Significance of A-to-I RNA editing of transcripts modulating pharmacokinetics and pharmacodynamics

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Abstract

RNA editing is a post-transcriptional process that alters the nucleotide sequence of RNA transcripts to generate transcriptome diversity. Among the various types of RNA editing, adenosine-to-inosine (A-to-I) RNA editing is the most frequent type of RNA editing in mammals. Adenosine deaminases acting on RNA (ADAR) enzymes, ADAR1 and ADAR2, convert adenosines in double-stranded RNA structures into inosines by hydrolytic deamination. Inosine forms a base pair with cytidine as if it were guanosine; therefore, the conversion may affect the amino acid sequence, splicing, microRNA targeting, and miRNA maturation. It became apparent that disrupted RNA editing or abnormal ADAR expression is associated with several diseases including cancer, neurological disorders, metabolic diseases, viral infections, and autoimmune disorders. The biological significance of RNA editing in pharmacokinetics/ pharmacodynamics (PK/PD)-related genes is starting to be demonstrated. The authors conducted pioneering studies to reveal that RNA editing modulates drug metabolism potencies in the human liver, as well as the response of cancer cells to chemotherapy agents. Awareness of the importance of RNA editing in drug therapy is growing. This review summarizes the current knowledge on the RNA editing that affects the expression and function of drug response-related genes. Continuing studies on the RNA editing that regulates pharmacokinetics/pharmacodynamics would provide new beneficial information for personalized medicine.

Keywords

RNA editing, Post-transcriptional regulation, Pharmacokinetics, Pharmacodynamics

Contents

Abbreviations

ABC, ATP-binding cassette transporter; ADAR, adenosine deaminase acting on RNA; ADH, alcohol dehydrogenase; AhR, aryl hydrocarbon receptor; AKR, aldo-keto reductase; ALS, amyotrophic lateral sclerosis; AZIN1, antizyme inhibitor 1; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; A-to-I, adenosine-to-inosine; BCRP, breast cancer resistance protein; CAR, constitutive androstane receptor; CES, carboxylesterase; COMT, catechol *O*-methyltransferase; CYP, cytochrome P450; DHFR, dihydrofolate reductase; dsRNA, double-stranded RNA; ER, estrogen receptor; FMO, flavin-containing monooxygenase; FXR, farnesoid X receptor; GluR2, glutamate receptor 2; GR, glucocorticoid receptor; HCC, hepatocellular carcinoma; HNF, human hepatocyte nuclear factor; LXR, liver X receptors; miRNA, microRNA; MRP, multidrug resistance-associated protein; NR, nuclear receptor; OAT, organic anion transporter; OATP, organic anion transporting polypeptide; OCT, organic cation transporter; OCTN, organic cation/carnitine transporter; PCA, prostate cancer antigen; PD, pharmacodynamics; PEPT, peptide transporter;

P-gp, P-glycoprotein; PK, pharmacokinetics; PPAR, peroxisome proliferator-activated receptor; Pre-miRNA, precursor microRNA, Pri-miRNA, primary microRNA; PXR, pregnane X receptor; SLC, solute carrier family; SLCO, solute carrier organic anion transporter; SULT, sulfotransferase; T2DM, Type 2 diabetes mellitus; TPMT, thiopurine *S*-methyltransferase; UGT, UDP-glucuronosyltransferase; URAT, urate transporter; UTR, untranslated region; VDR, vitamin D receptor

1. Introduction

Gene regulation occurs at every step in the central dogma. In addition to regulatory mechanisms that act on DNA and proteins, eukaryotic RNA transcripts are subjected to various processing, such as alternative splicing, nucleotide modification and localization. Among such regulatory mechanisms, RNA editing refers to sequence alterations, including insertion, deletion or nucleotide conversion, that offer diversity in the transcriptome. Adenosine-to-inosine (A-to-I) RNA editing is a predominant form of RNA editing in mammals (Fig. 1) (Nishikura, 2010). In 1987, an enzymatic activity that causes the unwinding of double-stranded RNA (dsRNA) was discovered in *Xenopus laevis* oocytes and embryos (Bass & Weintraub, 1987). Later, this reaction was found to be the hydrolytic deamination of adenosine to form inosine, i.e., A-to-I RNA editing, which is catalyzed by adenosine deaminase acting on RNA (ADAR) enzymes (Bass & Weintraub, 1988; Wagner et al., 1989). At that time, a limited number of RNA editing sites were discovered in the coding region of mRNA by comparing the sequences of genomic DNA and cDNA using Sanger sequencing (Sommer et al., 1991). Within the past 10 years, the progress of next-generation sequencing technologies has enabled the high-throughput identification of 4.5 million A-to-I RNA editing sites, not only in the coding region but also in the non-coding region (Picardi et al., 2017). Since the base-paring property of inosine is similar to that of guanosine, the conversion of nucleotides potentially affects gene function and expression, depending on the region where the editing event occurs. For genes associated with pharmacokinetics (PK) and pharmacodynamics (PD), the roles of RNA editing have just started being studied. The purpose of this review is to outline recent findings on the role of RNA editing of PK/PD-related genes and its potential pharmacotherapeutic implications.

2. ADAR enzymes

ADAR enzymes convert the adenosines in dsRNA structures into inosines by hydrolytic deamination at the C6 position (Kim et al., 1994; Gerber et al., 1997). A highly conserved deaminase domain in the C-terminal region and dsRNA-binding domains in the N-terminal

region of ADARs catalyze this reaction (Nishikura, 2016). In the ADAR family in vertebrates, there are three members, ADAR1, ADAR2, and ADAR3 (also called ADAR, ADARB1, and ADARB2, respectively) (Bass et al., 1997). The *ADAR1* gene encodes two isoforms, ADAR1 p110 (110 kDa protein) and ADAR1 p150 (150 kDa protein), using different transcription initiation sites and start codons. The former is constitutively expressed and is localized in the nucleus whereas the expression of the latter is induced by interferon and exists in both the nucleus and the cytoplasm (Patterson and Samuel, 1995; Desterro et al., 2003). ADAR2, which is also a ubiquitous form, is highly expressed in the brain and is localized in nucleus (Melcher et al, 1996b). The expression of ADAR3 is limited to the brain (Melcher et al., 1996b; Chen et al., 2000). ADAR1 (Kim et al., 1994) and ADAR2 (Melcher et al., 1996a) have A-to-I RNA editing ability whereas ADAR3 does not show editing activity (Melcher et al., 1996b; Herbert et al., 1997; Chen et al., 2000). For ADAR1 and ADAR2, homodimerization is required to exert their editing activities (Cho et al., 2003; Poulsen et al., 2006; Valente & Nishikura, 2007). Cho et al. (2003) have reported that ADAR3 is unable to homodimerize, which may account for its lack of editing activity. Although there is no strict sequence specificity for A-to-I editing, the surrounding nucleotides have some influence on recognition by ADARs. ADAR1 has a 5' neighbor preference (A = U > C > G), but no apparent 3' neighbor preference (Riedmann et al., 2008). The 5' neighbor preference of ADAR2 (A ≈ U > C = G) is similar to that of ADAR1 whereas ADAR2 has a 3' neighbor preference (U = G > C = A) (Polson & Bass, 1994). Some nucleotides are edited by either ADAR1 or ADAR2, and the others can be edited by both enzymes (Lehmann & Bass, 2000; Hartner et al., 2004).

Mice genetically lacking either ADAR1 or ADAR2 were generated. It has been reported that the ADAR1-null mouse dies with numerous tissue failures at the embryogenesis stages (E11.5-12.5) (Wang et al., 2004), and the ADAR2-null mouse dies with epileptic seizures at postnatal day 20 (Higuchi et al., 2000), suggesting that ADAR proteins are indispensable for life.

3. Functional significance of A-to-I RNA editing of coding genes and miRNAs

Following the conversion of adenosine into inosine, the nucleotide is interpreted as a guanosine, leading to a series of functional consequences depending on the site of A-to-I editing (Fig. 2) (Zipeto et al., 2015). Editing in the coding region of pre-mRNA may change the genetic code, resulting in a change in the amino acid sequence. Editing in the intron may affect splicing by generating or deleting alternative splice sites. The editing events within the 3'-untranslated region (3'-UTR) have the potential to create or destroy the binding site of microRNAs (miRNAs) (Borchert et al., 2009; Farajollahi & Maas, 2010; Deffit & Hundley, 2016).

miRNAs are short (~22-nucleotide), endogenous non-coding RNAs that regulate gene expression at the post-transcriptional level via base pairing with the target mRNA, resulting in gene silencing by either translational repression or mRNA degradation (Bartel, 2004). Nucleotides 2-8 at the 5'-end of the miRNA, called the seed sequence, are critical and sometimes sufficient for repressing the target translation (Lewis et al., 2005). The biogenesis of miRNA is a multi-step process. miRNAs are transcribed in the nucleus by RNA polymerase II as long primary transcripts (pri-miRNAs) containing a stem-loop structure. The pri-miRNAs are subsequently cleaved into 70-100 nt precursors (pre-miRNAs) by the microprocessor complex, which is composed of the RNase III, Drosha and DiGeorge syndrome critical region 8 (DGCR8). After they are exported into the cytoplasm by exportin 5, pre-miRNAs undergo secondary cleavage by Dicer and TAR RNA binding protein (TRBP), leading to mature miRNA duplexes, and then they are unwound into the guide strand form of mature miRNAs. The passenger strand, named miRNA*, is usually degraded, but it is sometimes functional. The stem-loop structures of pri-miRNAs and pre-miRNAs are favorable targets for ADARs (Luciano et al., 2004; Kawahara et al., 2007). The A-to-I change in a miRNA transcript can alter its processing by changing the ability of Drosha and Dicer to bind to pri-miRNA and pre-miRNA, thereby affecting miRNA expression. In other cases, A-to-I editing of the miRNA seed sequence could change its target selection or binding efficiency, although editing frequency in this region is low (Vesely et al., 2012; Ekdahl et al.,

2012; Alon et al., 2012). With the finding of miRNAs, RNA editing became recognized as a critically important regulator of gene expression.

In addition to A-to-I sequence change in miRNAs or their targets, ADAR regulates miRNA processing via an editing-independent mechanism (Nishikura et al., 2013). It has been reported that ADAR1 forms a complex with DGCR8 which is mutually exclusive with the DGCR8-Drosha complex, leading to suppression of processing of pri-miRNA to pre-miRNA (Nemlich et al., 2013; Chen et al., 2015). Ota et al. (2013) revealed that ADAR1 forms a heterodimer with Dicer to function like TRBP, a Dicer's partner. ADAR1 promotes Dicer-dependent pre-miRNA cleavage, resulting the increase of generation of miRNAs. Thus, ADAR has a potential to modulate global miRNA synthesis.

4. Databases of RNA editing sites

Recent advances in next-generation sequencing enabled us to identify global RNA editing sites. RNA editing sites have been compiled in databases such as DARNED (http://darned.ucc.ie/, Kiran & Baranov, 2010; Kiran et al., 2013), RADAR (http://rnaedit.com/, Ramaswami & Li, 2014), and REDIportal (http://srv00.recas.ba.infn.it/atlas/, Picardi et al., 2017). According to RADAR, almost all of the editing sites (99.83%) are located in non-coding regions, including introns and UTR in coding genes as well as non-coding RNA molecules (Ramaswami & Li, 2016). A limited number of genes are edited in the coding regions, and their effects on protein function have been demonstrated (Pinto et al. 2014). Despite the broad distribution of editing sites in the non-coding regions, their physiological and functional significances largely remain to be clarified. The databases can help us to search for editing sites that may have biological significance.

5. Diseases associated with RNA editing

Accumulating evidence has demonstrated that disrupted RNA editing or abnormal ADAR expression in humans is linked with several diseases, including cancer, neurological disorders,

metabolic diseases, viral infections, and autoimmune disorders (Table 1) (Slotkin and Nishikura, 2013). In this section, the studies on the RNA editing that is associated with a neurological disorder, cancers and a metabolic disease are summarized.

5.1. Neurological disorder

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder that is characterized by the selective death of motor neurons. Although mutations of the *superoxide dismutase* gene have been found in 20% of familial ALS patients (Rosen et al., 1993), the majority of ALS cases are sporadic. Glutamate receptor 2 (GluR2), a subunit of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor, is the first example that was found to be subjected to A-to-I editing in mammals (Sommer et al., 1991). The editing occurs in the coding region and causes an amino acid substitution, Q607R. This site is completely edited in neurons throughout life, starting in, from the embryonic stage onwards. Kawahara et al. (2004) found that its editing levels in the motor neurons from patients with sporadic ALS were lower than those in healthy subjects. Motor neurons with less edited receptors had increased Ca^{2+}, which resulted in cell death (Higuchi et al., 2000). Two research groups reported that the loss of editing of GluR2 is attributed to the decreased expression of ADAR2 (Kawahara & Kwak, 2005; Aizawa et al., 2010), although the reason behind the down-regulation of ADAR2 in ALS patients remains to be clarified. Hideyama et al (2010) have generated a conditional knockout mouse whose motor neurons lack ADAR2. These mice prematurely died with a neuronal defect (Hideyama et al., 2010), but the restoration of ADAR2 could rescue them, indicating that the editing of GluR2 is crucial for neuronal survival (Yamashita et al., 2013).

Interestingly, a research group reported that antidepressants, such as paroxetine and imipramine, have the potency to enhance the editing of GluR2 at the Q/R site by up-regulating ADAR2 expression (Sawada et al., 2009). Therefore, such drugs may have the potential to treat sporadic ALS. In addition, the Q607R substitution changes the affinity towards the Joro spider toxin (JSTX) isolated from *Nephila clavata*, which is one of the most

potent blockers of the AMPA receptor (Blaschke et al., 1993). Non-edited GluR2 has a higher affinity toward sJSTX than edited GluR2 (Iino et al., 1996; Savidge & Bristow, 1998). Therefore, inhibitors that are specific to the non-edited receptor may be an additional tool for ALS therapy.

5.2. Cancers

Tumor initiation and progression are the processes characterized by cellular changes in proliferation, differentiation and survival. Some genes and non-coding RNAs are involved in these processes as oncogenes or tumor suppressor genes. In addition, the effects of somatic mutations on cancer progression are well studied. RNA editing was also been found to be dysregulated in human cancers (Galeano et al., 2012). Interestingly, ADARs differentially function as oncogenes or tumor suppressive genes, depending on the cancer type, to cause hyper- or hypo-editing. Here, we summarize the studies demonstrating the role of A-to-I editing in cancer biology (Slotkin & Nishikura, 2013; Gallo & Locatelli, 2012).

Hepatocellular carcinoma (HCC) is the third most common cause of cancer related deaths in the world. Recent studies demonstrated that the dysregulation of ADARs in tumors, which causes bankrupted editing, underlies tumor progression (Chen et al., 2013; Chan et al., 2014). Patients with up-regulated ADAR1 have an increased risk of liver cirrhosis, postoperative recurrence, and poor prognoses (Chan et al., 2014). In that study, the authors found that the mRNA of antizyme inhibitor 1 (*AZIN1*) is edited by ADAR1, leading to an S367G amino acid substitution (from AGC [serine] to GGC [glycine]). AZIN1 inhibits the antizyme-mediated degradation of cycle regulatory proteins, such as ornithine decarboxylase and cyclin D1 (Coffino, 2001; Bercovich & Kahana, 2004; Newman et al, 2004; Kahana, 2009). The editing of AZIN1 mRNA resulted in the stabilization of AZIN1 protein and increased its binding to the antizymes to prevent the degradation of onco-proteins. Notably, the edited AZIN1 conferred a tumorigenic phenotype to HCC cells that presented as higher proliferation and invasive ability, indicating that ADAR1 functions as an oncogene by enhancing the activity of AZIN1. A recent study revealed that Ba/F3 cells (murine dependent pro-B cell line) stably

expressing edited AZIN1 shows a higher resistance to BMS536924, an IGF-1R inhibitor, than the cells expressing non-edited AZIN1 (Han et al., 2015). The authors found that the IC_{50} value of chemotherapy agents, such as paclitaxel, irinotecan and topotecan, were correlated with the editing levels of AZIN1 in 740 cell lines from the Cancer Cell Line Encyclopedia (Barretina et al., 2012), suggesting that RNA editing could modulate the sensitivity of cancer cells to the drug. Another study revealed that ADAR2 expression is elevated in HCC tissues and that ADAR2-mediated RNA editing of pre-miR-214 resulted in a decrease in the mature miR-214 level, leading to increased expression of its target Rab15, a member of the RAS oncogene family.

Melanoma is the most aggressive type of skin cancer. Shoshan et al. (2015) found that cyclic AMP-responsive element binding protein (CREB) negatively regulates ADAR1 expression and that ADAR1 inhibits melanoma tumor growth and metastasis. It has been reported that RNA editing by ADAR1 suppresses the maturation of pri-miR-455. The decrease in ADAR1 expression results in the decreased editing of pri-miR-455, an increased mature miR-455-5p level, and decreased levels of its target, the tumor suppressor cytoplasmic polyadenylation element-binding protein 1 (CPEB1) in melanoma progression.

Previous studies have revealed a decreased expression of ADAR2 in glioblastoma, which is the most aggressive type of brain cancer (Maas et al., 2001; Ishiuchi et al., 2002; Galeano et al., 2013). The restoration of ADAR2 in glioblastoma cells resulted in decreased proliferation and migration *in vitro*. As the biological mechanism, it has been reported that the phosphatase CDC14B, which regulates Skp2/p21/p27 and is involved in glioblastoma growth, is a target of ADAR2 (Galeano et al., 2013). Onco-miRNAs, pri-miR-21 and pri-miR-221/222 are edited by ADAR2, leading to a decrease in their mature miRNA levels (Tomaselli et al., 2015). The seed sequence of miR-376* is edited by ADAR2, but the editing is reduced in glioblastoma. Since non-edited miR-376* directly represses RAP2A (a member of RAS family) expression, the increase in the non-edited miR-376* level by a decreased editing level in glioblastoma facilitates the down-regulation of RAP2A (Choudhury et al., 2012). Interestingly, it has been revealed that hypo-editing of the coding region of GluR2 increases Ca^{2+} influx, leading to the

promotion of glioblastoma growth (Ishiuchi et al., 2002). Thus, these studies indicate the essential role of ADAR2 in glioblastoma, with ADAR2 acting on multiple targets that contribute to cancer progression.

The biological significance of RNA editing in cancer is not fully understood. Current knowledge of ADAR's impact on human cancer development and progression may provide new ideas for developing drugs for cancer therapy. A study demonstrated that 2'-*O*-methyl/locked nucleic acid mixmer antisense oligonucleotides against the target region of ADAR can be potent and selective inhibitors of RNA editing (Mizrahi et al., 2013). Site-selective editing inhibitors could have therapeutic potential in cancers, which are characterized by hyper-editing at specific sites.

5.3. Metabolic disease

Type 2 diabetes mellitus (T2DM) is a metabolic disease characterized by the abnormal secretion and uptake of insulin. The glucose-stimulated secretion of insulin from pancreatic β-cells plays a pivotal role in the pathology of T2DM (Ashcroft & Rorsman, 2012). It has been revealed that ADAR2 expression in pancreatic β-cells is up-regulated by glucose stimulation via the c-Jun amino-terminal kinase-1 pathway (Gan et al., 2006; Yang et al., 2012). Another study demonstrated that the knockdown of ADAR2 impairs the secretion of insulin via glucose stimulation, suggesting that ADAR2 is required for insulin secretion by the pancreatic β-cells (Yang et al., 2010). Although the responsible target(s) of ADAR2 for insulin secretion has not been uncovered, ADAR2-knockdowned β-cells exhibited a lower expression of two key molecules, Munc18-1 and synaptotamin-7, that are involved in vesicle exocytosis (Yang et al., 2010). Further studies are required to disclose the mechanism in which ADAR2 is associated with the glucose-stimulated insulin secretion in pancreatic β-cells.

6. RNA editing of human AhR, which modulates PK

As mentioned above, the roles of RNA editing in human diseases is becoming clear. However, the significance of RNA editing of the mRNAs that regulate drug responses is

largely unknown. Recently, we found that there is a large inter-individual difference (220-fold) in ADAR1 protein expression (whereas ADAR2 was not detected) in human livers (Nakano et al., 2016), which is the principal tissue of drug metabolism, indicating the possibility that interindividual differences in ADAR expression and RNA editing levels may affect the expression or function of drug metabolism-related genes.

In RADAR, many PK-related genes, including drug-metabolism enzymes, drug transporters and transcriptional factors, are registered as mRNAs that are subjected to RNA editing (Table 2). Almost all of the RNA editing sites are located in non-coding regions, such as introns and 3'-UTR, suggesting a possibility that the RNA editing events may affect the splicing or expression of the concerned genes. However, the biological significance of their RNA editing is not fully understood. Recently, we demonstrated the first evidence of the impact of RNA editing on the expression of drug-metabolism enzymes, which is described as follows.

AhR is a ligand-activated transcription factor that regulates the expression of xenobiotic-metabolizing enzymes, including CYP1A1, CYP1A2, CYP1B1, UDP-glucuronosyltransferase, glutathione *S*-transferase (Ramadoss et al., 2005). We found that there are 38 edited sites in the 3'-UTR of AhR mRNA in the human liver. ADAR1 is responsible for their editing, and ADAR1-mediated RNA editing negatively regulates AhR expression in human liver cells (Nakano et al., 2016) (Fig. 3). Interestingly, the down-regulation of the AhR attenuated 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-mediated induction of CYP1A1, a downstream gene of AhR, suggests that RNA editing affects the expression of P450s. For the underlying mechanism of the negative regulation of AhR, we found that RNA editing creates the binding site of miR-378 in the 3'-UTR of AhR. In the human liver samples, a significant inverse association was observed between the miR-378 and AhR protein levels, suggesting that the RNA editing-dependent down-regulation of AhR by miR-378 contributes to the variability in the constitutive hepatic expression of AhR. This is the first evidence to reveal that A-to-I RNA editing modulates the potency of xenobiotic metabolism in the human liver.

7. RNA editing of human DHFR, which modulates PD

RNA editing can occur in the transcripts of a drug target, possibly affecting the drug response (Streit & Decher, 2011; Decher et al., 2013). We recently found a clinical significance to the RNA editing of dihydrofolate reductase (DHFR) expression.

DHFR is a key enzyme of folate metabolism. It catalyzes the reduction of dihydrofolate to form tetrahydrofolate using NADPH as a cofactor. Tetrahydrofolate is essential for the *de novo* synthesis of purine and thymidylate, which are required for DNA synthesis, cell growth and proliferation. Therefore, DHFR is a target of the chemotherapeutic agents methotrexate and pemetrexed (Schweitzer et al., 1990; Fowler. 2001; Nazki et al., 2014). The efficacy of methotrexate in cancer cells is often limited by the acquisition of resistance. As one of the mechanisms of methotrexate resistance, the overexpression of DHFR protein is recognized. In addition to the known mechanism, we recently found that DHFR expression in breast cancer is positively regulated by RNA editing via DHFR mRNA stabilization. For the underlying mechanism of the positive regulation of DHFR, we found that RNA editing destroys the binding sites of miR-25-3p and miR-125a-3p in the 3'-UTR of DHFR. (Nakano et al., 2017) (Fig. 4). The up-regulation of DHFR enhanced cellular proliferation and resistance to methotrexate. The editing levels in breast cancer tissues were higher than in normal tissues, suggesting a possibility that hyper-editing of the DHFR transcript could be responsible for increased DHFR expression. ADAR1 may be a potential anti-tumor target for anti-folate compounds, including methotrexate.

8. Potential effects of editing in miRNA on PK and PD

We and other research groups have revealed the impacts of miRNA-mediated regulation on drug metabolism-related genes (Nakajima & Yokoi 2011; Li et al., 2016). For CYP enzymes, human CYP1A1 (Choi et al., 2012), CYP1B1 (Tsuchiya et al., 2006), CYP2A6 (Nakano et al., 2015a), CYP2B6 (Jin et al., 2016), CYP2C8 (Zhang et al., 2012), CYP2C9 (Riger et al., 2015; Yu et al., 2015a), CYP2C19 (Zhang et al., 2012; Yu et al., 2015b),

CYP2E1 (Mohri et al., 2010; Nakano et al., 2015b; Miao et al., 2016) and CYP3A4 (Pan et al., 2009; Wei et al., 2014; Shi et al., 2015) have been revealed to be regulated by miRNAs. Such accumulating evidence indicates that miRNA is an important determinant of drug efficacy and toxicity. However, it remains to be clarified how the expression of miRNAs that regulate PK/PD-related genes is regulated. As mentioned above, the RNA editing in miRNA molecules has the potential to change their expression and function. In Table 3, miRNAs that regulate PK-related genes and have RNA editing sites in their pre-miRNA are summarized. To further elucidate the impact of RNA editing on PK/PD, the effects of the RNA editing of pre-miRNAs on the expression of PK/PD-related genes that are the targets of miRNAs should be evaluated.

9. Conclusions

At present, the critical roles of RNA editing in physiological processes and their involvement in human diseases are becoming clear. The significance of RNA editing in the research field of PK/PD has only recently started to become clear, and this post-transcriptional modulation could be an additional solid factor that causes intra- and inter-individual differences in drug response. The issues that would make it possible for RNA editing to become a part of pharmacotherapy are as follows: (1) The impact of RNA editing on the expression or function of PK-related genes should be examined, as the functional significance of RNA editing of the mRNAs shown in Table 2 is unknown. (2) It should be investigated whether the change of RNA editing levels could affect PK/PD properties in vivo. (3) It should be determined to what extent ADARs expression and RNA editing levels change under physiological conditions (aging, hormones, diet, alcohol, smoking, environmental chemicals, stress, and drugs). (4) Trials to examine whether ADARs can be a therapeutic target should be conducted. The development of molecules to modulate ADAR expression or activity may be challenging. Studies to uncover the roles of RNA editing of the genes associated with pharmacotherapy would also provide useful information for personalized medicine.

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

References

Aizawa, H., Sawada, J., Hideyama, T., Yamashita, T., Katayama, T., Hasebe, N., et al. (2010). TDP-43 pathology in sporadic ALS occurs in motor neurons lacking the RNA editing enzyme ADAR2. *Acta Neuropathol* 120, 75-84.

Alon, S., Mor, E., Vigneault, F., Church, G. M., Locatelli, F., Galeano, F., et al. (2012). Systematic identification of edited microRNAs in the human brain. *Genome Res* 22, 1533-1540.

Ashcroft, F. M., & Rorsman, P. (2012) Diabetes mellitus and the β cell: the last ten years. *Cell* 148, 1160-1171.

Barretina, J., Caponigro, G., Stransky, N., Venkatesan, K., Margolin, A. A., Kim, S., et al. (2012). The Cancer Cell Line Encyclopedia enables predictive modelling of anticancer drug sensitivity. *Nature* 483, 603-607.

Bartel, D. P. (2004). MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 116, 281-297.

Bass, B. L., & Weintraub, H. (1987). A developmentally regulated activity that unwinds RNA duplexes. *Cell* 48, 607-613.

Bass, B. L., & Weintraub, H. (1988). An unwinding activity that covalently modifies its double-stranded RNA substrate. *Cell* 55, 1089-1098.

Bass, B. L., Nishikura, K., Keller, W., Seeburg, P. H., Emeson, R. B., O'Connell, M. A., et al. (1997). A standardized nomenclature for adenosine deaminases that act on RNA. *RNA*

3, 947-949.

Bercovich, Z., & Kahana, C. (2004). Degradation of antizyme inhibitor, an ornithine decarboxylase homologous protein, is ubiquitin-dependent and is inhibited by antizyme. *J Biol Chem* 279, 54097-54102.

Blaschke. M., Keller. B. U., Rivosecchi. R., Hollmann. M., Heinemann. S., & Konnerth. A. (1993). A single amino acid determines the subunit-specific spider toxin block of alpha-amino-3-hydroxy-5-methylisoxazole-4-propionate/kainate receptor channels. *Proc Natl Acad Sci USA* 90, 6528-6532.

Borchert, G. M., Gilmore, B. L., Spengler, R. M., Xing, Y., Lanier, W., Bhattacharya, D., et al. (2009) Adenosine deamination in human transcripts generates novel microRNA binding sites. *Hum Mol Genet* 18, 4801-4807.

Chan, T. H., Lin, C. H., Qi, L., Fei, J., Li, Y., Yong, K. J., et al. (2014). A disrupted RNA editing balance mediated by ADARs (Adenosine DeAminases that act on RNA) in human hepatocellular carcinoma. *Gut* 63, 832-843.

Chen, C. X., Cho, D. S., Wang, Q., Lai, F., Carter, K. C., & Nishikura, K. (2000). A third member of the RNA-specific adenosine deaminase gene family, ADAR3, contains both single- and double-stranded RNA binding domains. *RNA* 6, 755-767.

Chen, L., Li, Y., Lin, C. H., Chan, T. H., Chow, R. K., Song, Y., et al. (2013). Recoding RNA editing of AZIN1 predisposes to hepatocellular carcinoma. *Nat Med* 19, 209-216.

Chen, T., Xiang, J. F., Zhu, S., Chen, S., Yin, Q. F., Zhang, X. O., et al. (2015). ADAR1 is required for differentiation and neural induction by regulating microRNA processing in a catalytically independent manner. *Cell Res* 25, 459-476.

Cho, D. S., Yang, W., Lee, J. T., Shiekhattar, R., Murray, J. M., & Nishikura, K. (2003). Requirement of dimerization for RNA editing activity of adenosine deaminases acting on RNA. *J Biol Chem*. 278, 17093-170102.

Choi, Y. M., An, S., Lee, E. M., Kim, K., Choi, S. J., Kim, J. S., et al. (2012) CYP1A1 is a target of miR-892a-mediated post-transcriptional repression. *Int J Oncol* 41, 331-336.

Choudhury, Y., Tay, F. C., Lam, D. H., Sandanaraj, E., Tang, C., Ang, B. T., et al. (2012).

Attenuated adenosine-to-inosine editing of microRNA-376a* promotes invasiveness of glioblastoma cells. *J Clin Invest* 122, 4059-4076.

Coffino, P. (2001). Regulation of cellular polyamines by antizyme. *Nat Rev Mol Cell Biol* 2, 188-194.

Decher, N., Netter, M. F., & Streit, A. K. (2013). Putative impact of RNA editing on drug discovery. *Chem Biol Drug Des* 81, 13-21.

Deffit, S. N., & Hundley H. A. (2016). To edit or not to edit: regulation of ADAR editing specificity and efficiency. *Wiley Interdiscip Rev RNA*. 7, 113-127.

Desterro, J. M., Keegan, L. P., Lafarga, M., Berciano, M. T., O'Connell, M., & Carmo-Fonseca, M. (2003). Dynamic association of RNA-editing enzymes with the nucleolus. *J Cell Sci* 116, 1805-1818.

Doria, M., Neri, F., Gallo, A., Farace, M. G., & Michienzi, A. (2009). Editing of HIV-1 RNA by the double-stranded RNA deaminase ADAR1 stimulates viral infection. *Nucleic Acids Res* 37, 5848-5858.

Ekdahl, Y., Farahani, H. S., Behm, M., Lagergren, J., & Öhman, M. (2012) A-to-I editing of microRNAs in the mammalian brain increases during development. *Genome Res* 22, 1477-1487.

Farajollahi, S., & Maas, S. (2010). Molecular diversity through RNA editing: a balancing act. Trends Genet. 26, 221-230.

Fowler, B. (2001). The folate cycle and disease in humans. *Kidney Int Suppl* 78, 221-229.

Gaisler-Salomon, I., Kravitz, E., Feiler, Y., Safran, M., Biegon, A., Amariglio, N., Rechavi et al. (2014) Hippocampus-specific deficiency in RNA editing of GluA2 in Alzheimer's disease. *Neurobiol Aging* 35, 1785-1791.

Galeano, F., Rossetti, C., Tomaselli, S., Cifaldi, L., Lezzerini, M., Pezzullo, M., et al. (2013) ADAR2-editing activity inhibits glioblastoma growth through the modulation of the CDC14B/Skp2/p21/p27 axis. *Oncogene* 32, 998-1009.

Galeano, F., Tomaselli, S., Locatelli, F., & Gallo, A. (2012). A-to-I RNA editing: the "ADAR" side of human cancer. *Semin Cell Dev Biol* 23, 244-250.

Gallo, A., & Locatelli, F. (2012). ADARs: allies or enemies? The importance of A-to-I RNA editing in human disease: from cancer to HIV-1. *Biol Rev Camb Philos Soc* 87, 95-110.

Gan, Z., Zhao, L., Yang, L., Huang, P., Zhao, F., Li, W. (2006). RNA editing by ADAR2 is metabolically regulated in pancreatic islets and beta-cells. *J Biol Chem* 281, 33386-33394.

Gerber, A., O'Connell, M. A., & Keller, W. (1997). Two forms of human double-stranded RNA-specific editase 1 (hRED1) generated by the insertion of an Alu cassette. *RNA* 3, 453-463.

Haenisch, S., Laechelt, S., Bruckmueller, H., Werk, A., Noack, A., Bruhn, O., et al. (2011). Down-regulation of ATP-binding cassette C2 protein expression in HepG2 cells after rifampicin treatment is mediated by microRNA-379. *Mol Pharmacol* 80, 314-320.

Han, L., Diao, L., Yu, S., Xu, X., Li, J., Zhang, R., et al. (2015). The Genomic Landscape and Clinical Relevance of A-to-I RNA Editing in Human Cancers. *Cancer Cell* 28, 515-528.

Hartner, J. C., Schmittwolf, C., Kispert, A., Müller, A. M., Higuchi, M., & Seeburg, P. H. (2004). Liver disintegration in the mouse embryo caused by deficiency in the RNA-editing enzyme ADAR1. *J Biol Chem* 279, 4894-4902.

Herbert, A., Alfken, J., Kim, Y. G., Mian, I. S., Nishikura, K., & Rich, A. (1997). A Z-DNA binding domain present in the human editing enzyme, double-stranded RNA adenosine deaminase. *Proc Natl Acad Sci USA* 94, 8421-8426.

Hideyama, T., Yamashita, T., Suzuki, T., Tsuji, S., Higuchi, M., Seeburg, P. H., et al. (2010). Induced loss of ADAR2 engenders slow death of motor neurons from Q/R site-unedited GluR2. *J Neurosci*. 30, 11917-11925.

Higuchi, M., Maas, S., Single, F. N., Hartner, J., Rozov, A., Burnashev, N., et al. (2000). Point mutation in an AMPA receptor gene rescues lethality in mice deficient in the RNA-editing enzyme ADAR2. *Nature* 406, 78-81.

Iino, M., Koike, M., Isa, T., Ozawa, S. (1996). Voltage-dependent blockage of Ca(2+)-permeable AMPA receptors by joro spider toxin in cultured rat hippocampal neurones. *J Physiol* 496: 431-437.

Ishiuchi, S., Tsuzuki, K., Yoshida, Y., Yamada, N., Hagimura, N., Okado, H., et al., (2002). Blockage of Ca(2+)-permeable AMPA receptors suppresses migration and induces apoptosis in human glioblastoma cells. *Nat Med* 8, 971-978.

Jennewein, C., von Knethen, A., Schmid, T., & Brüne, B. (2010). MicroRNA-27b contributes to lipopolysaccharide-mediated peroxisome proliferator-activated receptor γ (PPARγ) mRNA destabilization. *J Biol Chem* 285, 11846-11853.

Jin,Y., Yu, D., Tolleson, W. H., Knox, B., Wang, Y, Chen, S., et al. (2016) MicroRNA hsa-miR-25-3p suppresses the expression and drug induction of CYP2B6 in human hepatocytes. *Biochem Pharmacol* 113, 88-96

Kahana, C. (2009) Antizyme and antizyme inhibitor, a regulatory tango. *Cell Mol Life Sci* 66, 2479-2488.

Karbiener, M., Fischer, C., Nowitsch, S., Opriessnig, P., Papak, C., Ailhaud, G., et al. (2009). microRNA miR-27b impairs human adipocyte differentiation and targets PPARγ. *Biochem Biophys Res Commun* 390, 247-251

Kawahara, Y., Ito, K., Sun, H., Aizawa, H., Kanazawa, I., & Kwak, S. (2004). Glutamate receptors: RNA editing and death of motor neurons. *Nature* 427, 801.

Kawahara, Y., & Kwak, S. (2005). Excitotoxicity and ALS: what is unique about the AMPA receptors expressed on spinal motor neurons? *Amyotroph Lateral Scler Other Motor Neuron Disord* 6, 131-144.

Kawahara, Y., Zinshteyn, B., Chendrimada, T. P., Shiekhattar, R., & Nishikura, K. (2007). RNA editing of the microRNA-151 precursor blocks cleavage by the Dicer-TRBP complex. *EMBO Rep* 8, 763-769.

Kida, K., Nakajima, M., Mohri, T., Oda, Y., Takagi, S., Fukami, T., et al. (2011). PPARα is regulated by miR-21 and miR-27b in human liver. *Pharm Res* 28, 2467-2476.

Kim, S. Y., Kim, A. Y., Lee, H. W., Son, Y. H., Lee, G. Y., Lee, J. W., et al. (2010). miR-27a is a negative regulator of adipocyte differentiation via suppressing PPARγ expression. *Biochem Biophys Res Commun* 392, 323-328

Kim, U., Wang, Y., Sanford, T., Zeng, Y., & Nishikura K. (1994). Molecular cloning of cDNA

for double-stranded RNA adenosine deaminase, a candidate enzyme for nuclear RNA editing. *Proc Natl Acad Sci USA* 91, 11457-11461.

Kiran, A., & Baranov, P. V. (2010). DARNED: a DAtabase of RNa EDiting in humans. *Bioinformatics* 26, 1772-1776.

Kiran, A. M., O'Mahony, J. J., Sanjeev, K., Baranov, P. V. (2013). Darned in 2013: inclusion of model organisms and linking with Wikipedia. *Nucleic Acids Res* 41, D258-D261 (Database issue).

Kwak, S., & Kawahara, Y. (2005). Deficient RNA editing of GluR2 and neuronal death in amyotropic lateral sclerosis. *J Mol Med* 83, 110-120.

Lehmann, K. A., & Bass, B. L. (2000). Double-stranded RNA adenosine deaminases ADAR1 and ADAR2 have overlapping specificities. *Biochemistry* 39, 12875-12884.

Lewis, B. P., Burge, C. B., & Bartel, D. P. (2005). Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *Cell* 120, 15-20.

Li, M. P., Hu, Y. D., Hu, X. L., Zhang, Y. J., Yang, Y. L., Jiang, C., et al. (2016). MiRNAs and miRNA Polymorphisms Modify Drug Response. *Int J Environ Res Public Health* 13, E1096.

Li. Z., Hu. S., Wang. J., Cai. J., Xiao, L., Yu, L., et al. (2010). MiR-27a modulates MDR1/P-glycoprotein expression by targeting HIPK2 in human ovarian cancer cells. *Gynecol Oncol* 119, 125-130.

Lin, Q., Gao, Z., Alarcon, R. M., Ye, J., & Yun, Z. (2009). A role of miR-27 in the regulation of adipogenesis. *FEBS J* 276, 2348-2358.

Liu, H., Wu, X., Huang, J., Peng, J., & Guo, L. (2015). miR-7 modulates chemoresistance of small cell lung cancer by repressing MRP1/ABCC1. *Int J Exp Pathol* 96, 240-247.

Liu, Q., Jiang, L., Liu, W. L., Kang, X. J., Ao, Y., Sun, M., et al. (2006). Two novel mutations and evidence for haploinsufficiency of the ADAR gene in dyschromatosis symmetrica hereditaria. *Br J Dermatol* 154, 636-642.

Liu, W. H., Chen, C. H., Yeh, K. H., Li, C. L., Wu, Y. J., Chen, D. S., et al. (2013). ADAR2-

mediated editing of miR-214 and miR-122 precursor and antisense RNA transcripts in liver cancers. *PLoS One* 8, e81922.

Luciano, D. J., Mirsky, H., Vendetti, N. J., & Maas, S. (2004). RNA editing of a miRNA precursor. *RNA* 10, 1174-1177.

Maas, S., Patt, S., Schrey, M., & Rich, A. (2001). Underediting of glutamate receptor GluR-B mRNA in malignant gliomas. Proc Natl Acad Sci USA. 98: 14687-14692.

Margaillan, G., Lévesque, É., & Guillemette, C. (2015). Epigenetic regulation of steroid inactivating UDP-glucuronosyltransferases by microRNAs in prostate cancer. *J Steroid Biochem Mol Biol* 155, 85-93.

Melcher, T., Maas, S., Herb, A., Sprengel, R., Higuchi, M., & Seeburg, P. H. (1996a). RED2, a brain-specific member of the RNA-specific adenosine deaminase family. *J Biol Chem* 271, 31795-31798.

Melcher, T., Maas, S., Herb, A., Sprengel, R., Seeburg, P. H., & Higuchi, M. (1996b). A mammalian RNA editing enzyme. *Nature* 379, 460-464.

Miao, L., Yao, H., Li, C., Pu, M., Yao, X., Yang, H. et al. (2016) A dual inhibition: microRNA-552 suppresses both transcription and translation of cytochrome P450 2E1. *Biochim Biophys Acta* 1859, 650-662.

Miyamura, Y., Suzuki, T., Kono, M., Inagaki, K., Ito, S., Suzuki, N., et al. (2003). Mutations of the RNA-specific adenosine deaminase gene (DSRAD) are involved in dyschromatosis symmetrica hereditaria. *Am J Hum Genet* 73, 693-699.

Mizrahi, R. A., Schirle, N. T., & Beal, P. A. (2013) Potent and selective inhibition of A-to-I RNA editing with 2'-O-methyl/locked nucleic acid-containing antisense oligoribonucleotides. *ACS Chem Biol* 19, 832-839.

Mohri, T., Nakajima, M., Fukami, T., Takamiya, M., Aoki, Y., & Yokoi, T. (2010). Human CYP2E1 is regulated by miR-378. *Biochem Pharmacol* 79, 1045-1052

Morabito, M. V., Abbas, A. I., Hood, J. L., Kesterson, R. A., Jacobs, M. M., Kump, D. S., et al. (2010). Mice with altered serotonin 2C receptor RNA editing display characteristics of Prader-Willi syndrome. *Neurobiol Dis* 39, 169-80.

Nakajima. M., & Yokoi. T. (2011). MicroRNAs from biology to future pharmacotherapy: regulation of cytochrome P450s and nuclear receptors. *Pharmacol Ther* 131, 330-337.

Nakano, M., Fukami, T., Gotoh, S., & Nakajima, M. (2017) A-to-I RNA editing up-regulates human dihydrofolate reductase in breast cancer. *J Biol Chem* 292, 4873-4884.

Nakano, M., Fukami, T., Gotoh, S., Takamiya, M., Aoki, Y., & Nakajima, M. (2016). RNA editing modulates human hepatic aryl hydrocarbon receptor expression by creating microRNA recognition sequence. *J Biol Chem* 291, 894-903.

Nakano, M., Fukushima, Y., Yokota, S., Fukami, T., Takamiya, M., Aoki, Y., et al. (2015a). CYP2A7 pseudogene transcript affects CYP2A6 expression in human liver by acting as a decoy for miR-126*. *Drug Metab Dispos* 43, 703-712.

Nakano, M., Mohri, T., Fukami, T., Takamiya, M., Aoki, Y., McLeod, H. L., et al. (2015b). Single-Nucleotide Polymorphisms in Cytochrome P450 2E1 (CYP2E1) 3'-Untranslated Region Affect the Regulation of CYP2E1 by miR-570. *Drug Metab Dispos* 43, 1450-1457.

Nazki, F. H., Sameer, A. S. & Ganaie, B. A. (2014). Folate: metabolism, genes, polymorphisms and the associated diseases. *Gene* 533, 11-20.

Nemlich, Y., Greenberg, E., Ortenberg, R., Besser, M. J., Barshack, I., Jacob-Hirsch, J., et al. (2013). MicroRNA-mediated loss of ADAR1 in metastatic melanoma promotes tumor growth. *J Clin Invest* 123, 2703-2718.

Newman, R. M., Mobascher, A., Mangold, U., Koike, C., Diah, S., & Schmidt, M. (2004). Antizyme targets cyclin D1 for degradation. A novel mechanism for cell growth repression. *J Biol Chem* 279, 41504-41511.

Nishikura, K. (2016). A-to-I editing of coding and non-coding RNAs by ADARs. *Nat Rev Mol Cell Biol* 17, 83-96.

Nishikura, K. (2010). Functions and regulation of RNA editing by ADAR deaminases. *Annu Rev Biochem* 79, 321-349.

Nishikura, K., Sakurai, M., Ariyoshi, K., & Ota, H. (2013). Antagonistic and stimulative roles of ADAR1 in RNA silencing. *RNA Biol* 10, 1240-1247.

Ota, H., Sakurai, M., Gupta, R., Valente, L., Wulff, B. E., Ariyoshi, K., et al. (2013). ADAR1 forms a complex with Dicer to promote microRNA processing and RNA-induced gene silencing. *Cell* 153, 575-589.

Pan, Y. Z., Gao, W., & Yu, A. M. (2009). MicroRNAs regulate CYP3A4 expression via direct and indirect targeting. *Drug Metab Dispos* 37, 2112-2117.

Patterson, J. B., & Samuel, C. E. (1995). Expression and regulation by interferon of a double-stranded-RNA-specific adenosine deaminase from human cells: evidence for two forms of the deaminase. *Mol Cell Biol* 15, 5376-5388.

Picardi, E., D'Erchia, A. M., Giudice. L. C., & Pesole, G. (2017). REDIportal: a comprehensive database of A-to-I RNA editing events in humans. *Nucleic Acids Res* 45:D750-D757 (Database issue).

Pinto, Y., Cohen, H. Y., & Levanon, E. Y. (2014). Mammalian conserved ADAR targets comprise only a small fragment of the human editosome. *Genome Biol* 15, R5.

Polson, A. G., & Bass, B. L. (1994). Preferential selection of adenosines for modification by double-stranded RNA adenosine deaminase. *EMBO J* 13, 5701-5711.

Poulsen, H., Jorgensen, R., Heding, A., Nielsen, F. C., Bonven, B., & Egebjerg, J. (2006). Dimerization of ADAR2 is mediated by the double-stranded RNA binding domain. *RNA* 12, 1350-1360.

Ramadoss, P., Marcus, C., & Perdew G. H. (2005). Role of the aryl hydrocarbon receptor in drug metabolism. *Expert Opin Drug Metab Toxicol* 1, 9-21.

Ramaswami, G., & Li, J. B. (2014). RADAR: a rigorously annotated database of A-to-I RNA editing. *Nucleic Acids Res* 42, D109-D113 (Database issue).

Ramaswami, G., & Li, J. B. (2016). Identification of human RNA editing sites: A historical perspective. *Methods* 107, 42-47.

Rice, G., Patrick, T., Parmar, R., Taylor, C. F., Aeby, A., Aicardi, J., et al. (2007). Clinical and molecular phenotype of Aicardi-Goutieres syndrome. *Am J Hum Genet*. 81: 713-725.

Riedmann, E. M., Schopoff, S., Hartner, J. C., & Jantsch, M. F. (2008). Specificity of ADAR-mediated RNA editing in newly identified targets. *RNA* 14, 1110-1118.

Rieger, J. K., Reutter, S., Hofmann, U., Schwab, M., & Zanger, U. M. (2015) Inflammation-associated microRNA-130b down-regulates cytochrome P450 activities and directly targets CYP2C9. *Drug Metab Dispos* 43, 884-888.

Rosen, D. R., Siddique, T., Patterson, D., Figlewicz, D. A., Sapp, P., Hentati, A., et al. (1993). Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature* 362, 59-62.

Salameh, A., Lee, A. K., Cardó-Vila, M., Nunes, D. N., Efstathiou, E., Staquicini, F. I., et al. (2015). PRUNE2 is a human prostate cancer suppressor regulated by the intronic long noncoding RNA PCA3. *Proc Natl Acad Sci USA* 112, 8403-8408.

Savidge, J. R., & Bristow, D. R. (1998). Ca2+ permeability and joro spider toxin sensitivity of AMPA and kainate receptors on cerebellar granule cells. *Eur J Pharmacol*. 351, 131-138.

Sawada, J., Yamashita, T., Aizawa, H., Aburakawa, Y., Hasebe, N., & Kwak, S. (2009). Effects of antidepressants on GluR2 Q/R site-RNA editing in modified HeLa cell line. *Neurosci Res* 64, 251-258.

Schweitzer, B. I., Dicker, A. P., & Bertino, J. R. (1990). Dihydrofolate reductase as a therapeutic target. *FASEB J* 4, 2441-2452.

Shang, Y., Zhang, Z., Liu, Z., Feng, B., Ren, G., Li, K., et al. (2014). miR-508-5p regulates multidrug resistance of gastric cancer by targeting ABCB1 and ZNRD1. *Oncogene* 33, 3267-3276.

Shi, Y., Liu, Y., Wei, Z., Zhang, Y., Zhang, L., Jiang, S., et al. (2015). Hsa-miR-27a is involved in the regulation of CYP3A4 expression in human livers from Chinese Han population. *Pharmacogenomics* 16, 1379-8136.

Shoshan, E., Mobley, A. K., Braeuer, R. R., Kamiya, T., Huang, L., Vasquez, M. E., et al. (2015). Reduced adenosine-to-inosine miR-455-5p editing promotes melanoma growth and metastasis. *Nat Cell Biol* 17, 311-321.

Slotkin, W., & Nishikura, K. (2013). Adenosine-to-inosine RNA editing and human disease. *Genome Med* 5, 105.

Sommer, B., Köhler, M., Sprengel, R., & Seeburg, P. H. (1991). RNA editing in brain controls a determinant of ion flow in glutamate-gated channels. *Cell* 67, 11-19.

Streit, A. K., & Decher, N. (2011). A-to-I RNA editing modulates the pharmacology of neuronal ion channels and receptors. *Biochemistry (Mosc)* 76, 890-899.

Tojo, K., Sekijima, Y., Suzuki, T., Suzuki, N., Tomita, Y., Yoshida, K., et al. (2006). Dystonia, mental deterioration, and dyschromatosis symmetrica hereditaria in a family with ADAR1 mutation. *Mov Disord*. 21, 1510-1513.

Tomaselli, S., Galeano, F., Alon, S., Raho, S., Galardi, S., Polito, V. A., et al. (2015). Modulation of microRNA editing, expression and processing by ADAR2 deaminase in glioblastoma. *Genome Biol* 16, 5.

Tsuchiya, Y., Nakajima, M., Takagi, S., Taniya, T., & Yokoi, T. (2006) MicroRNA regulates the expression of human cytochrome P450 1B1. *Cancer Res* 66, 9090-9098

Vesely, C., Tauber, S., Sedlazeck, F. J., Haeseler, A., & Jantsch, M. F. (2012). Adenosine deaminases that act on RNA induce reproducible changes in abundance and sequence of embryonic miRNAs. *Genome Res*. 22, 1468-1476.

Valente, L. & Nishikura, K. (2007). RNA binding-independent dimerization of adenosine deaminases acting on RNA and dominant negative effects of nonfunctional subunits on dimer functions. *J Biol Chem* 282, 16054-16061.

Wagner, R. W., Smith, J. E., Cooperman, B. S., & Nishikura, K. (1989). A double-stranded RNA unwinding activity introduces structural alterations by means of adenosine to inosine conversions in mammalian cells and *Xenopus* eggs. *Proc Natl Acad Sci USA* 86, 2647-2651.

Wang, Q., Miyakoda, M., Yang, W., Khillan, J., Stachura, D. L., Weiss, M. J., et al. (2004). Stress-induced apoptosis associated with null mutation of ADAR1 RNA editing deaminase gene. *J Biol Chem* 279, 4952-4961.

Wei, Z., Jiang, S., Zhang, Y., Wang, X., Peng, X., Meng, C., et al. (2014). The effect of microRNAs in the regulation of human CYP3A4: a systematic study using a mathematical model. *Sci Rep* 4, 4283.

Werk, A. N., Bruckmueller, H., Haenisch, S., & Cascorbi, I. (2014). Genetic variants may play an important role in mRNA-miRNA interaction: evidence for haplotype-dependent downregulation of ABCC2 (MRP2) by miRNA-379. *Pharmacogenet Genomics* 24, 283-291.

Wijayakumara, D. D., Hu, D. G., Meech, R., McKinnon, R. A., & Mackenzie, P. I. (2015). Regulation of Human UGT2B15 and UGT2B17 by miR-376c in Prostate Cancer Cell Lines. *J Pharmacol Exp Ther* 354, 417-425.

Yamashita, T., Chai, H. L., Teramoto, S., Tsuji, S., Shimazaki, K., Muramatsu, S., et al. (2013). Rescue of amyotrophic lateral sclerosis phenotype in a mouse model by intravenous AAV9-ADAR2 delivery to motor neurons. *EMBO Mol Med* 5, 1710-1719.

Yang, L., Huang, P., Li, F., Zhao, L., Zhang, Y., Li, S., et al. (2012). c-Jun amino-terminal kinase-1 mediates glucose-responsive upregulation of the RNA editing enzyme ADAR2 in pancreatic beta-cells. *PLoS One* 7, e48611.

Yang, L., Zhao, L., Gan, Z., He, Z., Xu, J., Gao, X., et al. (2010). Deficiency in RNA editing enzyme ADAR2 impairs regulated exocytosis. *FASEB J* 24, 3720-3732.

Yang, T., Zheng, Z. M., Li, X. N., Li, Z. F., Wang, Y., Geng, Y. F., et al. (2013). MiR-223 modulates multidrug resistance via downregulation of ABCB1 in hepatocellular carcinoma cells. *Exp Biol Med* 238, 1024-1032.

Yan, L., Liu, J., Zhao, Y., Nie, Y., Ma, X., Kan, Q., et al. (2017). Suppression of miR-628-3p and miR-641 is involved in rifampin-mediated CYP3A4 induction in HepaRG cells. *Pharmacogenomics* 18, 57-64.

Yu, D., Green, B., Marrone, A., Guo, Y., Kadlubar, S., Lin, D., et al. (2015a). Suppression of CYP2C9 by microRNA hsa-miR-128-3p in human liver cells and association with hepatocellular carcinoma. *Sci Rep* 5, 8534.

Yu, D., Green, B., Tolleson, W. H., Jin, Y., Mei, N., Guo, Y., et al. (2015b). MicroRNA hsa-miR-29a-3p modulates CYP2C19 in human liver cells. *Biochem Pharmacol* 98, 215-223.

Zhang, S. Y., Surapureddi, S., Coulter, S., Ferguson, S. S., & Goldstein, J. A. (2012). Human

CYP2C8 is post-transcriptionally regulated by microRNAs 103 and 107 in human liver. *Mol Pharmacol* 82, 529-540.

Zhao, J. J., Lin, J., Yang, H., Kong, W., He, L., Ma, X., et al. (2008). MicroRNA-221/222 negatively regulates ERα and associates with tamoxifen resistance in breast cancer. *J Biol Chem* 283, 31079-31086.

Zhu, H., Wu, H., Liu, X., Evans, B. R., Medina, D. J., Liu, C. G., et al. (2008). Role of MicroRNA miR-27a and miR-451 in the regulation of MDR1/P-glycoprotein expression in human cancer cells. *Biochem Pharmacol* 76, 582-588.

Zipeto, M. A., Jiang, Q., Melese, E., & Jamieson, C. H. (2015). RNA rewriting, recoding, and rewiring in human disease. *Trends Mol Med* 21, 549-559.

Table 1. RNA editing-associated diseases

Disease	ADAR involved	Target	Reference
Amyotrophic lateral sclerosis (ALS)	ADAR2	GluR2	Kawahara et al., 2004 Kwak & Kawahara, 2005 Hideyama et al., 2010
Hepatocellular carcinoma	ADAR1	AZIN1	Chan et al., 2014
	ADAR2	miR-214	Liu et al., 2013
Melanoma	ADAR1	miR-455-5p	Shosan et al., 2015
Glioblastoma	ADAR2	CDC14B	Galeano et al., 2013
	ADAR2	miR-376*	Choudhury et al., 2012
	ADAR2	miR-21/221/222	Tomaselli et al., 2015
	ADAR2	GluR2	Ishiuchi et al., 2002
Prostate cancer	ADAR1	PRUNE2/PCA3	Sarameh et al., 2015
Type 2 diabetes mellitus	ADAR2	Unknown	Gan et al., 2006 Yang et al., 2010
Prader-Willi syndrome (PWS)	ADAR1/ADAR2	5-HT_{2C}-R	Morabito et al., 2010
Dyschromatosis symmetrica hereditaria (DSH)	ADAR1	Unknown	Miyamura et al., 2003 Tojo et al., 2006 Liu et al., 2006
Aicadi-Goutières syndrome (AGS)	ADAR2	Unknown	Rice et al., 2007
Alzheimer's disease	ADAR2	GluR2	Gaisler-Salomon et al., 2014
HIV-1 infection	ADAR1	Viral p24 Gag	Doria et al., 2009

Table 2. Drug metabolizing enzymes, transporters, and transcriptional factors that are subjected to RNA editing

Drug metabolizing enzyme	Genic region	Transporter	Genic region	Transcriptional factor	Genic region
ADH1B	Intron, 3'-UTR	BCRP (ABCG2)	Intron	AhR	3'-UTR
ADH1C	Coding region	MRP2 (ABCC2)	Intron	CAR (NR1I3)	Intron
AKR1C1	Intron, 3'-UTR	OAT2 (SLC22A2)	Intron	FXR (NR1H4)	Intron
AKR1C2	Intron	OAT3 (SLC22A8)	Coding region	GR (NR3C1)	Intron
CES2	3'-UTR	OATP1A2 (SLCO1A2)	Intron	LXRα (NR1H3)	Intron
COMT	Intron	OATP1B1 (SLCO1B1)	Intron	PXR (NR1I2)	Intron
CYP1A2	Intron, 3'-UTR	OATP2B1 (SLCO2B1)	Intron	VDR	Intron, 3'-UTR
CYP1B1	Intron	OCT1 (SLC22A1)	Intron		
CYP2B6	Intron, 3'-UTR	OCTN2 (SLC22A5)	Intron		
CYP2C8	5'-UTR, intron	PEPT1 (SLC15A1)	Intron, 3'-UTR		
CYP2C9	Intron	P-gp (ABCB1)	Intron		
CYP2C18	Intron	URAT1 (SLC22A12)	Intron		
CYP2D6	Intron				
CYP2E1	Intron				
CYP3A4	Intron				
CYP3A7	Intron				
FMO1	Intron				
FMO2	Intron				
FMO3	Intron				
FMO4	Intron				
FMO5	5'-UTR, coding region, intron				
SULT1A1	Intron, 3'-UTR				
TPMT	Intron, 3'-UTR				
UGT2B15	Intron				

From RADAR (http://rnaedit.com/search/)

Table 3. miRNAs that regulate PK-related genes and have RNA editing sites in their pre-miRNAs

miRNA	Pre-miRNA	RNA editing site[a]	Target gene	Reference
miR-7	Pre-miR-7-2	+41	P-gp (ABCB1)	Liu et al., 2015
miR-27a	Pre-miR-27a	+17, +24	CYP3A4	Shi et al., 2015
			P-gp (ABCB1)	Zhu et al., 2008 & Li et al.,2010
			PPARγ	Lin et al., 2009 & Kim et al., 2010
miR-27b	Pre-miR-27b	+13	CYP1B1	Tsuchiya et al., 2006
			CYP3A4	Pan et al., 2009
			PPARα	Kida et al., 2011
			PPARγ	Karbiener et al., 2009 & Jennewein et al., 2010
			VDR	Pan et al., 2009
miR-222	Pre-miR-222	+10	ER	Zhao et al., 2008
miR-223	Pre-miR-223	+91	P-gp (ABCB1)	Yang et al., 2013
miR-379	Pre-miR-379	+10	MRP2 (ABCC2)	Haenisch et al., 2011 & Werk et al., 2014
miR-508	Pre-miR-508	+31	P-gp (ABCB1)	Shang et al., 2014
miR-532	Pre-miR-532	+34	CYP3A4	Wei et al., 2014
miR-641	Pre-miR-641	+24, +30, +31	CYP3A4	Yan et al., 2017
miR-376c	Pre-miR-376c	+48, +50	UGT2B15	Margaillan et al., 2015 & Wijayakumara et al., 2015
			UGT2B17	Margaillan et al., 2015 & Wijayakumara et al., 2015

These pre-miRNAs are registered in RADAR (http://rnaedit.com/search/).

[a]The numbering denotes the 5' end of the pre-miRNA as +1.

Figure Legends

Fig. 1. A-to-I RNA editing, which refers to the deamination of adenosine to inosine in the RNA molecule. Adenosine deaminase acting on RNA (ADAR) enzymes convert adenosine to inosine by hydrolytic deamination. Inosine is recognized by the cellular machinery as if it were guanosine.

Fig. 2. Functional significance of A-to-I RNA editing. A-to-I editing occurs in double-stranded RNA structure. Editing in the coding region of pre-mRNA may change the amino acid sequence. Editing in the intron may affect splicing by generating or deleting alternative splice sites. The editing events within the 3'-untranslated region (3'-UTR) have the potential to create or destroy the binding site of miRNAs. The A-to-I change in a miRNA transcript can alter its processing, thereby affecting miRNA expression. A-to-I editing of the miRNA seed sequence could change its target selection or binding efficiency.

Fig. 3. The down-regulation of aryl hydrocarbon receptor (AhR) expression by RNA editing. The 3'-UTR of AhR is edited by ADAR1, and the edited sequence of AhR is recognized by miR-378 in human liver cells. This mechanism affects the expression and induction of drug-metabolizing enzymes that are downstream of AhR.

Fig. 4. The up-regulation of dihydrofolate reductase (DHFR) expression by RNA editing. The 3'-UTR of DHFR is edited by ADAR1, and the edited DHFR mRNA can escape from repression by miR-25-3p and miR-125a-3p, leading to an increase in DHFR expression in breast cancer cells with high ADAR1 expression. This mechanism affects cellular proliferation and resistance to methotrexate.

Figure 1

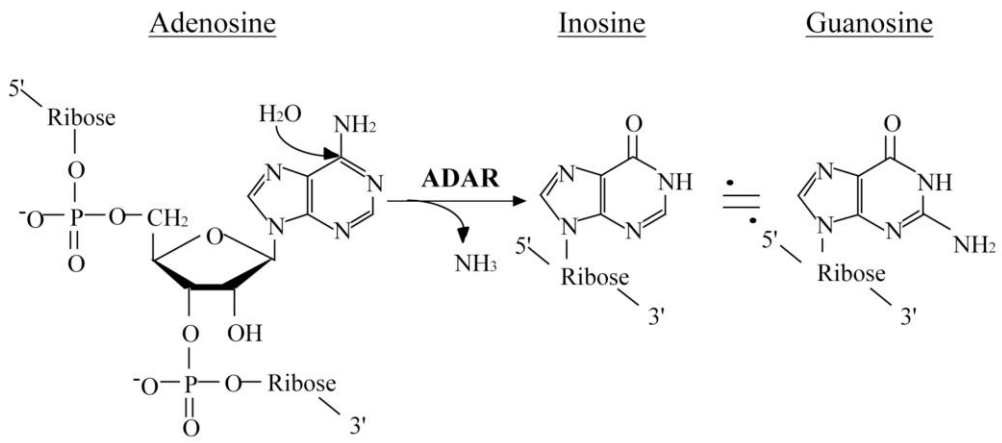

Adenosine
Inosine
Guanosine
5'
Ribose
O
-O—P—O—CH2
O
O
H2O
NH2
N
N
N
N
O
OH
-O—P—O—Ribose
O
3'
ADAR
NH3
O
NH
5'
Ribose
3'
O
NH
NH2
5'
Ribose
3'

Figure 2

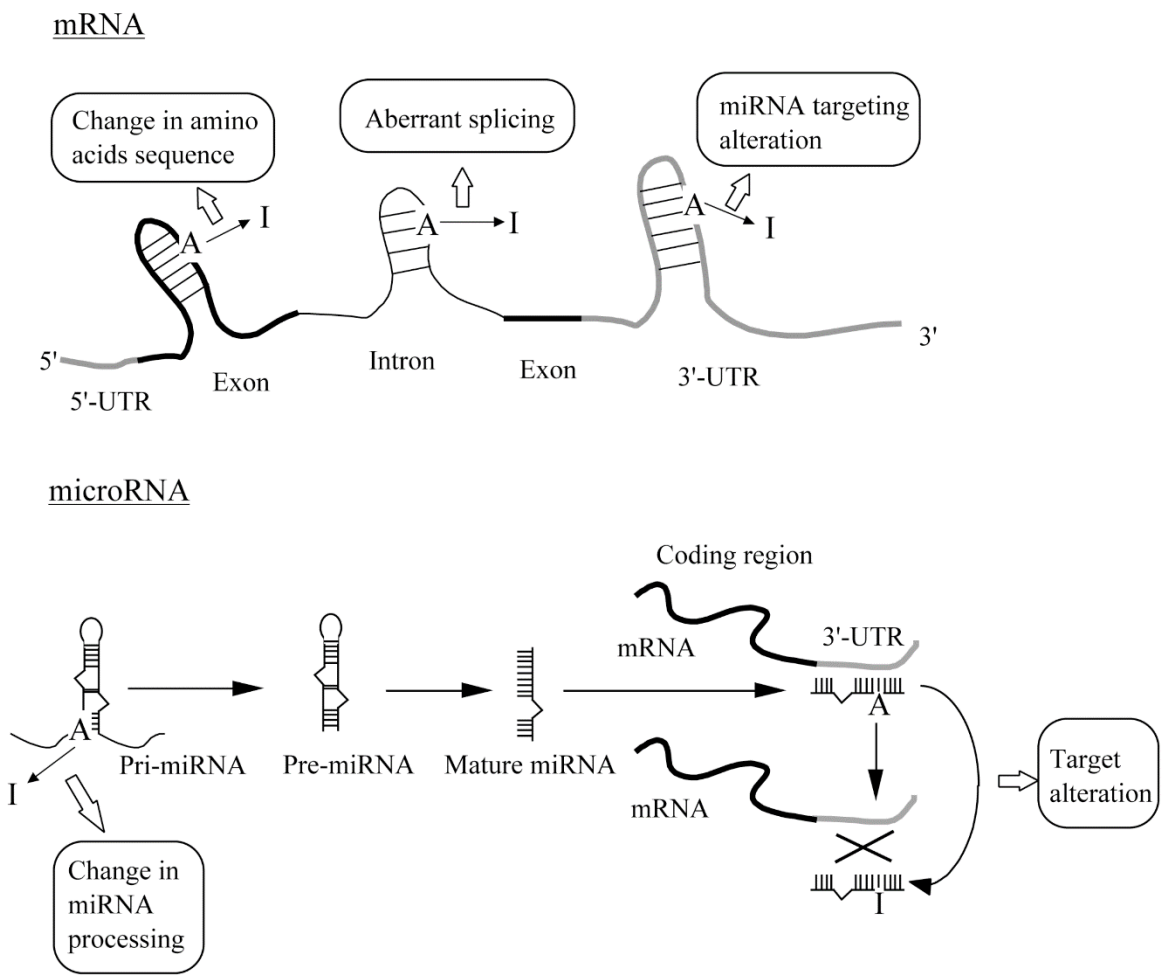

mRNA
Change in amino acids sequence
Aberrant splicing
miRNA targeting alteration
A
I
A
I
A
I
5'
3'
5'-UTR
Exon
Intron
Exon
3'-UTR
microRNA
Coding region
mRNA
3'-UTR
A
I
Pri-miRNA
Pre-miRNA
Mature miRNA
mRNA
Target alteration
Change in miRNA processing
I

Figure 3

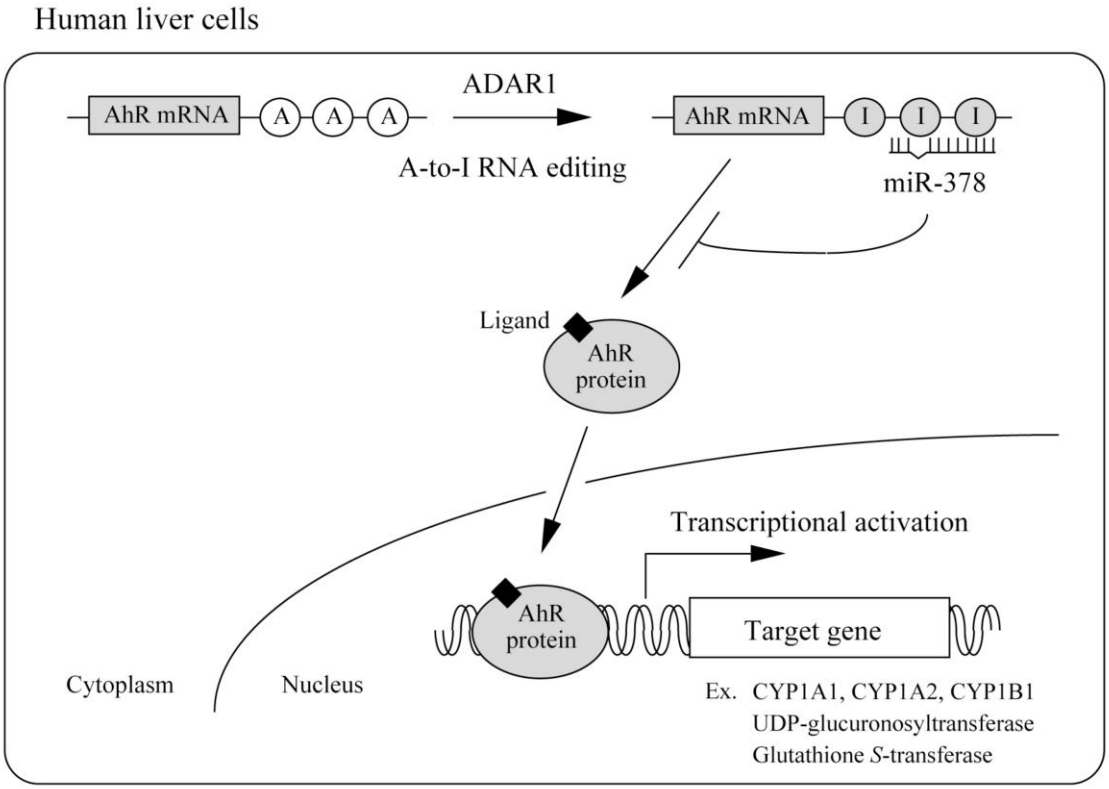
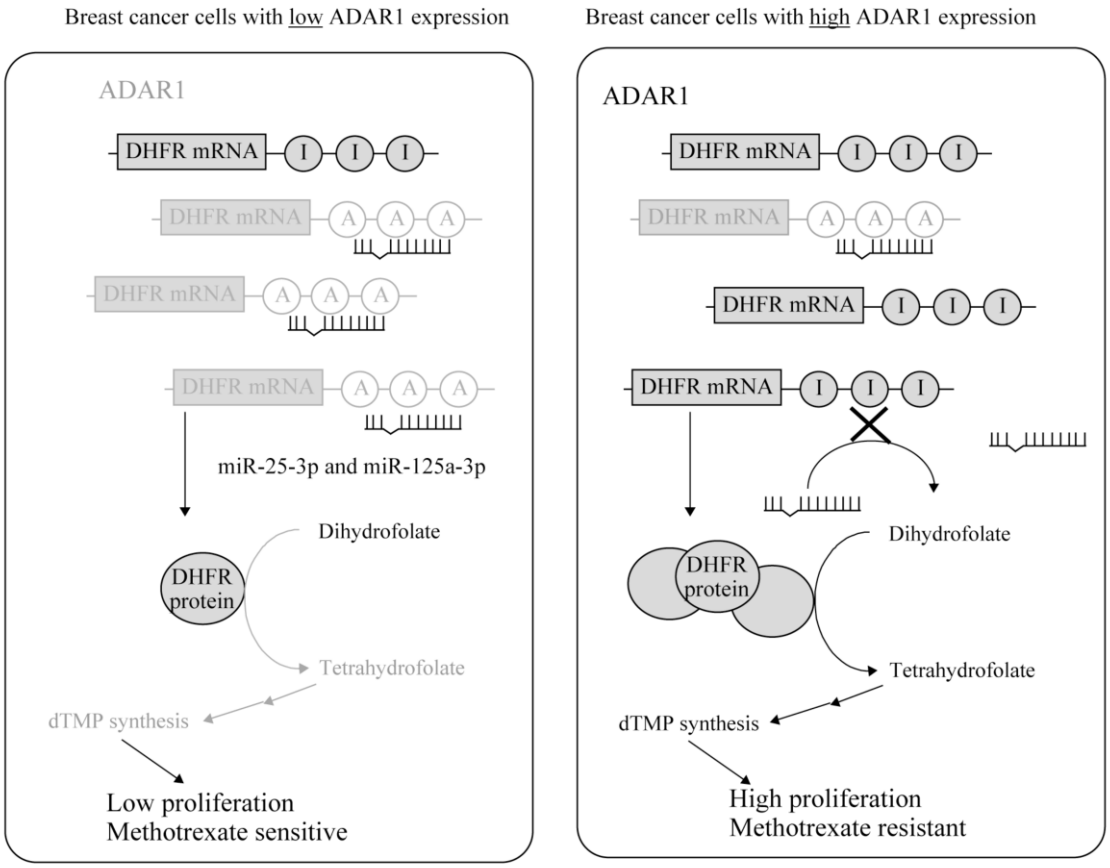

Human liver cells
AhR mRNA
A
A
A
ADAR1
A-to-I RNA editing
AhR mRNA
I
I
I
miR-378
Ligand
AhR protein
Transcriptional activation
AhR protein
Target gene
Cytoplasm
Nucleus
Ex. CYP1A1, CYP1A2, CYP1B1
UDP-glucuronosyltransferase
Glutathione S-transferase

Figure 4


Breast cancer cells with low ADAR1 expression
ADAR1
DHFR mRNA
I
I
I
DHFR mRNA
A
A
A
DHFR mRNA
A
A
A
DHFR mRNA
A
A
A
miR-25-3p and miR-125a-3p
Dihydrofolate
DHFR protein
Tetrahydrofolate
dTMP synthesis
Low proliferation
Methotrexate sensitive
Breast cancer cells with high ADAR1 expression
ADAR1
DHFR mRNA
I
I
I
DHFR mRNA
A
A
A
DHFR mRNA
I
I
I
DHFR mRNA
I
I
I
Dihydrofolate
DHFR protein
Tetrahydrofolate
dTMP synthesis
High proliferation
Methotrexate resistant