Multi-tissue transition of A-to-I RNA editing pattern and its regulatory relevance in transcription, splicing, and translation during development

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Abstract

Previous studies have shown that A-to-I RNA editing can occur in various organs and tissues of normal physiological conditions. However, the dynamics of RNA editing and its functional relevance in multiple tissues and organs during the embryo-to-adult transition in mammals remains to be elucidated. Herein, we performed a comprehensive analysis of RNA sequencing and ribosome profiling of six mouse tissues at embryonic and adult stages, to elucidate the tissue- and stage-specific landscape of A-to-I RNA editing. Our result identified transcriptome-wide A-to-I RNA editing in six tissue types. Furthermore, differential expression was concurrently observed in a set of distinct differential RNA editing genes at both mRNA and protein levels across different tissues. Gene function and pathway enrichment analysis indicated that these genes with both differential editing and expression were involved in not only tissue-specific biological functions, but also common fundamental processes of post-transcriptional and post-translational modification. Further analysis showed a dynamic interaction between A-to-I RNA editing and alternative splicing in cell survival, death, signal transduction, and cell-cell interactions during development. Overall, our study demonstrates the potential role played by A-to-I editing during development, providing new insight into the effects of RNA editing within both transcriptional and translational landscape on it.

Keywords: A-to-I editing, transcription, translation, alternative splicing, development

1. Introduction

RNA editing is a post-transcriptional mechanism that alters the transcript sequence and can increase transcript and protein diversities [1]. Adenosine-to-inosine (A-to-I) RNA editing, catalyzed by the adenosine deaminases acting on RNA (*ADAR*s) family, is a canonical type of RNA editing in mammals [2, 3]. A-to-I RNA editing is involved in diverse biological processes, depending on the editing location. RNA editing profiles may vary among tissues and can also dynamically regulate critical biological processes, such as brain development, cancers, and metabolic disorders [4-7].

Mammals have almost identical genome sequences but exhibit great functional diversity in different tissues [8-11]. Correspondingly, A-to-I RNA editing in different tissues and developmental stages may also present complex landscapes and functions [12]. Therefore, the study of A-to-I RNA editing during mammalian development could not only reveal the dynamic regulation of RNA editing but also help unravel the complex mechanisms of this process. Previous studies have shown that A-to-I editing plays a crucial role in brain and neurological development, especially in the context of ion channels, neurotransmitter receptors, and synaptic plasticity, and contributes to the complexity and adaptability of the nervous system [4, 13]. Recent genome-wide characterization of RNA editing highlighted roles of high editing events of glutamatergic synapse during retinal development [14]. However, most of these studies have focused on the analysis of RNA editing in single tissues or organs, while further investigation is needed to compare RNA editing and their functional relevance to expression processes such as transcription, splicing, and translation across different tissues or organs.

In the current study, we performed comprehensive transcriptome and translatome analysis of six types of mouse tissues at both embryonic and adult stages. Our research findings suggested the substantial regulatory role of A-to-I RNA editing in transcription,

splicing and translation, providing new insight into the potential effects of RNA editing on both transcriptional and translational landscape during development.

2. Materials and methods

2.1. Dataset download

Reads of RNA sequencing (RNA-seq) and ribosome profiling (Ribo-seq) from a dataset (GSE94982) were downloaded from the NCBI Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo) database. The dataset contains six types of tissue samples from wild-type C57BL/6 mice at embryonic day (E) 15.5 and postnatal day (P) 42 (N = 2 each tissue group per time point), including ectoderm-derived brain and retina, mesoderm-derived heart and kidney, as well as endoderm-derived liver and lung. The tissue collection, RNA extraction, and library construction and sequencing were performed as described in the original study [15].

2.2. Data processing

The raw sequencing reads of RNA-Seq and Ribo-seq were processed using FASTP (version 0.23.4) to remove adapters and low-quality reads [16]. The obtained Clean data were mapped to the mouse reference genome (UCSC mm10) using RNA STAR (Version 2.7.0e) [17], generating alignment files in BAM format. SAMtools (Version 1.9) was used to eliminate multiple mappings and optical duplications of reads, retaining reads uniquely mapped to the mouse genome [18]. GATK (Version 4.1.3) was used to recalibrate the base quality scores of the BAM files by following the best practices suggested by the software [19].

2.3. Identification and annotation of A-to-I editing sites

VarScan (version 2.4.3) was utilized to call variants to identify single nucleotide variations (SNVs) [20]. Given that inosine (I) is interpreted as guanosine (G) during reverse transcription and translation, A-to-I RNA editing is also referred to as A-to-G RNA editing [21, 22]. Filtering was performed using a standard bioinformatic pipeline described in our previous study [23, 24].

Annotation was carried out using the Ensembl Variation Effect Predictor (VEP) [25]. Briefly, only SNVs that met the following criteria were kept: base quality ≥ 25, total sequencing depth ≥ 10, alternative allele depth ≥ 2, and alternative allele frequency (AAF) ≥ 1%. SNVs that met the following criteria were filtered and removed unless annotated in the REDIportal V2.0 database [26, 27]: (1) located within the mitochondria DNA, simple repeat sequences, or homopolymer runs ≥ 5 nucleotides (nt); (2) located within six nt of splice junctions or one nt of insertions-deletions (INDELs); (3) annotated in the dbSNP database as known variant types; (4) with an AAF equal to 100% or ranging between 40% and 60% in over 90% of samples; (5) covered by high-quality reads < 10, or edited reads < 2, or editing levels < 1%.

2.4. Identification of differential RNA editing sites

Generalized Linear Models (GLM) and Likelihood Ratio Tests (LRT) were used to compare RNA editing levels between embryonic and adult stages. Differential RNA editing (DRE) sites were identified using the following criteria: (1) differential editing levels ≥ 1%; (2) false discovery rate (FDR) < 0.05 [28].

2.5. Quantification of gene expression

FeatureCounts (Version 2.0.1) was utilized to extract pseudo-counts of gene expression from BAM files [29]. Subsequently, EdgeR (Version 3.7) was used to calculate transcripts per kilobase million (TPM) values for each gene, and differential gene expression analysis was carried out by the Generalized Linear Model (GLM) [30]. *P*-values < 0.05 were determined to be statistically significant. The correlation coefficients (*r*) between RNA editing level and gene expression were calculated using Pearson correlation analysis.

2.6. Enrichment analysis of gene function and pathways

Enrichr (https://maayanlab.cloud/Enrichr/) [31] and DAVID online prediction tools (https://david.ncifcrf.gov/tools.jsp) [32] was used to perform Gene Ontology (GO) biological process enrichment analysis. Items with *P*-value < 0.05 were considered to be statistically

significant.

2.7. Prediction and mapping of RNA binding proteins binding sites

RBPmap website (http://rbpmap.technion.ac.il) was used to predict the binding patterns of RNA binding proteins (RBPs) to differential A-to-I RNA editing sites [33]. The results of RBPs binding prediction were visualized with WordCloud package (Version 2.6).

2.8. Analysis of differential alternative splicing

The replicate multivariate analysis of transcript splicing (rMATS) (Version 4.0.2) was used to detect differential alternative splicing (DAS) events across different tissues [34]. The obtained alternative splicing (AS) events were classified into five categories: skipped exon (SE), retained intron (RI), alternative 5′ splice sites (A5SS), alternative 3′ splice site (A3SS) and mutually exclusive exons (MXE). For each AS event, inclusion and exclusion junction reads were averaged from replicates and used to calculate the Inclusion Level Difference (ΔPSI). DAS events were identified using the following criteria: (1) | ΔPSI | > 10%; (2) *P*-values < 0.05.

3. Results

3.1. Tissue-specific A-to-I RNA Editing across mouse development

From the RNA-seq data of six tissues at E15.5 and P42, we identified a total of 618 high-confidence A-to-I RNA editing sites in 317 genes in brain, 501 high-confidence A-to-I RNA editing sites in 265 genes in the heart, 503 high-confidence A-to-I RNA editing sites in 254 genes in the kidney, 479 high-confidence A-to-I RNA editing sites in 235 genes in the liver, 660 high-confidence A-to-I RNA editing sites in 323 genes in the lung, 853 high-confidence A-to-I RNA editing sites in 404 genes in the retina **(Figure 1A; Supplementary Table S1)**. Analysis of the functional categories showed that the majority (68.5-86.4%) of them were 3′-untranslated region (3′UTR) RNA editing. Missense RNA editing constituted to only a small proportion (2.7 -7.3%) of the total variants **(Figure 1B,**

Supplementary Figure S1A). Sorting intolerant from tolerant (SIFT) analysis predicted 15.4 - 33.3% of the missense variants could potentially impact function of the encoded protein **(Figure 1C, Supplementary Figure S1B)**. Motif analysis showed that G was suppressed at one base upstream but preferred at one base downstream of the editing sites **(Figure 1D)**, consistent with the reported sequence preference of *ADAR* editing.

To investigate the dynamic regulation of A-to-I RNA editing, we compared the existence of editing sites and edited genes between E15.5 and P42 in different tissues. As shown in **Figure 2A and Supplementary Figure S2**, generally more editing sites and edited genes at P42 than E15.5 were observed across different tissues, except for the heart.

3.2. Differential A-to-I RNA Editing between embryonic and adult stages

To systematically analyze RNA editing events during development, we then analyzed differential RNA editing (DRE) between embryonic and adult stages. We identified 186, 110, 117, 112, 208, 294 DRE sites in these six tissues, respectively **(Figure 2B; Supplementary Table S2)**. The top 50 DRE sites in different tissues ranked by p-values are shown in **Figure 3**. In addition, we found that most of the DRE sites were up-regulated at P42 compared to E15.5. Consistent with the overall RNA editing landscape, most of the DRE sites were also found in 3UTR **(Figure 2C)**, and showed similar sequence motifs across all tissues **(Supplementary Figure S3A)**.

We then evaluated the expression of *Adar*s across different tissues at both stages. Our findings revealed a dramatic increase in Adarb1 expression during the development of the brain and retina at both transcriptional and translational levels **(Figure 2E)**. *Adar* was significantly up-regulated in the brain at the translational level but not at the transcriptional level and significantly up-regulated in the retina at the transcriptional level but not the translational level **(Figure 2D)**. In addition, we observed a significant positive correlation between the mean editing level and *Adarb1* mRNA expression both in the brain and retina

(*Pearson*'s r = 0.99, P = 0.006 and *Pearson*'s r = 0.97, P = 0.029, respectively) **(Figure 2F, G)**. We then identified *Adarb1*-correlated DRE sites by evaluating the correlation of their editing levels with *Adarb1* mRNA expression levels in the brain and retina. 11 *Adarb1*-correlated DRE sites in 11 genes. 18 ones in 13 genes were identified in the brain and retina, respectively, all of which sites were significantly up-regulated and positively correlated with *Adarb1* expression (**Supplementary Figure S4, S5**). These observations indicated that *Adarb1* may be the primary editing enzyme of A-to-I editing sites in the brain and retina. However, we failed to observe a significant correlation between editing levels and *ADAR* expression in the other four tissues **(Supplementary Figure S3B, C)**.

3.3. Functional enrichment analysis of differential A-to-I RNA Editing genes

To explore the biological function of tissue-specific A-to-I RNA editing in development, we performed enrichment analysis of DRE genes in each tissue, respectively. GO analysis indicated that these DRE genes were not only enriched for certain tissue-specific biological functions but also various routine biological processes related to development, such as 'positive regulation of growth hormone secretion', 'regulation of mitotic cell cycle', 'RNA-dependent DNA biosynthetic process' and 'phosphate-containing compound metabolic process' **(Figure 2H; Supplementary Table S3)**.

3.4. Potential effects of A-to-I RNA editing on transcription and translation

To explore the landscape function of A-to-I RNA editing at transcriptional and translational levels, we compared the DRE genes and differentially expressed genes (DEGs) identified by mRNA-Seq or Ribo-seq in different tissues **(Figure 4A; Supplementary Table S4)**. Our results revealed that 46, 18, 40, 37, 59, and 58 DRE genes were differentially expressed both in mRNA-Seq and Ribo-Seq in the brain, heart, kidney, liver, lung, and retina, respectively **(Figure 4B)**. We then evaluated these DRE genes with differential expression for significant functional enrichment in at least two tissue types. DRE genes with up-regulated expression

were enriched in routine biological processes of protein modification and metabolic regulation, such as 'protein-containing complex localization', 'dephosphorylation', 'regulation of lipid metabolic process', and 'glycerolipid metabolic process', whereas DRE genes with down-regulated expression were enriched in biological processes related to DNA and RNA dynamics, such as 'DNA conformation change', 'DNA replication', 'mRNA export from nucleus' and 'RNA localization' **(Figures 4C, D; Supplementary Table S5)**.

In addition, it was also noticed that 59, 40, 20, 14, 37, and 51 DRE genes were differentially expressed at the transcription level only but not at the translation level in the brain, heart, kidney, liver, lung, and retina, respectively **(Supplementary Figure S6A)**. We then evaluated these DRE genes with differential RNA expression for significant functional enrichment in at least two tissue types. Likewise, our enrichment analysis indicated that these DRE genes, with transcriptional differential expression only, were enriched for biological processes involved in the regulation of cellular processes and metabolic processes, such as 'cellular component disassembly', 'negative regulation of cell motility', 'thioester metabolic process' and 'nucleoside bisphosphate metabolic process', while these DRE genes with down-regulated expression were mainly enriched for biological processes involved in DNA regulation and gene silencing, such as 'positive regulation of DNA repair', 'positive regulation of DNA metabolic process', 'regulation of gene silencing' and 'positive regulation of posttranscriptional gene silencing' **(Supplementary Figure S6B, C; Supplementary Table S4)**.

3.5. RNA binding prediction of differential A-to-I RNA editing sites

To gain insights into the potential functional impact of RNA editing, we utilized the RBPmap database to predict the binding patterns of RBP overlapping with differential A-to-I RNA editing sites across different tissues **(Supplementary Figure S7)**. **Figure 5** illustrates the top 10 RBPs with the highest binding frequencies to differential A-to-I RNA editing sites across

different tissues. Notably, RNA binding motif protein 45 (*RBM45*), Heterogeneous nuclear ribonucleoprotein A0 (*HNRNPA0*), Serine and arginine-rich splicing factor 10 (*SRSF10*), Unk Zinc Finger (*UNK*), CCR4-NOT transcription complex subunit 4 (*CNOT4*) were identified across all six tissues. This suggests that these RBPs may play a crucial and ubiquitous role in regulating RNA editing, indicating potential functional significance across gene expression and cellular processes.

3.6. Interaction between A-to-I RNA editing and alternative splicing

To gain insights into the interplay between A-to-I RNA editing and alternative splicing, we used rMATS to identify alternative splicing (AS) events and differential alternative splicing (DAS) events in different tissues. We found that 39-54% of genes with A-to-I RNA editing also exhibited alternative splicing in these six tissues, suggesting a potential interaction between A-to-I RNA editing and alternative splicing **(Figure 6A)**. Then, we identified DAS events and genes between embryonic and adult stages **(Figure 6B; Supplementary Table S6)**. Amongst the six tissues, the brain and retina exhibit the largest numbers of DAS events and genes. Comparing DRE genes and DAS genes, we found that 4-40 DAS genes were also differentially edited in these six tissues during development **(Figure 6C)**. Enrichment analysis indicated that these genes were involved in various routine biological processes related to development, such as 'negative regulation of programmed necrotic cell death', 'positive regulation of cytoplasmic transport', 'fibroblast growth factor receptor signaling pathway', and 'neuron cell-cell adhesion.' This suggests that RNA editing and alternative splicing were closely intertwined in cellular processes such as survival, death, signal transduction, and cell-cell interactions during development **(Figure 6D)**.

4. Discussion

ADAR-mediated A-to-I RNA editing could be crucial for the mammalian development of various organs and tissues [35]. However, the dynamic regulation of RNA editing during the

mammalian embryo-to-adult transition remains to be further elucidated. In the current study, we performed a transcriptome-wide RNA editing analysis of six mouse tissues to study the tissue- and stage-specific landscape of A-to-I RNA editing during development. Our analysis revealed dramatic RNA editing changes in the development from embryonic into adult stages across different tissues, most of which exhibited significantly increased editing levels and were involved in potential biological functions related to development.

Among these DRE sites, 61 were found to be missense RNA editing. Notably, significant up-regulation of two missense editing c.284A>G (p.K95R) and c.232A>G (p.R78G) in the insulin-like growth factor binding protein 7 (*Igfbp7*) gene were observed in the mouse brain, heart and kidney at the adult stage compared to the embryonic stage. These two conserved missense sites were also found in other mammals, such as humans and pigs [36]. *Igfbp7* encodes a member of the soluble proteins that bind insulin growth factor (IGF) and suppress downstream signaling, thereby inhibiting protein synthesis, cell growth, and survival [36]. Recent studies have observed that K95R and R78G editing in *IGFBP7* showed a high level in the adult brain and cardiovascular tissues, and are reported to be up-regulated in atherosclerosis and cardiomyopathies [37, 38]. R/G substitutions in the IGF binding domain may disrupt the interaction between the protein and its IGF ligands [39]. In addition, the R78G and K95R recordings have been reported to possibly modulate its susceptibility to proteolysis [40]. Our results also found another missense editing c.14A>G (p.Q5R) in the bladder cancer-associated protein (*Blcap*) gene was observed in the mouse heart, kidney, and retina. The editing level of this site decreased in the heart and kidney yet increased in the retina during the development from the embryo to adult. *Blcap* encodes a tumor suppressor that prevents tumorigenesis by promoting apoptosis and inhibiting cell proliferation. RNA over-editing of *BLCAP* can activate the Akt/mTOR signaling pathway by regulating the phosphorylation level, thus promoting the progression of hepatocellular carcinoma [41] and

cervical carcinogenesis [42]. In addition, missense editing in Neuroligin 2 (*Nlgn2*) c.406A>G (p.N136D) was also observed to be up-regulated in the brain, lung, and retina in adult mice compared to the embryo, which probably abolishes an N-glycosylation target site in Nlgn2 [43] and thus might affect neurotransmission. *Nlgn2* encodes a transmembrane scaffolding protein and promotes synapse development by forming trans-synaptic bridges spanning the synaptic cleft [44]. Moreover, *Nlgn2* has been reported to be associated with neurological and psychiatric disorders, such as schizophrenia [45], autism [46], and anxiety [47]. The functional relevance of most of these missense DRE sites in development, especially those in *Igfbp7* and *Nlgn2*, remains poorly understood.

Interestingly, our results showed that up-regulated RNA editing in a subset of long intergenic non-coding RNA (lincRNA), such as small nucleolar RNA host gene 11 (*Snhg11*), H19 imprinted maternally expressed transcript (*H19*), and maternally expressed 3 (*Meg3*) during development. One intronic editing variant in *Snhg11* and two exonic editing variants in *Meg3* were significantly up-regulated in the brain in adult mice. Seven intronic editing variants in *Snhg11* and four exonic editing variants in *Meg3* were significantly up-regulated in the retina in adult mice **(Supplementary Table S2)**. *Snhg11* has been reported to promote cell proliferation, migration, apoptosis, and autophagy during cancer development [48, 49]. Recent studies implicated that *Snhg11* also plays a neuroprotective role in neuronal injury [50]. Previous studies have suggested *Meg3* not only contributed to cancer development but is also associated with the pathological stages of cancer and the prognosis of cancer patients [51]. Recent studies have shown that *Meg3* regulates AMPA receptor expression on the surface of primary cortical neurons and plays a vital role in the nervous system [52]. The hyper editing in *Snhg11* and *Meg3* and differential RNA editing in both genes suggest a potential role of these two lincRNA genes during brain and retina development.

In mammals, A-to-I editing is catalyzed by *ADAR*s, including *ADAR1*, *ADARB1* and

ADARB2 [53]. Our findings revealed that *Adarb1* could be primarily responsible for the dynamics of A-to-I editing during the development of the brain and retina, compared to *Adar* and *Adarb2*. However, the expression of *Adar*, *Adarb1*, and *Adarb2* have no significant difference in the other four tissues. These results suggest that the *ADAR* family exhibits tissue-specific mediation in A-to-I RNA editing, highlighting the vital role of *Adarb1* in regulating A-to-I RNA editing activity during central nervous system (CNS) development.

Dynamic regulation of transcription and translation plays a key role in embryonic development and maintenance of adult tissues [54, 55]. This regulation involves multiple dimensions, with RNA modification standing out as a crucial facet in this intricate process [56, 57]. Previous studies have shown that A-to-I editing could play an important role in regulating transcriptome diversity [58]. However, the impact of RNA editing on translation during mammalian development remains largely unexplored. Ribo-seq can reveal the intricacies of translation dynamics, enabling exploration of the specific regions of mRNA that are actively undergoing translation by ribosomes [59]. Our results revealed certain differentially edited genes with differential expression at both mRNA and protein levels, which were primarily involved in both tissue-specific and routine biological processes related to development. Previous studies have shown that A-to-I editing is crucial in neurological development [13]. Ectoderm-derived brain and retina are important components of the CNS [60]. Our results consistently showed that DRE might contribute to brain and retina development by regulating the expression of genes related to synaptic vesicles and neurotransmitters, highlighting its crucial role in CNS development.

Overlapping of differentially edited sites and RBP binding sites provides a potential link between RNA editing and RBP functions in development, such as the regulation of pre-mRNA splicing and high-order RNA structures [61]. Our study identified a set of 5 core RBPs with the highest binding frequencies to DRE sites across different tissues, including

RBM45, *HNRNPA0*, *SRSF10*, *UNK*, and *CNOT4*. *RBM45* encodes an RBP involved in neural development, and its pathological aggregation is closely related to neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS) and frontotemporal lobar dementia (FTLD) [62]. *SRSF10* encodes an atypical member of serine/arginine-rich (SR) proteins [63], and has been reported to influence glucose, fat, and cholesterol metabolism, embryonic heart development, and neural processes by controlling alternative splicing of specific transcripts [64]. *CNOT4* encodes an evolutionarily conserved E3 ubiquitin ligase [65]. *CNOT4* has been demonstrated to play synergistic biochemical functions during development and is required for post-implantation embryo development and meiosis progression during spermatogenesis [66]. Our study suggested the involvement of these RBPs in the downstream effects of differential RNA editing during development. Further experimental and functional studies will be needed to further understand the interplay between these RBPs and RNA editing during development.

Previous studies have shown that A-to-I editing of pre-mRNA before RNA splicing could be closely related to alternative pre-mRNA splicing [61, 67]. However, a global understanding of the interplay between RNA editing and alternative splicing during development is still missing. Our study systematically compared differential A-to-I RNA editing genes and differential alternative splicing genes in six tissues, revealing their closely intertwined in cell survival, death, signal transduction, and cell-cell interactions during development, providing a new insight into the relationship between RNA editing and alternative splicing.

In conclusion, our study suggested a transition of A-to-I RNA editing pattern across multiple tissues and its regulatory relevance in transcription, splicing, and translation during development. Further investigation will be needed to evaluate the role and mechanisms of RNA editing within both transcriptional and translational landscapes.

Funding

This study was supported in part by grants from the National Natural Science Foundation of China (no. 31671311), the "Six Talent Peak" Plan of Jiangsu Province (no. SWYY-127), the Program for High-Level Entrepreneurial and Innovative Talents of Jiangsu Province, Natural Science Foundation of Guangdong Province/Guangdong Basic and Applied Basic Research Foundation (2019A1515012062), Taihu Lake Talent Plan, and Fundamental Research Funds for the Central Universities (JUSRP51712B and JUSRP1901XNC).

CRediT authorship contribution statement

Jia-Qi Pan: Conceptualization, Data curation, Formal analysis, Writing - original draft. **Xu-Bin Pan:** Conceptualization, Methodology, Software. **Yan-Shan Liu**: Methodology, Software, Supervision. **Yun-Yun Jin:** Methodology, Project administration, Supervision. **Jian-Huan Chen:** Conceptualization, Supervision, Funding acquisition, Writing – review & editing.

Declaration of Competing Interest

All authors declare that they have no known financial or personal relationships that might influence the publication of this paper.

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Figure Legends

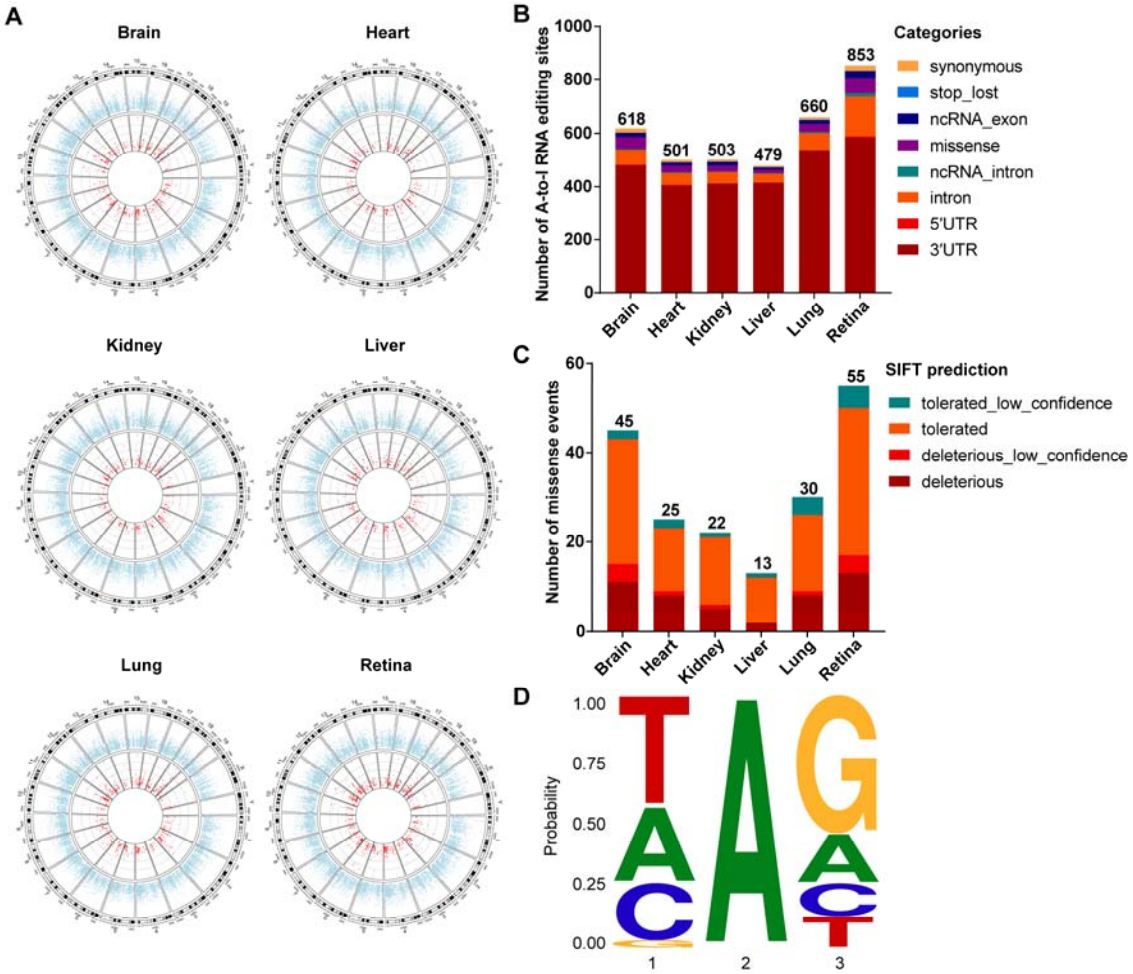

Figure. 1. Transcriptome-wide analysis of A-to-I RNA editing in different tissues from embryonic (E15.5) and adult (P42) mice. (A) Circos plot of gene expression and A-to-I RNA editing sites in all six tissues. The outer circle denotes the mean expression level of genes, and the inner circle denotes the A-to-I RNA editing sites. **(B)** Functional categories of A-to-I RNA editing variants in all six tissues. **(C)** SIFT prediction of missense A-to-I RNA editing variants. **(D)** Motif context around A-to-I RNA editing sites.

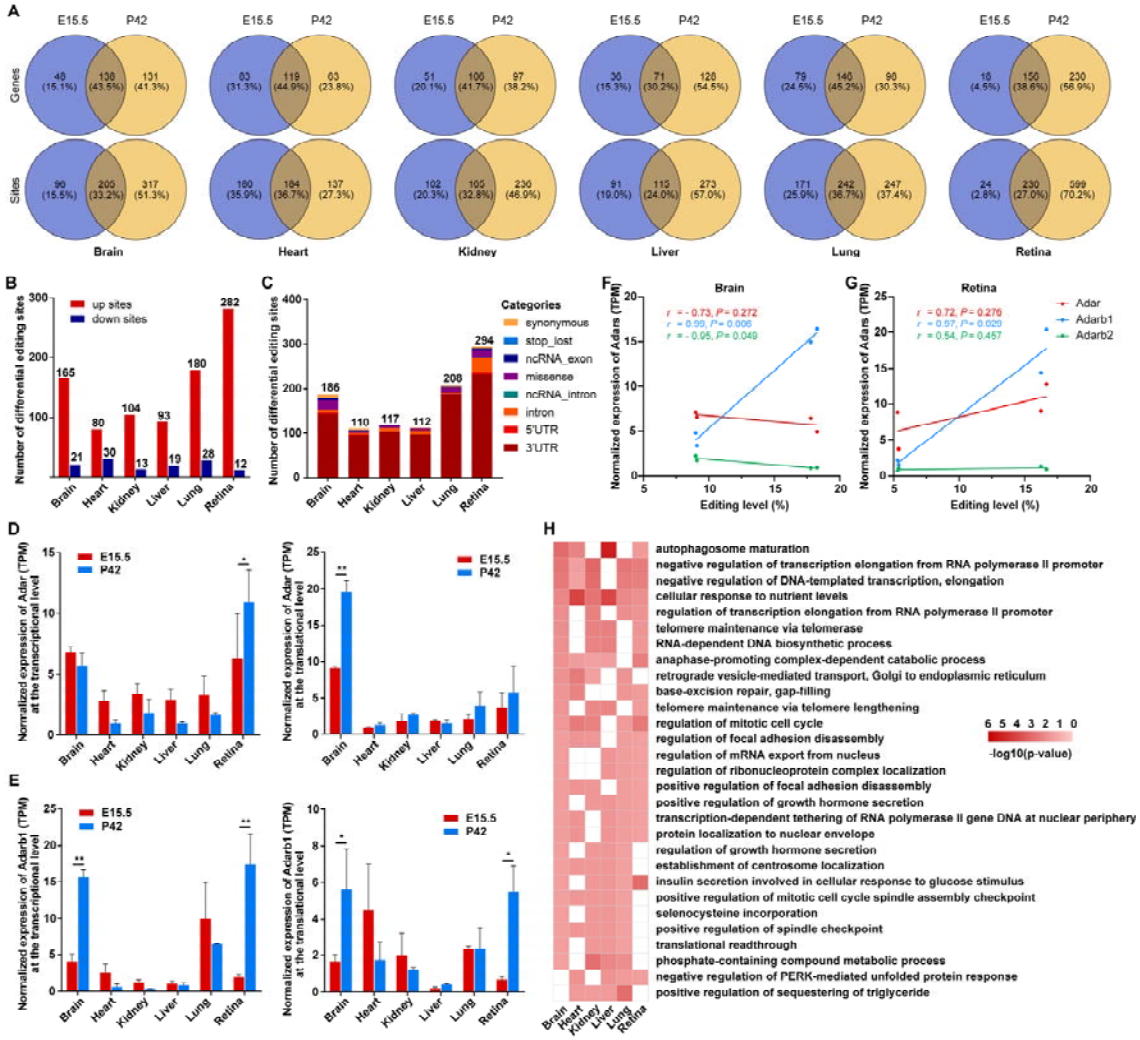

Figure. 2. Differential A-to-I RNA editing in different tissues between embryonic (E15.5) and adult (P42) mice. (A) Venn plots of genes and sites with A-to-I RNA editing at embryonic and adult stages. **(B)** Bar plot showing the number of DRE sites between embryonic and adult stages. **(C)** Functional categories of DRE sites. **(D)** Normalized expression of *Adar* at the transcriptional (left) and translational level (right). **(E)** Normalized expression of *Adarb1* at the transcriptional (left) and translational level (right). **:* $P < 0.01$; *: $P < 0.05$. *Pearson*'s correlation analysis between editing levels and normalized expression of *Adars* at the transcriptional level in the brain **(F)** and the retina **(G)**. **(H)** Heatmap showing terms from GO items enriched by differential edited genes in ≥ three tissues.

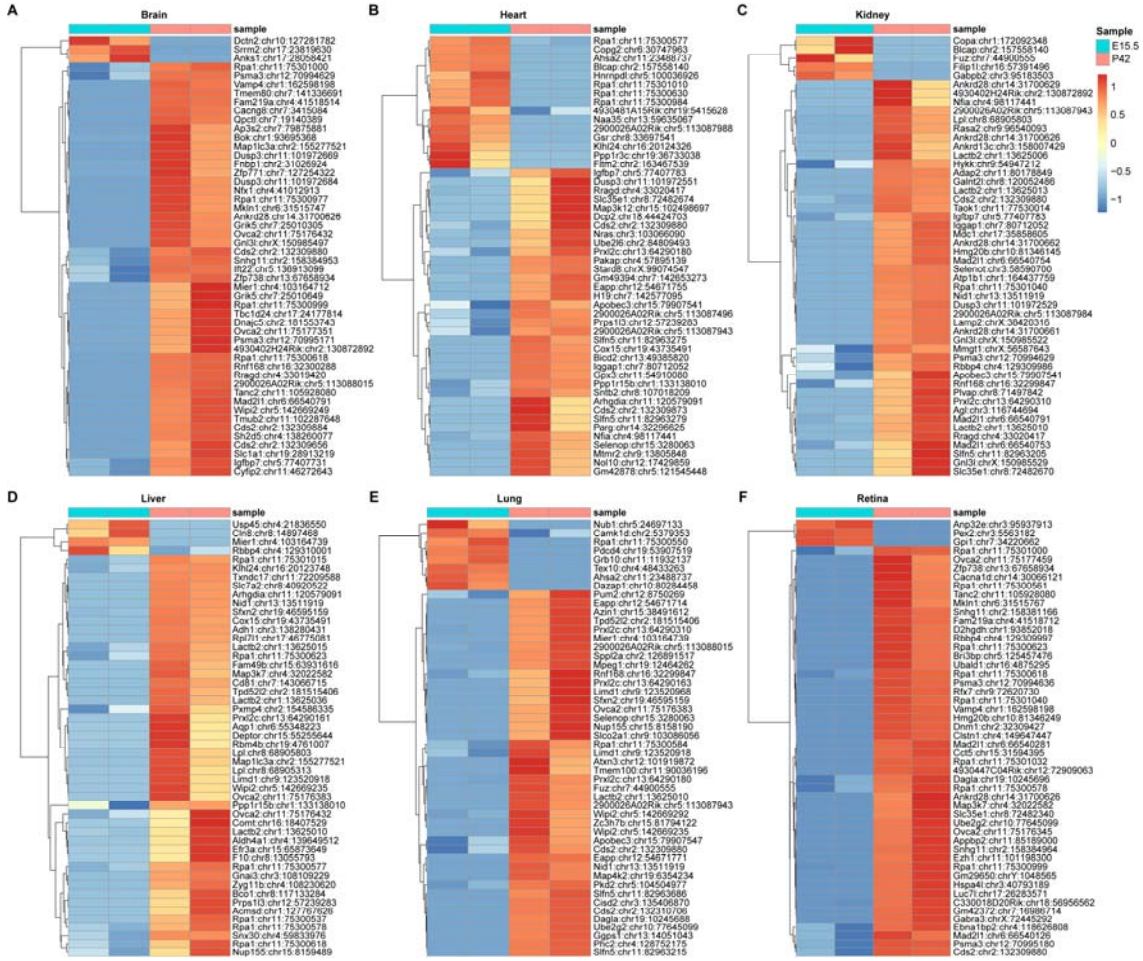

Figure. 3. A-to-I DRE sites in different tissues during development. Heatmap showing the top 50 editing levels of DRE sites in different tissues during development.

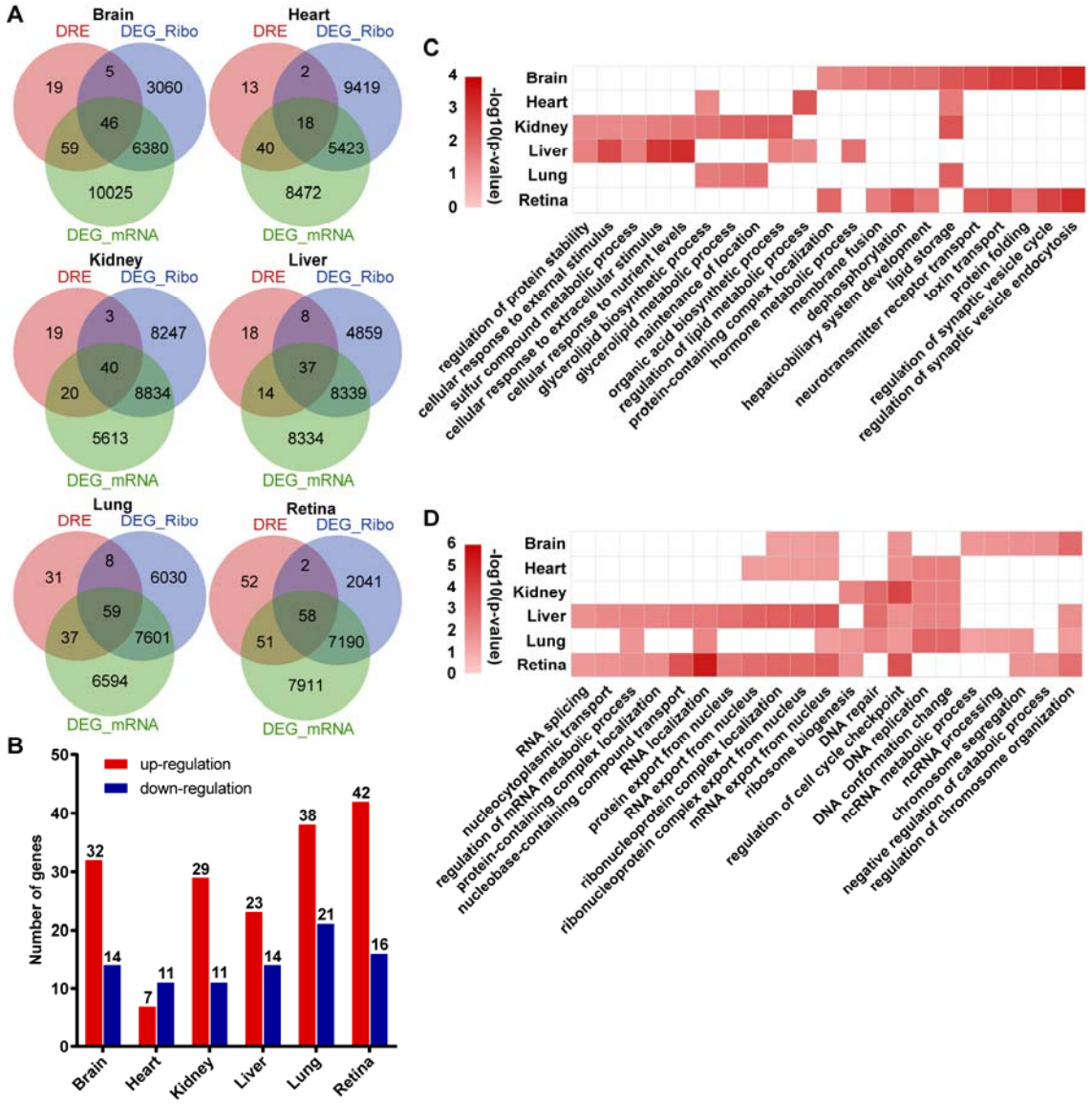

Figure. 4. Possible functional impact of A-to-I DRE on transcription and translation during development. (A) Venn plots comparing genes with DRE and DEGS identified by mRNA-Seq (DEG_mRNA) or Ribo-seq (DEG_Ribo) across different tissues. **(B)** Bar plot showing the number of DRE genes differentially expressed both in mRNA-Seq and Ribo-Seq. Heatmap showing the enriched GO terms for DRE genes with up-regulated **(C)** and down-regulated expression **(D)** in at least two tissue types.

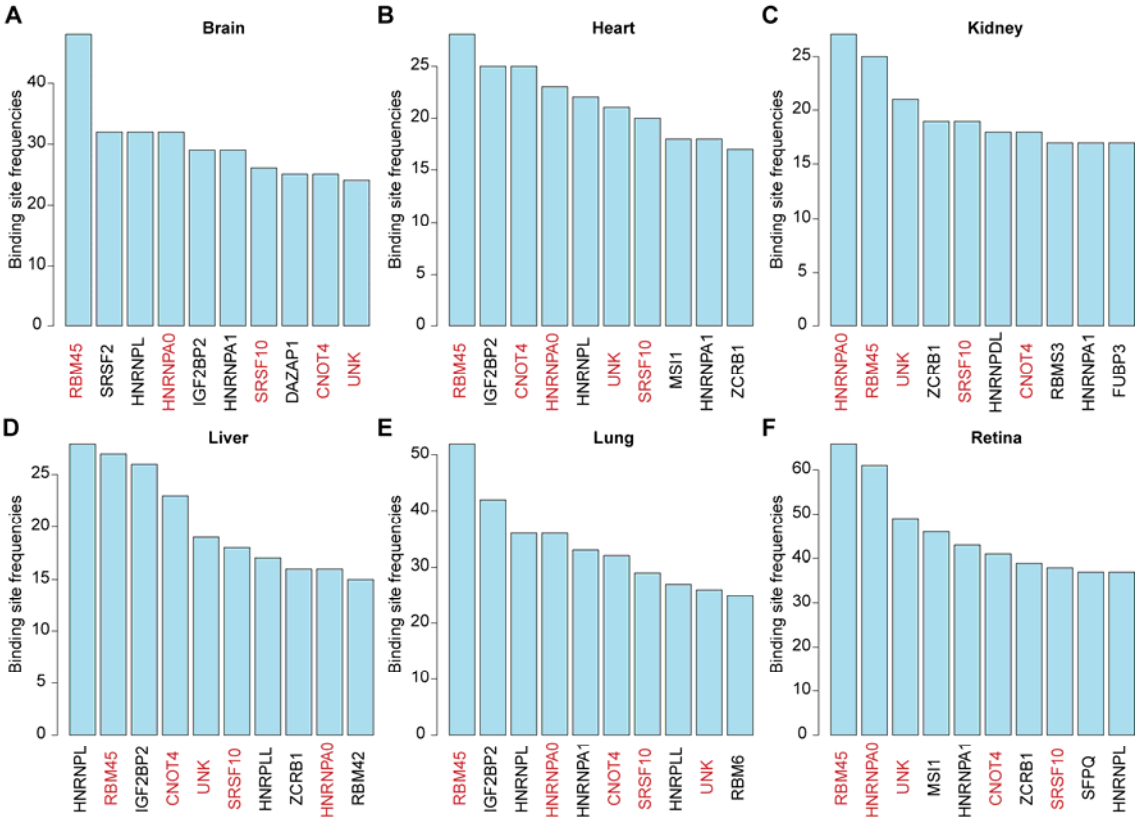

Figure. 5. The top 10 frequent RNA binding proteins (RBPs) ranked by their binding frequencies to DRE sites in different tissues.

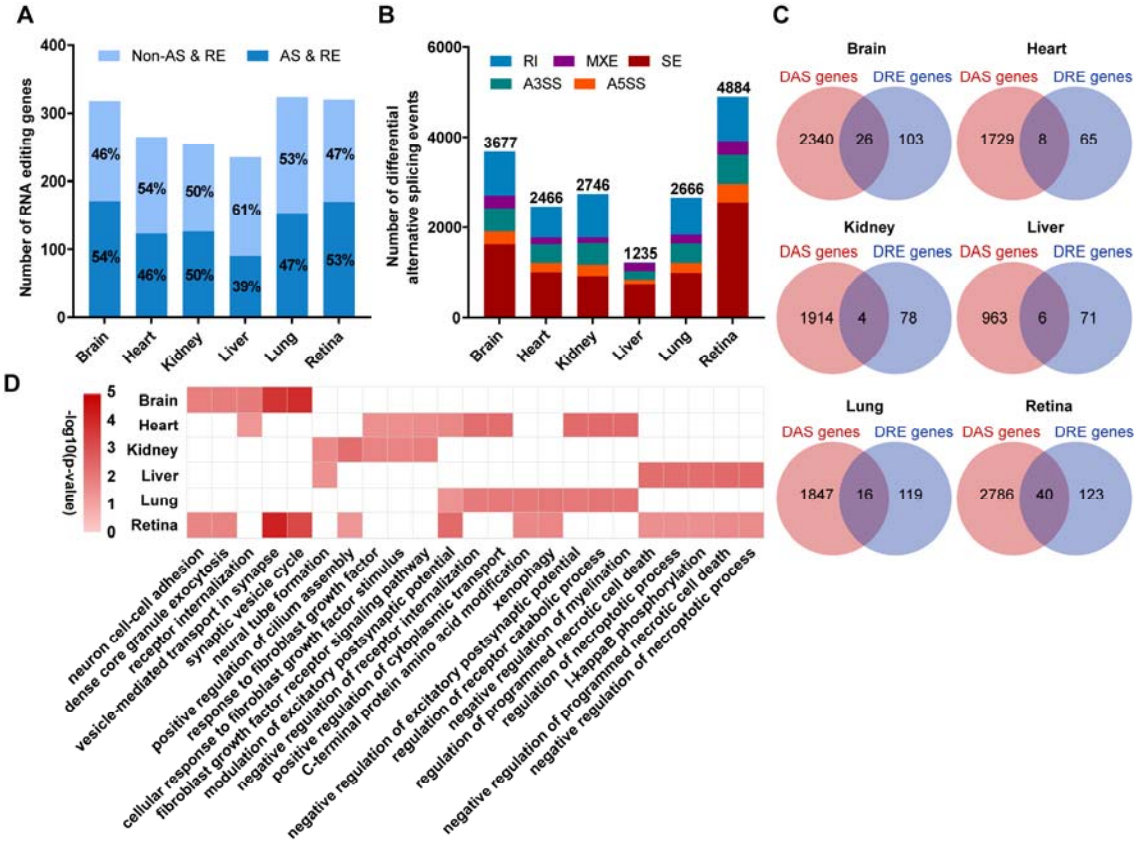

Figure. 6. Developmental interrelation between A-to-I RNA editing and alternative splicing. (A) Proportion and number of edited genes with and without alternative splicing (AS) in different tissues. **(B)** Bar plot comparing the number of differential alternative splicing (DAS) events between embryonic and adult stages. **(C)** Venn plots of DAS genes and DRE genes in different tissues. **(D)** Heatmap showing the enriched GO terms for DAS genes with differential editing in at least two tissue types. RI, retained intron; MXE, mutually exclusive exons; A3SS, alternative 3′ splice sites; A5SS, alternative 5′ splice sites; SE, skipped exon.