression profiles, protein functions, splicing patterns, and microRNA regulations of tumor genes. Our functional A-to-I RNA editing events (https://ccsm.uth.edu/CAeditome/) will help better understand the cancer pathology from the A-to-I RNA editing aspect.

Introduction

Adenosine-to-inosine (A-to-I) RNA editing is the most common RNA editing type in humans, constituting nearly 90% of all RNA editing events. Recently, increasing evidence has revealed a significant contribution of RNA editing to tumorigenesis through multiple mechanisms [1,2], including alteration of protein-coding capacity, generation of diverse protein isoforms, and change of cellular fate of RNA and its likelihood of being translated. Specifically, A-to-I RNA editing in coding sequences can result in the functional alterations of proteins that have roles in tumors. For example, an A-to-I RNA editing of *SLC22A3*, resulting in the substitution of asparagine 72 to aspartate, drives early tumor invasion and metastasis in familial esophageal cancer [3]. A study of gastric cancer has

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reported that editing at codon 241 of *PODXL* confers a loss-of-function phenotype that neutralizes the tumorigenic ability of the unedited gene [4]. Also, A-to-I RNA editing can modulate splicing to generate diverse isoforms associated with cancer. In acute myeloid leukemia, an experiment *in vitro* provided the evidence of aberrant intron-retaining splice variant caused by the hyper-editing of *PTPN6*, which is potentially involved in leukemogenesis [5]. *STAT3β*, the tumor regression-associated isoform, is preferentially induced by an A-to-I RNA editing event residing in proximity to the alternatively spliced exon [6]. Besides, microRNAs (miRNAs) and the three prime untranslated regions (3′-UTRs) of mRNAs can also undergo A-to-I RNA editing, which may affect their interactions in cancer. For example, an RNA editing site in miR-200b has been reported to switch the functional roles of this miRNA in terms of cell migration and invasion from suppression to promotion [7]. The edited mature miR-455-*5* caused the reduction of tumor growth and metastasis by promoting tumor suppressor gene (TSG) *CPEB1* in melanoma [8]. As shown in these examples, the systematic and intensive analyses of A-to-I RNA editing will provide critical evidence and novel therapeutic targets in human cancers.

To date, there are several pan-cancer editing landscapes covering the functional annotations of RNA editing events from the aspects of clinical associations [9–11], protein recoding [9], and miRNA regulations [7,11]. For these aspects, they either provided limited candidates for each cancer type, or included partial analyses of RNA editing. For a more comprehensive map of functional A-to-I RNA editing events, in this study, we performed a systematic and intensive bioinformatics analysis pipeline (**Figure 1**) for all the samples across 33 cancer types from The Cancer Genome Atlas (TCGA), similar as that was used in the data analyses of Alzheimer's disease from our recent study [12]. All the analyses were cooperative to point out 24,236 functional A-to-I RNA editing candidates and present their potential roles in the scenarios of tumorigenesis. From the analyses, we confirmed the possible functions of the well-known R/G editing (*GRIA2*, CAediting_390714) in neurological and brain tumors, expanded the roles of *BLCAP* Q/R editing (CAediting_1426931) in carcinogenesis promotion in pan-cancers, and re-addressed the tumorigenic control potential of edited miR-589-3p (CAediting_524911) through dysregulations of tumor genes (TGs). In addition, we also studied two another novel and promising functional RNA editing events. One case (CAediting_1478179) was up-edited in diverse cancers and may confer its pathological function through the intervention in miRNA regulation on the TG of *APOL1*. Another event (CAediting_543208) occurred only in tumor samples for multiple cancer types and may enhance the ability of *IGFBP3* to inhibit tumor cell growth. All these discoveries are available at https://ccsm.uth.edu/CAeditome/. This database provides novel knowledge of tumorigenesis and lists potential targets for cancer and drug research communities.

Results

RNA editing candidates are abnormally edited in cancers

The changes of editing frequencies in tumors, along with diverse stages of tumor pathology and across different tumor survival statuses, can reveal aberrant RNA editing events probably responsible for tumor occurrence, progression, and poor survival. In this work, after comparing editing frequencies between tumor samples and controls, we identified 23,844 RNA editing events in 869 TGs showing tumor-specific frequencies (**Figure 2**A–F, Figures S1–S4; Table S1). Next, through the correlation studies of editing frequencies with tumor stages, we found 701 RNA editing events in 158 TGs which were significantly associated with tumor progression (Figure 2G–I, Figures S5 and S6; Table S1). Then the survival analysis discovered 272 RNA editing events in 99 TGs which might affect the survival risks of cancer patients (Figure 2J–L, Figures S7 and S8; Table S1). Among the 23,904 functional RNA editing events, we selected two candidates to show the effects of A-to-I RNA editing on tumors.

One is an RNA editing event in Chr22:36266650 (CAediting_1478179) of the *APOL1* gene in the cancer type of kidney renal clear cell carcinoma (KIRC). This event showed significantly higher editing frequencies in tumor samples than controls with a P value of 8.08E−05 (Figure 2F), as well as higher editing frequencies in more severe tumor samples (t-test: P = 2.42E−08; Spearman test: P = 1.71E−10 and R = 0.28) (Figure 2I) and a high risk of KIRC survival [Kaplan–Meier (KM) analysis: P = 3.21E−02; Cox proportional–hazards regression (COX) analysis: P = 4.75E−02 and hazard ratio (HR) = 3.42] (Figure 2L). Its existence in the 3′-UTR of *APOL1* seems to cause the up-regulation of the edited gene (t-test: P = 1.04E−14 and $\log_2$ fold change (FC) = 1.86; Pearson test: P = 7.44E−06 and R = 0.20) (**Figure 3**A and B) from the loss of original miR-7151-3p binding targets detected by TargetScan [13] and miRanda [14]. Due to the up-regulated expression of this gene in KIRC tumor samples compared with controls (P = 6.42E−21 and $\log_2$ FC = 2.04) (Figure 3C), and its inducing role in autophagy [15], we suggest this RNA editing event as a potential biomarker of KIRC progression and survival (Figure 3D).

Another editing candidate locates in Chr7:45916046 (CAediting_543208) of the *IGFBP3* gene as shown in Figure S9. For the KIRC cancer type, this event occurred only in tumor samples (246/535 *vs.* 0/72) and was up-edited in the samples with higher stages (t-test: P = 2.14E−02; Spearman test: P = 3.04E−04 and R = 0.23) (Figure 2C). Moreover, it seems to be linked with the up-regulated expression of *IGFBP3* (t-test: P = 2.18E−09 and $\log_2$ FC = 0.61; Pearson test: P = 2.06E−02 and R = 0.15). Because this gene was up-regulated in tumor samples (P = 1.81E−90 and $\log_2$ FC = 3.47), and seemed to act in an autocrine action to suppress tumor cell growth [16], we may carefully suggest the role of this RNA editing event in enhancing the protective functions of *IGFBP3* against cancer progression.

RNA editing candidates are potential factors to affect TG expression

From the analyses mentioned above, we found that the frequencies of several RNA editing events were significantly associated with the expression of their host genes involved in tumors. For systematic analysis of the potential contributions of these RNA editing events to the expression levels of the edited genes, we performed a differentially expressed gene (DEG) analysis between RNA-edited and non-edited tumor samples,

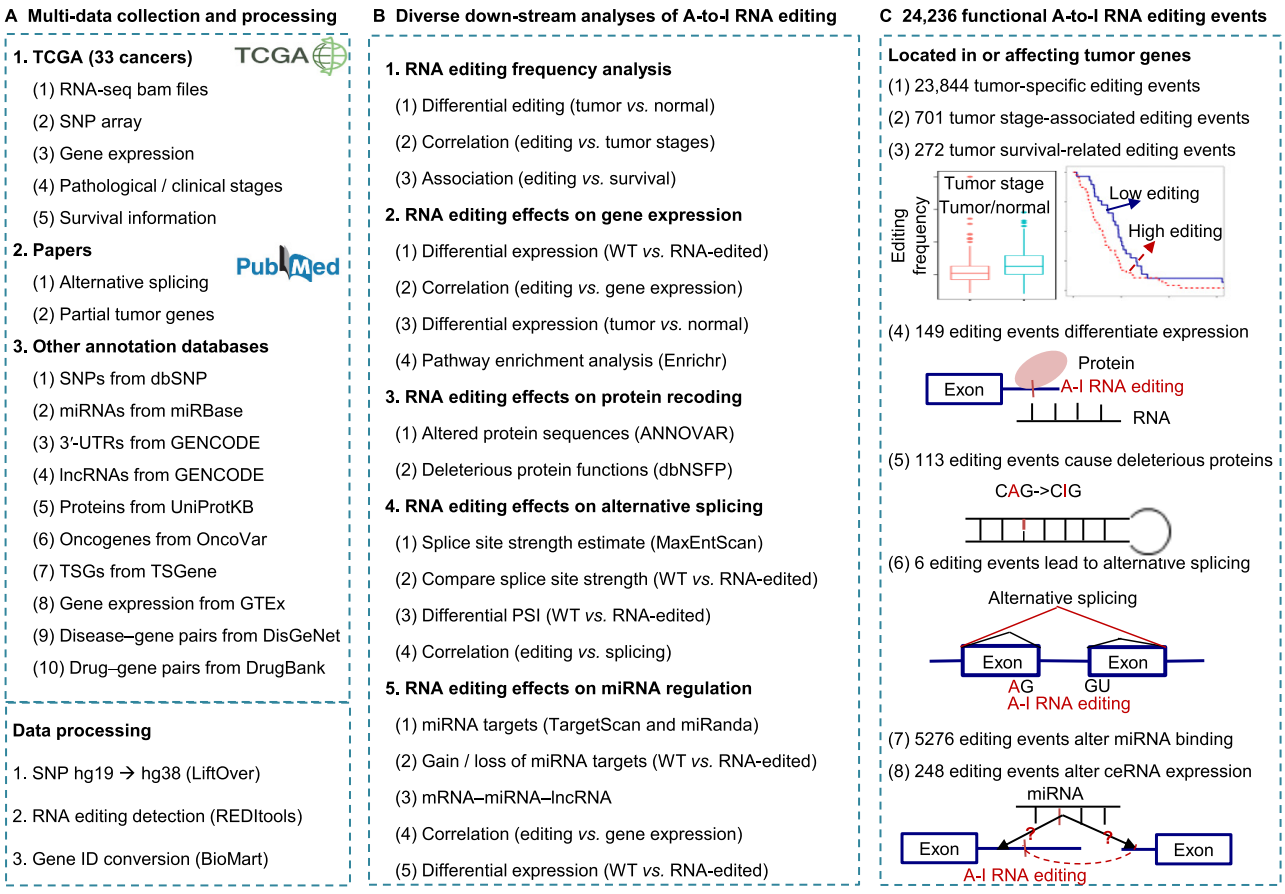

Figure 1 The flowchart to identify functional A-to-I RNA editing events in cancers

A. The collection and pre-processing of multi-omics data across 33 cancer types. **B.** Diverse down-stream analyses of A-to-I RNA editing events. **C.** The potentially functional A-to-I RNA editing events related to tumorigenesis. A-to-I, adenosine-to-inosine; TCGA, The Cancer Genome Atlas; RNA-seq, RNA sequencing; SNP, single-nucleotide polymorphism; miRNA, microRNA; 3′-UTR, 3′-untranslated region; lncRNA, long non-coding RNA; TSG, tumor suppressor gene; GTEx, Genotype-Tissue Expression; WT, wild-type.

as well as a correlation analysis between gene expression and editing frequency (Figures S10 and S11; Table S1). Then, the A-to-I RNA editing-affected DEGs that overlapped with the DEGs between tumor samples and controls were used for following enrichment analysis, to understand the probably involved pathways and biological functions of these RNA editing events in cancers.

As shown in Figure 3E–F, we first discovered 2780 and 333 RNA editing events that would cause the up- and down-regulated expression of 967 and 174 edited genes, respectively. Of them, 651 genes were also abnormally expressed in tumor samples compared with controls, including 55 TGs possibly affected by 149 A-to-I RNA editing events. Combining the potential promotion or inhibition roles of these edited genes in cancers, we could infer the possible functions of these RNA editing events related to tumorigenesis, such as CAediting_1478179 of *APOL1* and CAediting_543208 of *IGFBP3* mentioned above.

Besides, Figure S12 presents a well-known R/G editing case in Chr4:157360142 position (CAediting_390714) of *GRIA2* (syn. *GluA2*) in the cancer type of pheochromocytoma and paraganglioma (PCPG). Its editing frequency was positively associated with the expression of *GRIA2* (*t*-test: P = 5.04E−13 and $\log_2$ FC = 1.02; Pearson test: P = 1.34E−02 and R = 0.20). Due to the up-regulated expression of this gene in tumors (P = 4.64E−23 and $\log_2$ FC = 5.11), and its possible roles in proliferation stimulation, apoptosis resistance, migration, and invasion in cancer cell lines [17], this editing event in *GRIA2* may be a pathological biomarker for the PCPG cancer type, which was also supported by 146 edited PCPG tumor samples and non-edited normal samples.

The following enrichment analysis for these 651 edited DEGs revealed immune- and replication-related biological functions and processes (Figure 3G). Specifically, activation of innate and adaptive immune response through the regulation of viral defense and interferon-alpha production is benefit for cancer immunotherapy [18,19]. The replication processes related to ribosome, endoplasmic reticulum, kinetochore, and so on are important for tumor proliferation and cancer risks [20–22]. Moreover, for each cancer type, the enrichment analysis also discovered some tumor-related Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways as shown in Figure S13 and Table S2. For example, targeting apoptosis is a promising therapy to eliminate cancer cells [23], antigen processing and presentation pathway (APP) is the cellular

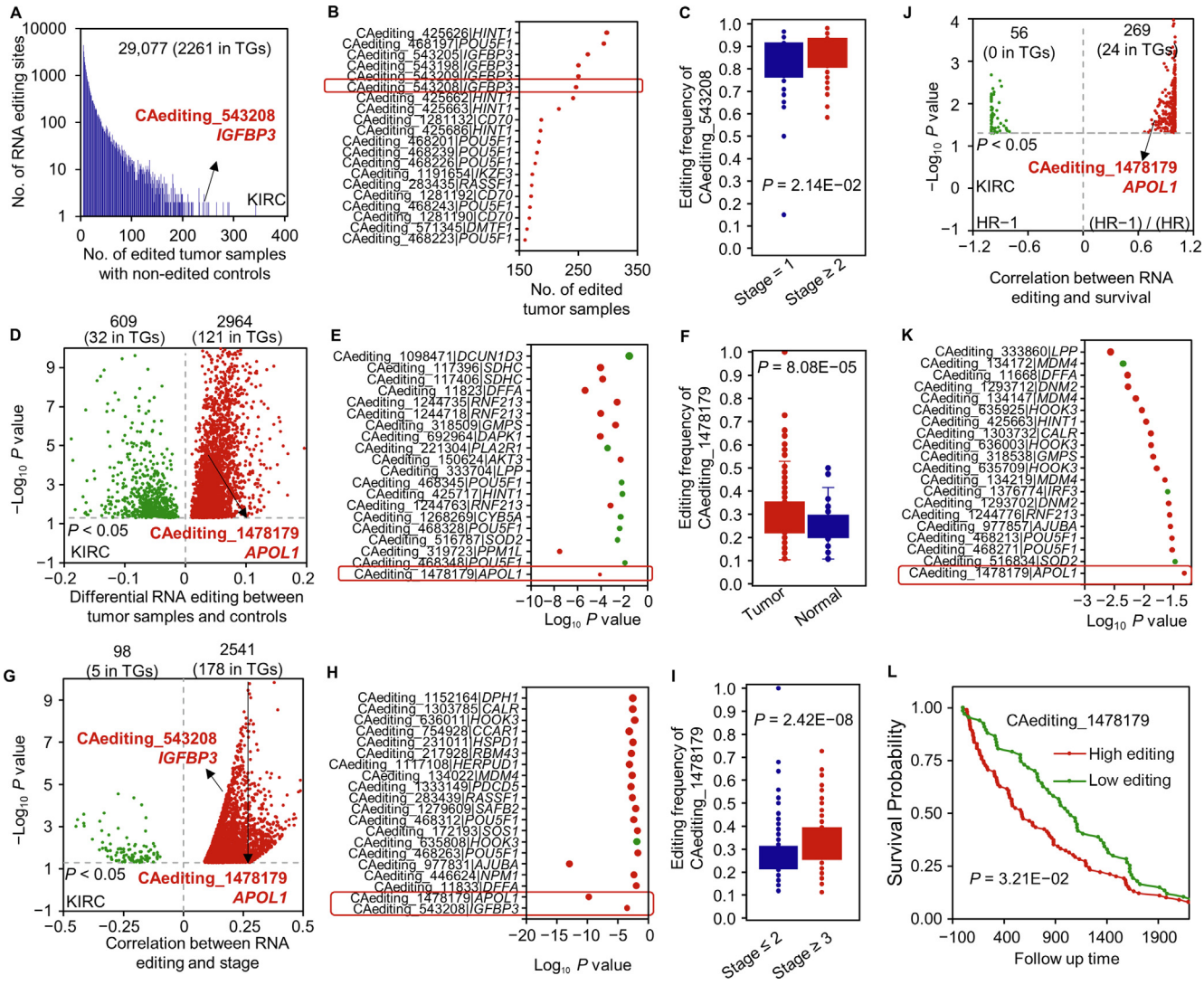

Figure 2 RNA editing frequency analysis

A. KIRC-specific A-to-I RNA editing events with more than five edited tumor samples and non-edited normal controls. The histogram presents the distribution of this kind of RNA editing events along with the number of edited tumor samples. **B.** The bubble plot introduces a part of KIRC-specific RNA editing events in TGs which are not edited in normal controls. **C.** One significant case of *IGFBP3* occurred only in 246 tumor samples for the KIRC cancer type, also showing higher editing frequencies in more severe KIRC tumors. **D.** KIRC-specific A-to-I RNA editing events showing differential editing frequencies in KIRC tumors compared with controls. The volcano plot presents the differences of editing frequencies between tumor samples and controls. **E.** The bubble plot introduces a part of KIRC-specific RNA editing events in TGs which are differentially edited in tumor samples compared with normal controls. **F.** One significant case in *APOL1* showed higher editing frequencies in KIRC tumor samples. **G.** KIRC stage-associated A-to-I RNA editing events. The volcano plot presents the correlations of editing frequencies with tumor stages. **H.** The bubble plot introduces a part of KIRC stage-associated RNA editing events in TGs. **I.** One significant case in *APOL1* showed higher editing frequencies in more severe KIRC tumors. **J.** KIRC survival-related A-to-I RNA editing events. The volcano plot presents the correlations of editing frequencies with cancer survival. **K.** The bubble plot introduces a part of KIRC survival-related RNA editing events in TGs. **L.** One significant case in *APOL1* showed higher editing frequencies in the poorer survival group. The analysis results of editing frequencies for other cancer types are displayed in Figures S1–S8. KIRC, kidney renal clear cell carcinoma; TG, tumor gene; HR, hazard ratio.

mechanism that determines direct interactions between cancer cells and adaptive immune system [24], and sphingolipids metabolic network provides regulatory nodes for controlling tumor growth and proliferation in response to cellular stress [25]. Then, the DEG-associated RNA editing events probably affect these pathways or processes involved in cancers.

RNA editing candidates may reshape their protein functions in tumorigenesis

RNA editing events in coding regions can alter amino acid sequences and have a chance to affect protein functions. To study this, we first selected A-to-I RNA editing sites in

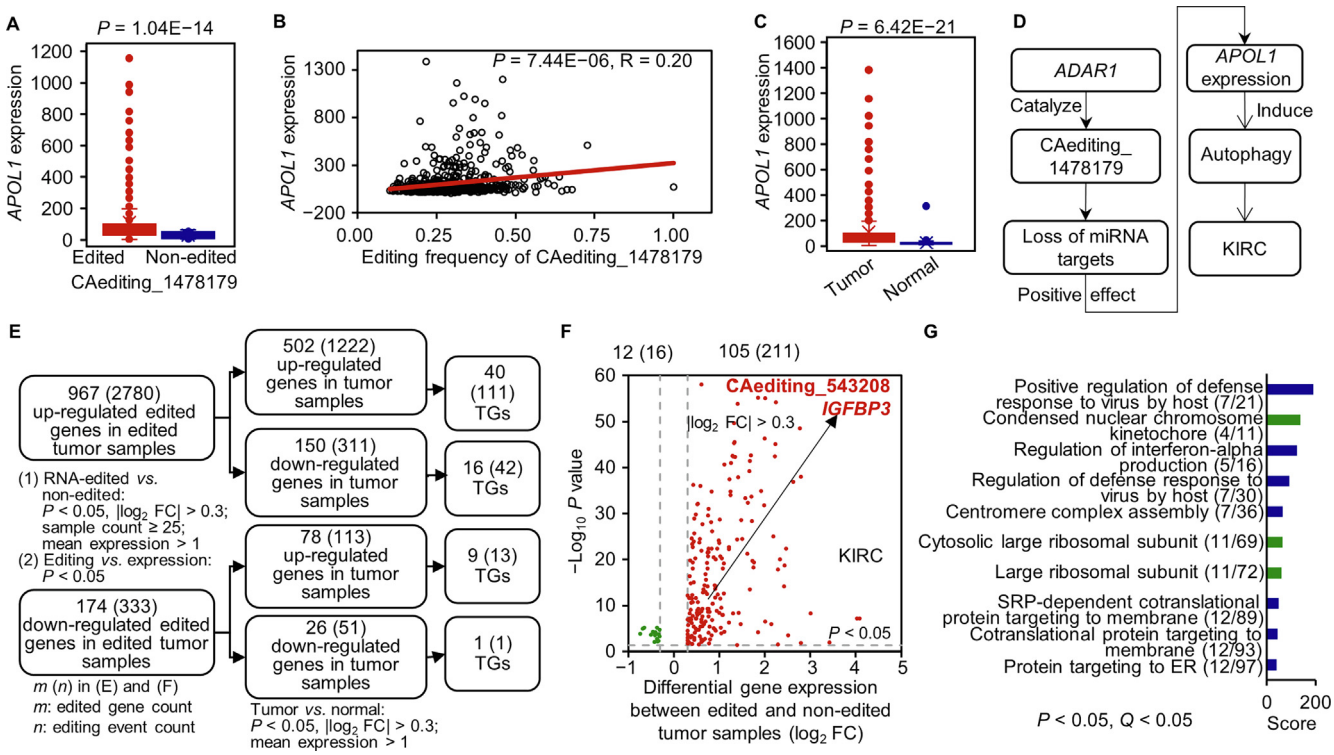

Figure 3 The effects of A-to-I RNA editing events on gene expression

A. *APOL1* was up-regulated in the RNA-edited group. **B.** The expression levels of *APOL1* were positively associated with the frequencies of the CAediting_1478179 editing event. **C.** *APOL1* was abnormally expressed in KIRC tumor samples compared with controls. **D.** The CAediting_1478179 editing event seems to be a potential biomarker for the KIRC cancer type, because it caused the loss of original miRNA binding targets to induce the up-regulated expression of *APOL1*, which may interfere in the autophagy function of this gene in cancer. **E.** The analysis procedure for the effects of A-to-I RNA editing events on gene expression. First, we performed a DEG analysis between RNA-edited and non-edited tumor samples, as well as a correlation analysis between gene expression and editing frequency to identify the DEGs whose expression levels were probably affected by A-to-I RNA editing. Then, we overlapped these genes with the DEGs identified in tumor samples compared to normal controls, to focus on RNA editing effects on the aberrantly expressed genes in cancers, especially the TGs. **F.** The overlapping DEGs in the KIRC cancer type. **G.** The overlapping DEGs were enriched in the immune- and replication-related functions and processes. The RNA editing effects on gene expression in other cancer types are presented in Figures S10–S13. FC, fold change; DEG, differentially expressed gene.

protein-coding sequences and identified 3785 non-synonymous and 121 stop-loss editing events (**Figure 4**A). Out of these, 1128 RNA editing sites were recognized to have impacts on the biological functions of 491 proteins by at least one of the annotation tools such as SIFT, Polyphen2, and PROVEAN. Among them, 113 A-to-I RNA editing events may reshape the functions of 52 tumor-related proteins (Figure 4B).

One RNA editing candidate in the position of Chr20:37519161 (CAediting_1426931) leads to the Q/R changes of key YXXQ motif in the BLCAP protein (Figure 4C). This editing event reverses the inhibition ability of BLCAP to STAT3, facilitating the cancer-initiating and progressing events [26]. Its roles in carcinogenesis promotion were also supported by (1) its abnormal editing cases in pan-cancers, such as the higher editing frequencies in the cancer types of breast invasive carcinoma (BRCA; P = 4.30E−03) (Figure 4D) and KIRC (P = 4.33E−02), (2) positive associations with tumor stages for bladder urothelial carcinoma (BLCA; P = 3.03E−02 and R = 0.29), and (3) mere occurrence in tumor samples of BLCA (55/411 *vs.* 0/19), colon adenocarcinoma (COAD; 176/471 *vs.* 0/41), head and neck squamous cell carcinoma (HNSC; 9/501 *vs.* 0/44), cholangiocarcinoma (CHOL; 10/36 *vs.* 0/9), and rectum adenocarcinoma (READ; 70/167 *vs.* 0/10).

Another well-known R/G editing event (CAediting_390714) in the coding region of the GRIA2 protein mediates the fast excitatory synaptic transmission [27] and may affect the functions of this gene in tumor cell growth, migration, and invasion [17]. Its roles in cancers were also supported by its differential editing frequencies in glioblastoma multiforme (GBM; P = 1.78E−02) (Figure S3). Therefore, the other 111 RNA editing events may also possibly be involved in cancers through modifying the functions of tumor-related proteins, which deserve to be studied further.

RNA editing candidates are probable regulators of alternative splicing in TGs

RNA editing sites in the alternatively spliced exon regions can differentiate splice site strength and eventually affect the selection of splicing positions. To study this, we focused on the editing sites locating around the exon junction boundaries

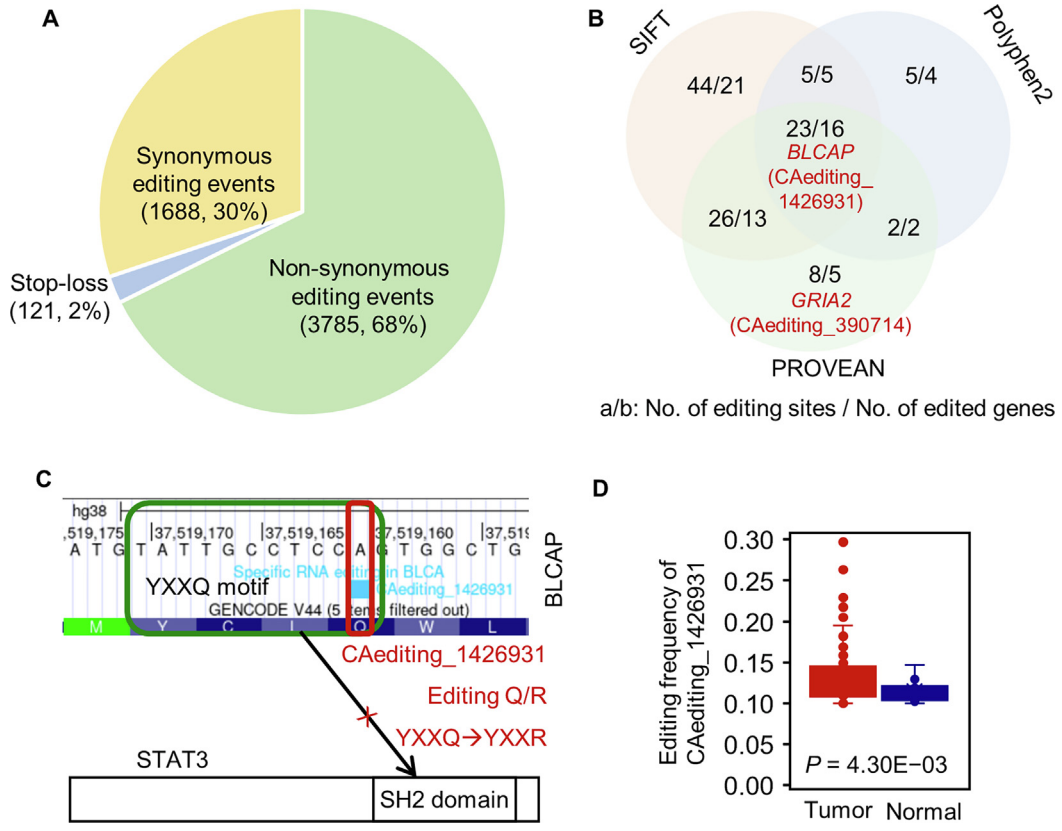

Figure 4 The effects of A-to-I RNA editing events on protein recoding

A. There are 3785 non-synonymous and 121 stop-loss editing events causing the changes of amino acid sequences. **B.** 113 A-to-I RNA editing events conferred their deleterious effects on 52 tumor-related proteins assessed by SIFT, Polyphen2, and PROVEAN. Among these, there were 12 proteins with different RNA editing events whose effects were predicted to be diverse by these three tools. **C.** The Q/R editing in the key YXXQ motif of the BLCAP protein reverses the inhibition ability of BLCAP to STAT3, potentially facilitating the cancer-initiating and progressing events. **D.** The hypothesis in (C) was supported by its higher editing frequency in breast invasive carcinoma.

for all the 33 cancer types. In total, we identified 3600 RNA editing sites in the 3′-acceptor splice site (3′-ss) regions and 1779 RNA editing sites in the 5′-donor splice site (5′-ss) regions. They present diverse impacts on the splice site strength of 1957 genes, due to their different locations in the splicing sequences (**Figure 5**A). Among these editing events, 79 cases have verified their effects on alternative splicing (Figure 5B), through the differential percent spliced in (PSI) values in RNA-edited samples and significant correlations of PSI values with editing frequencies. Out of them, 6 A-to-I RNA editing events may have an opportunity to be involved in autophagy reduction [15], tumor growth [28], cell proliferation [17,29], cancer metastasis [17,29], toxicity mediation [30], and so on, because they altered the splicing patterns of TGs.

One case of them is the well-known R/G editing (CAediting_390714) in *GRIA2*. It altered the canonical splicing pattern of AG-GU and caused a reduction of the 5′-donor splice site strength ($5.37 - 8.23 = -2.86$) to potentially induce the intron retention (157360143:157361009) for the isoforms of *GRIA2* in the PCPG cancer type (Figure 5C–E). According to all the analyses mentioned above, this editing event may play important roles in neurological or brain cancers through altering the excitatory synaptic transmission by its contributions to protein function changes, alternative splicing, and dysregulation of *GRIA2* (Figure S12).

RNA editing candidates likely intervene in miRNA regulation on TGs

RNA editing sites in the binding targets of miRNAs or their seed regions would alter miRNA–RNA interactions and possibly affect the expression of miRNA-regulated genes. Through miRNA target prediction for wild-type (WT) and RNA-edited transcripts, we identified 96,278 RNA editing sites in 7930 transcripts, which were presumed to potentially create 402,284 new miRNA targets and eliminate 291,685 original ones (**Figure 6**A). These altered miRNA–target interactions further conferred their effects on the expression of 14,107 regulated genes (Table S1) other than the edited genes. Taking into account the functions of all these genes in tumor, we eventually selected 248 functional A-to-I RNA editing candidates, which may likely intervene in miRNA regulation on 436 TGs to play their roles in tumorigenesis (Figure 6B).

For example, an RNA editing site (CAediting_1478179) in the 3′-UTR of *APOL1*-201/202/205/206 isoforms would lead to the loss of original binding targets of miR-7151-3p (Figure 6C). The lost regulation seems to cause the increased expression of *APOL1* and indirectly lead to the reduced expression of *ZNF280D* with long non-coding RNA (lncRNA) transcripts in uterine corpus endometrial carcinoma (UCEC), due to their competing relationships (Figure 6D). For these

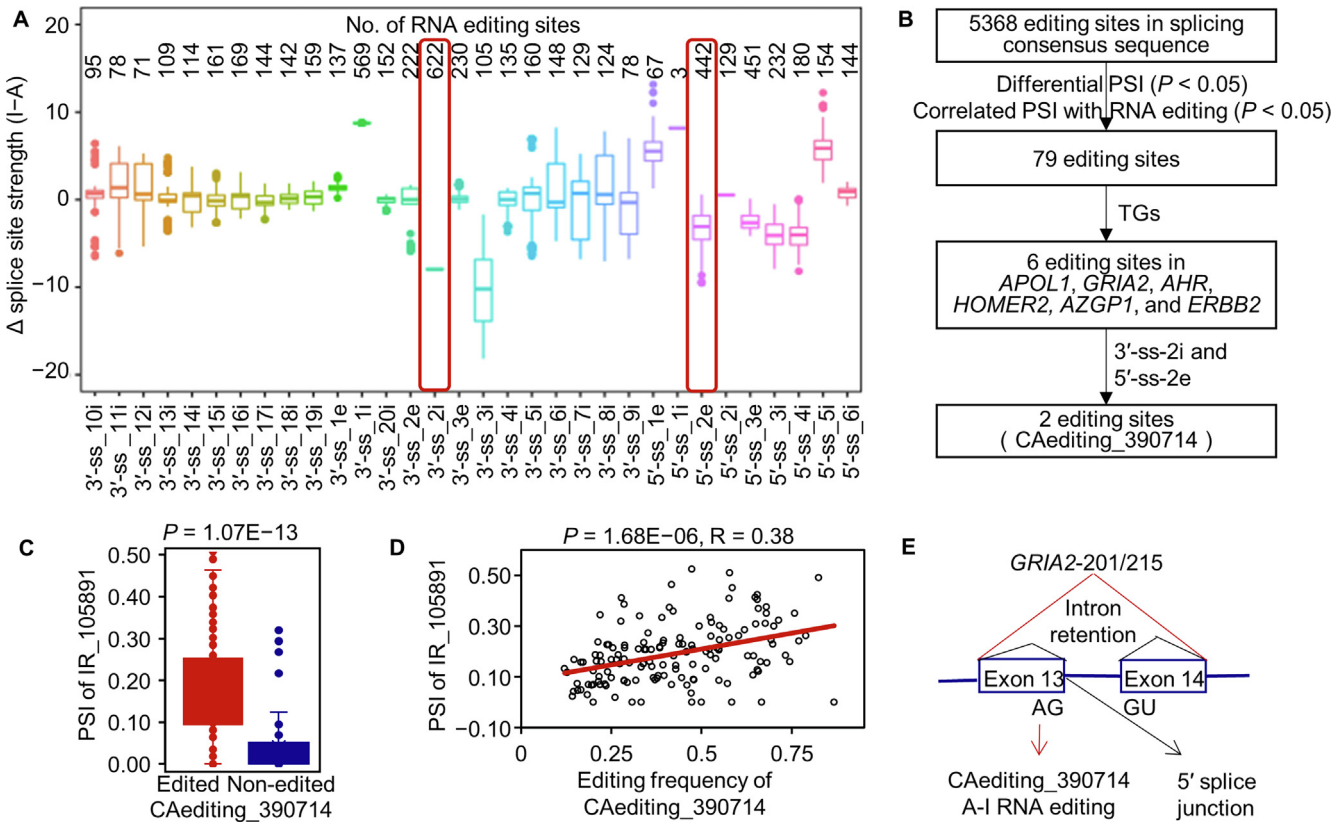

Figure 5 The effects of A-to-I RNA editing events on alternative splicing

A. The distribution of altered splice site strength caused by RNA editing events in the different positions of splicing regions. Individual RNA editing site may belong to different groups according to different exons. **B.** The analysis procedure for the effects of A-to-I RNA editing events on alternative splicing. **C.** The intron retention event (IR_105891) was mostly occurred in the RNA-edited group of PCPG. **D.** The intron retention event (IR_105891) was associated with the frequency of the editing event (CAediting_390714) in PCPG. **E.** A hypothesis that the R/G editing in *GRIA2* may alter the canonical splicing pattern of AG-GU to induce the intron retention for the isoforms of tumor-related *GRIA2* in PCPG. 3′-ss, 3′-acceptor splice site; 5′-ss, 5′-donor splice site; PSI, percent spliced in; PCPG, pheochromocytoma and paraganglioma.

two genes, several recent studies reported the induction function of *APOL1* in autophagy [15] to probably promote tumor cell growth and proliferation [31], and the fusion possibility of *ZNF280D* with the TSG of *STK3* [32,33] to involve in cancer. Besides, the DEG analysis also revealed the up-regulation of *APOL1* and down-regulation of *ZNF280D* in the UCEC tumor samples (Figure 6E). Therefore, we may infer CAediting_1478179 as a progression biomarker for the UCEC cancer type. It was also supported by the significantly higher frequencies of this editing event in the tumor samples, along with more severe tumor statuses and poorer survival probability (Figure 6F).

Another RNA editing example located in Chr7:5495852 (CAediting_524911) of miR-589-3p. It altered the miRNA binding target from original *DLEU1* to *LEFTY1* (Figure S14). The lost miRNA regulation led to the up-regulated expression of *DLEU1*, whereas the gained interactions caused the down-regulated expression of *LEFTY1* in the RNA-edited testicular germ cell tumor (TGCT) samples. From previous studies, we found that *DLEU1* is one lncRNA produced from the 13q14.3 tumor suppressor locus and regulates the NF-kB signaling pathway, which plays crucial roles in cancer initiation and progression [34,35]. We thus inferred a tumor suppressor role of *DLEU1* in TGCT because of its location in tumor suppressor locus, functions related to cancer, and also down-regulated expression in TGCT and more severe tumor samples. In addition, *LEFTY1*, a key gene in the Nodal pathway, was reported to be specifically associated with germ cell pluripotency, the presence of carcinoma *in situ*, and TGCT [36]. Moreover, this gene was also verified to be up-regulated in TCGT and along with more severe tumor samples. Due to the functions and expression alterations of these two genes, we could speculate that the edited miR-589-3p might potentially alleviate TGCT tumor condition, through the up-regulation of *DLEU1* and down-regulation of *LEFTY1*. In conclusion, these two editing candidates represent the potential functions of A-to-I RNA editing events in cancers through altering the miRNA regulation on TGs.

Discussion

Through measuring and analyzing RNA editing events in human pan-cancers, we could provide 24,236 potentially functional A-to-I RNA editing candidates (Table S3). They either were abnormally edited in cancers or might alter the original expression profiles, protein functions, splicing patterns, or miRNA regulation of TGs. Considering the contributions of

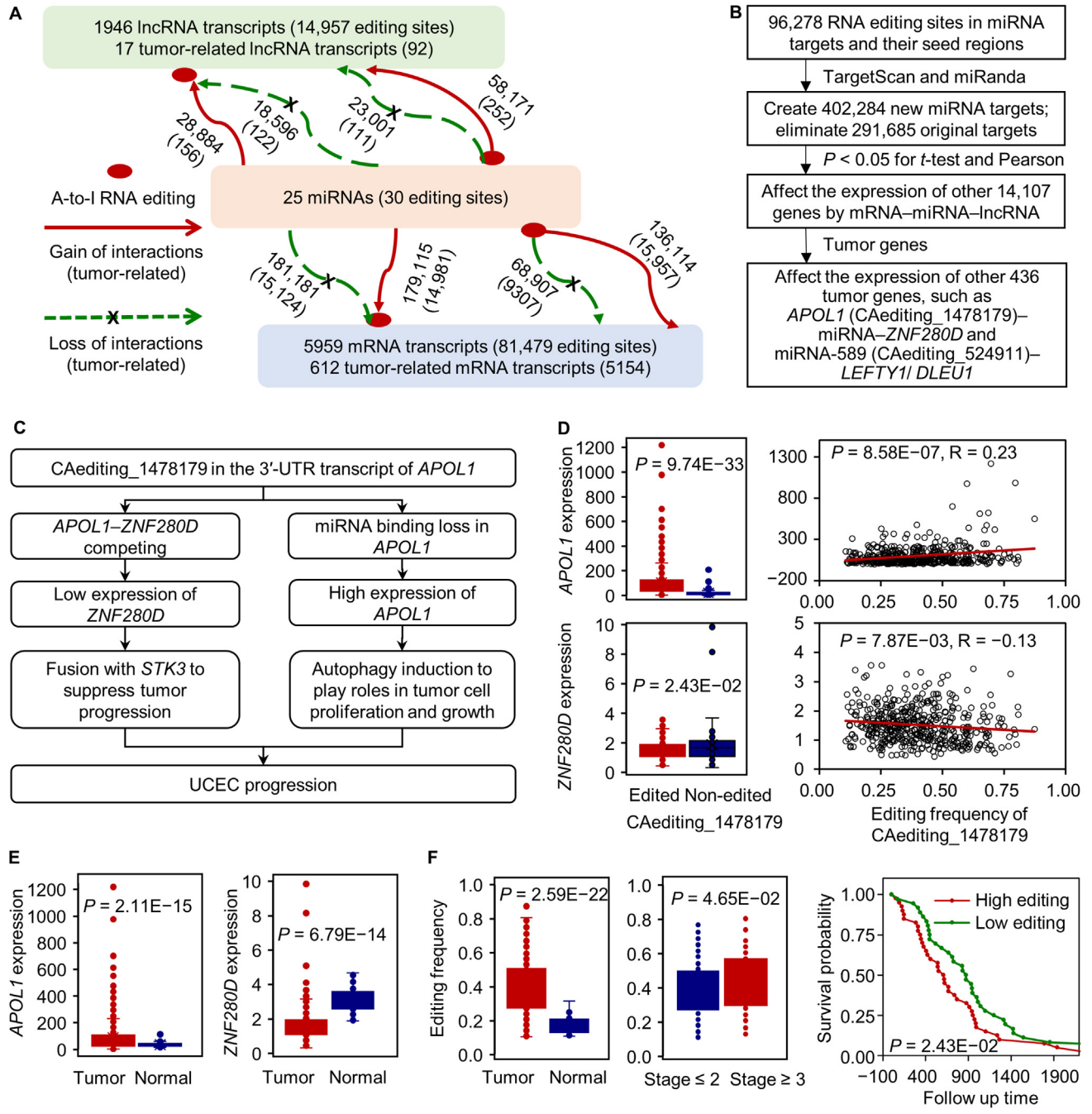

Figure 6 The effects of A-to-I RNA editing events on miRNA regulation

A. RNA editing events in the 3′-UTRs of mRNAs, lncRNAs, and miRNA seed regions led to the changes of miRNA–target interactions. Individual RNA editing site may locate in both of protein-coding or non-coding transcripts due to their possible overlaps. **B.** The analysis procedure for the effects of A-to-I RNA editing events on miRNA regulation. **C.** One significant RNA editing event (CAediting_1478179) in the 3′-UTR of *APOL1* likely intervened in the miRNA regulation on two TGs (*APOL1* and *ZNF280D*) to play its roles in the UCEC progression. **D.** The RNA editing event (CAediting_1478179) caused the loss of original miRNA binding target on *APOL1*. The altered miRNA regulation resulted in the increased expression of *APOL1* and indirectly led to the reduced expression of competing *ZNF280D* gene. **E.** *APOL1* and *ZNF280D* showed differential expression in UCEC, revealing their potential roles and functions in UCEC. **F.** Analyses uncovered CAediting_1478179 in the 3′-UTR of *APOL1* as a probably pathological biomarker of UCEC. Another significant case shown in panel B is described in Figure S14. UCEC, uterine corpus endometrial carcinoma.

these TGs to glutamine metabolism [17], modified immunity [26], selective autophagy [15], DNA damage responses [37], and so on, we may infer that the functional A-to-I RNA editing events may play important roles in tumorigenesis. In the future, the appearance of more functional RNA editing candidates will be accompanied by the increase of TGs. The

possible RNA editing events and their functions were all archived in CAeditome database (https://ccsm.uth.edu/CAeditome/).

Among them, five events were explored in detail to introduce their potential functions related to cancers in this study. They are CAediting_390714 of *GRIA2*, CAediting_1426931 of *BLCAP*, CAediting_1478179 of *APOL1*, CAediting_543208 of *IGFBP3*, and CAediting_524911 of miR-589-3p. The R/G editing in *GRIA2* (CAediting_390714) was studied deeply in previous work for its involvements in the desensitization of AMPA receptor (AMPAR) channels [27], AMPAR-mediated neurotransmission, and neurodevelopmental deficits [38,39]. For its potentials in cancers, one previous study validated its roles in tumor survival, cell viability, and targeted therapeutics [9]. In our study, for this editing event, we discovered its anomalously lower editing frequencies in GBM, and positive associations with aberrant expression profiles and alternative splicing values of *GRIA2* in PCPG. Due to the roles of this gene in proliferation stimulation, apoptosis resistance, migration, and invasion in cancer cell lines [17], we may suggest the possible bi-functions of this RNA editing event in neurological and brain tumors. As for the Q/R editing in *BLCAP* (CAediting_1426931), it was abnormally edited in multiple cancer types, such as the mere occurrence in the tumor samples of BLCA, COAD, HNSC, CHOL, and READ, higher editing frequencies in BRCA and KIRC, and positive associations with BLCA tumor stages. The analyses in our study expanded its roles of carcinogenesis promotion in pan-cancers from the cervical cancer reported in previous literature [26]. The third RNA editing event (CAediting_1478179) seems to be a novel and promising pathological biomarker for various cancer types including cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), COAD, and esophageal carcinoma (ESCA). Especially, it displayed remarkably up-regulated editing frequencies in tumors, more severe tumor samples, and poorer survival groups for the cancer types of KIRC (tumor *vs.* normal: P = 8.08E−05; editing *vs.* stage: P = 1.71E−10 and R = 0.28; editing *vs.* survival: P_{KM} = 3.21E−02, P_{COX} = 4.75E−02, and HR = 3.42), lung adenocarcinoma (LUAD) (tumor *vs.* normal: P = 1.52E−25; editing *vs.* stage: P = 6.59E−03 and R = 0.12; editing *vs.* survival: P_{KM} = 2.36E−02, P_{COX} = 6.81E−03, and HR = 3.88), and UCEC (tumor *vs.* normal: P = 2.59E−22; editing *vs.* stage: P = 4.32E−02 and R = 0.10; editing *vs.* survival: P_{KM} = 2.43E−02, P_{COX} = 1.50E−02, and HR = 5.74). Moreover, we discovered that it caused the loss of original miRNA binding targets to potentially induce the up-regulated expression of *APOL1*, which was supported by their positive associations in pan-cancers, such as for KIRC (P = 7.44E−06 and R = 0.20), LUAD (P = 6.78E−10 and R = 0.27), and UCEC (P = 8.58E−07 and R = 0.23). Thus, this RNA editing event may confer its pathological function in cancers through its intervention in miRNA regulation on the TG of *APOL1*. Another novel RNA editing event (CAediting_543208) occurred only in the tumor samples for multiple cancer types, especially for KIRC (246/535 *vs.* 0/72). It may enhance the inhibition ability of tumor cell growth through its positive impacts on the TSG of *IGFBP3* [16]. The last RNA editing event (CAediting_524911) was located in the seed region of miR-589-3p to potentially modify its original regulations on many TGs. For example, the edited miR-589-3p altered the expression levels of *DLEU1* and *LEFTY1*, which thus may alleviate TGCT tumor condition. Moreover, another evidence in one previous study also validated the regulatory potential of this RNA editing event (CAediting_524911) on two genes of *PCDH9* and *ADAM12* to control glioblastoma cell migration and invasion [40].

Of the five edited genes introduced in this study, three were discovered to be linked with tumor-related phenotypes from DisGeNET (January 2021, v.7.0) [41]. Specifically, *APOL1* is associated with neoplasm-related nephrotic syndrome and common focal segmental glomerulosclerosis form of kidney disease, *IGFBP3* plays important roles in multiple cancers, and *GRIA2* is related to neurological or brain diseases. Their possible relationships with these disorders may be partially attributed to the RNA editing events in them, which may also affect the effectiveness of probable drugs targeting them. In total, we discovered 6717 edited genes associated with 9203 different types of diseases in DisGeNET, and 1586 edited genes targeted by 1674 approved drugs from DrugBank (January 2021, v.5.1.8) [42]. The functions of A-to-I RNA editing events in these genes will be useful for exploring the pathological mechanisms of related diseases and providing novel knowledge to design the targeted drugs.

For the two novel RNA editing biomarkers in *APOL1* and *IGFBP3*, we also performed replication analyses in the lung squamous cell carcinoma (LUSC) samples from Cancer Cell Line Encyclopedia (CCLE) [43]. The analysis results (Figure S15) also supported the associations between these two editing events and their host genes mentioned in this study. Moreover, because CCLE contained metastasis information for each sample, we selected one RNA editing biomarker (CAediting_279186) in the metastasis-related gene of *RHOA* [44] for further validation. As shown in Figure S16, this editing event was negatively associated with *RHOA* expression. Given the abnormal expression of *RHOA* in primary tumors and metastasis samples, we could annotate the potential functions of this RNA editing event in cancer metastasis. The hypothesis was also partially held up after the analyses of this event in another metastasis dataset, MET500 [45].

Besides the five RNA editing events introduced in detail, there are also several potential candidates archived in CAeditome database that were validated or proposed in previous studies. For example, in our study, the analysis of RNA editing events in protein-coding regions identified I164V in *COPA* (CAediting_115738), S367G in *AZIN1* (CAediting_655260), and I635V in *COG3* (CAediting_962851). These three RNA editing events were also abnormally edited in tumor samples, correlated with tumor severity, associated with cancer survival, and possible factors to affect the expression of their host genes in multiple cancer types. Their functions in tumorigenesis have been validated in cell lines and mice models by other groups [9,46–48]. In addition, the analysis of RNA editing events in miRNA seed regions uncovered altered miR-200b regulation associated with CAediting_393. This event leads to the gain or loss of many miRNA binding targets, including that in *LIFR*, *ZEB1*, and *ZEB2*. Although the expression of these three genes was not significantly associated with the frequencies of this editing event as shown in different cell lines previously [7,49], we still included it as a potentially functional A-to-I RNA editing candidate, because it probably dysregulated other tumor-related genes (Download Page in CAeditome database). For example, *ACSL6* was down-regulated to possibly interfere in the metabolites of fatty acids, abnormality

of which is one of the cancer hallmarks [50]. Another gene *PKP1* was inhibited to prevent the survival and metastasis of cancer cells by decreasing cluster formation in circulatory system [51]. Moreover, there are also other RNA editing events whose functions in cancers were bio-experimentally validated, including H241R editing (CAediting_604339) in *PODXL* [4], K242R/K242E (CAediting_1062027/CAediting_1062028) editing in *NEIL1* [52], and two editing events (CAediting_442015/CAediting_442019) in the 3′-UTR of *GM2A* [53]. The other potential RNA editing biomarkers are waiting for the validation by cancer research communities.

In summary, this study proposed a transcriptome-wide and cancer-wide map for the functions of individual A-to-I RNA editing events. It will provide the chances to understand cancer pathology from the A-to-I RNA editing aspect and list potential biomarkers and therapeutic targets for cancer and drug research communities. However, during the analyses, the complex regulatory mechanisms associated with RNA editing pointed out two possible studies in the future.

Firstly, we noted the possible interactions of multiple RNA editing events and their co-effects on the downstream genes or regulations. For example, three RNA editing events were all in together to confer their effects on the expression of *APOL1*, which were deciphered by the least absolute shrinkage and selection operator regression method as shown in Figure S17. In the future, to uncover the co-regulation of RNA editing events, we will propose an RNA editing weighted gene expression network and evaluate its usefulness in various clinical scenarios such as survival prognosis.

On the other hand, we confirmed the deamination functions of three editing enzymes on 11,948 RNA editing events in this study ($P < 0.05$ and $R > 0.3$) (Figure S18). However, we should not ignore that there are another 28,062 RNA editing events showing no statistical associations with all the three enzymes. It revealed the possible multi-regulators of A-to-I RNA editing. From previous literature, these diverse regulatory mechanisms probably include genetic variations [54], splicing efficiency [55], and RNA binding proteins [56]. For a functional RNA editing candidate (CAediting_1478179 of *APOL1*) proposed in this study, we also discovered its differential editing frequencies among the genotyping groups of three single-nucleotide polymorphisms (SNPs) in the KIRC cancer type (Figure S19). For the impacts of genetic variants on RNA editing events, recently, Leng Han group has published a database named GPEdit [57]. The studies on the other potential regulators of A-to-I RNA editing are our further research plans.

Materials and methods

Detection of A-to-I RNA editing

For all the 11,056 RNA sequencing samples across 33 cancer types in TCGA (Table S1), we first detected RNA editing candidates by the script of REDItoolKnown.py (REDItools v.1.2.1) [58] with default settings (*i.e.*, minimal read coverage = 10; minimal quality score = 30; and minimal mapping quality score = 255) and the hg38 reference files (GENCODE v.22) same as that used in the Genomic Data Commons (GDC) data harmonization and generation pipelines. These detected candidates were then checked for their reliability. Only the candidates, which occurred in the REDIportal database (January 2021) [59] but did not belong to SNPs (dbSNP151 and Genome-Wide Human SNP Array 6.0 in hg38 version converted by LiftOver [60]), were covered by more than three edited reads, and showed editing frequencies higher than 0.1, were considered as reliable editing sites. Eventually, we selected one kind of RNA editing types, A-to-I RNA editing, for further analysis, because of its abundance in humans.

For all the detected A-to-I RNA editing events, we analyzed their distributions in diverse genomic locations and repeats by ANNOVAR [61], and evaluated the stability alterations of edited transcripts by RNAfold (ViennaRNA v.2.4.17) [62]. The consistency of these analysis results (Figures S20 and S21) with previous publications [10,12] revealed the reliability of A-to-I RNA editing detection in this study. Moreover, all these events covered a high ratio of RNA editing sites (72.49%) detected in one previous study [9] using a different pipeline as shown in Figure S22. It described the consistency of our RNA editing detection pipeline with others. In addition, the more RNA editing sites detected in our study provided an opportunity for a more comprehensive map of functional A-to-I RNA editing events in cancers.

Analysis of A-to-I RNA editing frequencies

To uncover potential A-to-I RNA editing events related to tumors, we first compared their editing frequencies between tumor samples and normal controls across 33 cancer types. Then we defined a tumor-specific RNA editing event if it only occurred in tumors with more than 5 edited samples or showed significantly differential editing frequency ($P < 0.05$) in tumor samples. Next, we analyzed the correlations between editing frequency and tumor stage (pathologic stage or clinical stage, $P < 0.05$), to identify tumor progression-associated RNA editing events. Third, we performed KM and COX analyses to determine the A-to-I RNA editing events ($P < 0.05$ for both results) that may affect tumor survival. Last, we focused on the RNA editing events in 1615 tumor-related genes, including driver oncogenes from OncoVar [63], TSGs from TSGene 2.0 [64], and some other genes reported to be associated with tumors in previous literature [15,17], in order to further analyze the possible effects of A-to-I RNA editing in cancers.

Analysis of A-to-I RNA editing effects on gene expression and pathways

To study the effects of A-to-I RNA editing events on gene expression, we performed the analyses of DEGs between RNA-edited and non-edited tumor samples ($P < 0.05$ and $|\log_2 FC| > 0.3$) by *t*-test and Pearson correlations between editing frequency and corresponding gene expression ($P < 0.05$) across 33 cancer types. The dysregulated genes in RNA-edited tumor groups and also along with the changes of editing frequency were inferred to be potentially affected by A-to-I RNA editing. The genes which overlapped with the DEGs identified in tumors compared with controls (*t*-test: $P < 0.05$ and $|\log_2 FC| > 0.3$) were further studied

by Enrichr [65], to assess the probably involved cellular processes of A-to-I RNA editing in cancers.

Analysis of A-to-I RNA editing effects on protein recoding and functions

For A-to-I RNA editing events in coding regions, we used ANNOVAR to detect the changes of amino acid sequences caused by the non-synonymous and stop-loss editing sites. These alterations are shown in lollipop figures with editing sites and UniProt KnowledgeBase (UniProtKB) protein IDs [66] converted from BioMart [67]. Then, their deleterious effects on protein functions were assessed by SIFT, Polyphen2, and PROVEAN (dbNSFP v.4.1a).

Analysis of A-to-I RNA editing effects on alternative splicing of pre-mRNAs

To study the effects of A-to-I RNA editing events on splicing, we first overlapped them with 5′-ss and 3′-ss regions around detected exons [68]. The 5′-ss region is a 9-mer region of 3 nt in the exon and 6 nt in the intron, whereas the 3′-ss region is a 23-mer region of 3 nt in the exon and 20 nt in the intron based on a previous splicing study [69]. MaxEntScan method proposed in that study was also used here to estimate the changes of splice site strength for sequences being edited. These splicing alterations were further validated by the comparisons of PSI values between RNA-edited and non-edited tumor samples ($P < 0.05$) and the correlations of PSI values with corresponding editing frequencies ($P < 0.05$), to discover the reliable effects of A-to-I RNA editing events on splicing patterns.

Analysis of A-to-I RNA editing effects on miRNA regulation

For the WT and RNA-edited 3′-UTRs of mRNAs, lncRNAs, and miRNA seed regions, we used TargetScan (v.7.0) and miRanda (v.3.3a) to detect miRNA binding targets. Based on the predicted miRNA–lncRNA/mRNA 3′-UTR interactions, we defined the gain of miRNA binding targets as the interactions existing in the RNA-edited sequences but not in the WT sequences supported by both tools and *vice versa* for the loss of miRNA binding targets. Furthermore, we checked the expression of miRNA-regulated genes between RNA-edited and non-edited tumor groups ($P < 0.05$) and also along with the changes of editing frequencies ($P < 0.05$), to discover the altered miRNA regulation caused by these A-to-I RNA editing events.

Data availability

All the analysis results involved in this study are available at https://ccsm.uth.edu/CAeditome/.

Competing interests

The authors have declared no competing interests.

CRediT authorship contribution statement

Sijia Wu: Conceptualization, Data curation, Methodology, Investigation, Software, Formal analysis, Writing – original draft, Funding acquisition. **Zhiwei Fan:** Visualization. **Pora Kim:** Conceptualization, Visualization, Validation, Writing – review & editing. **Liyu Huang:** Supervision, Project administration, Resources, Funding acquisition. **Xiaobo Zhou:** Supervision, Project administration, Resources. All authors have read and approved the final manuscript.

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Supplementary material

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