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Minireview

Adenosine-to-Inosine RNA Editing and N^6-Methyladenosine Modification Modulating Expression of Drug Metabolizing Enzymes

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ABSTRACT

Interindividual differences in the expression and activity of drug metabolizing enzymes including cytochrome P450, UDP-glucuronosyltransferase, and esterases cause variable therapeutic efficacy or adverse events of drugs. As the major mechanisms causing the variability in the expression of drug metabolizing enzymes, transcriptional regulation by transcription factors, epigenetic regulation including DNA methylation, and posttranscriptional regulation by microRNA are well known. Recently, adenosine-to-inosine RNA editing and methylation of adenosine at the N^6 position on RNA have emerged as novel regulators of drug metabolism potency. In this review article, the current knowledge of these two prevalent types of posttranscriptional modification mediated modulation of drug metabolism involved genes is introduced.

SIGNIFICANCE STATEMENT

Elucidation of the significance of adenosine-to-inosine RNA editing and N^6-methyladenosine in the regulation of drug metabolizing enzymes is expected to lead to a deeper understanding of interindividual variability in the therapeutic efficacy or adverse effects of medicines.

Introduction

There are large interindividual differences in the expression and activity of drug metabolizing enzymes including cytochrome P450 (P450), UDP-glucuronosyltransferase (UGT), and esterases, causing variable therapeutic efficacy or adverse events of drugs. The expression of drug metabolizing enzymes is regulated in each step, transferring genetic information from genomic DNA to RNA and then to protein. Transcriptional regulation by transcription factors including aryl hydrocarbon receptor (AhR), pregnane X receptor (PXR), constitutive androstane receptor (CAR), and hepatocyte nuclear factor 4α (HNF4α) is widely known (Zanger and Schwab, 2013). As well as such transcriptional regulation, posttranscriptional regulation has been recognized to contribute to interindividual differences in the expression of drug metabolizing enzymes. In the past decade, it became apparent that microRNAs (miRNAs), endogenous ∼22-nucleotide noncoding RNAs, posttranscriptionally modulate the expression of P450, UGT, and related transcription factors, and their significance in interindividual differences in drug metabolizing enzyme expression has been broadly recognized (Nakano and Nakajima, 2018a; Li et al., 2019).

In addition to posttranscriptional regulation by trans-acting factors such as miRNAs and RNA binding proteins, hundreds of distinct posttranscriptional modifications contribute to the regulation of the expression and function of proteins (Fu et al., 2014; Boccaletto et al., 2018). Two prevalent types of RNA modifications are adenosine-to-inosine (A-to-I) RNA editing and methylation of adenosine at the N^6 position (m^6A modification), which are observed in 38% and 25% of human genes (Picardi et al., 2017; Zhang et al., 2021). These epitranscriptomic regulations can modulate RNA processing, affecting protein expression and function (Maity and Das, 2016; Nishikura, 2016). Recently, we demonstrated that A-to-I RNA editing and m^6A modification modulate the expression of drug metabolizing enzymes, including P450, UGT, and esterase. This review describes the current knowledge of these posttranscriptional modifications and their significance in the regulation of drug metabolism.

A-to-I RNA Editing

In 1987, a phenomenon in which double-stranded RNA (dsRNA) is unwound was found in *Xenopus laevis* oocytes and embryos (Bass and Weintraub, 1987). Later, this phenomenon was found to be caused by hydrolytic deamination of adenosine to convert to inosine, which occurs in human cells as well as *Xenopus laevis* (Bass and Weintraub, 1988;

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ABBREVIATIONS: A-to-I, adenosine-to-inosine; ADAR, adenosine deaminase acting on RNA; AhR, aryl hydrocarbon receptor; ALKBH5, alkB homolog 5; CAR, constitutive androstane receptor; CES, carboxylesterase; CSE, cigarette smoke extract; DAA, 3-deazaadenosine; dsRNA, double-stranded RNA; FTO, fat mass and obesity–associated; HNF4α, hepatocyte nuclear factor 4α; m^6A, N^6-methyladenosine; METTL, methyltransferase-like; miRNA, microRNA; P450, cytochrome P450; pri-miRNA, primary microRNA; PXR, pregnane X receptor; UGT, UDP-glucuronosyltransferase; UTR, untranslated region; YTH, YT521-B homology.

Wagner et al., 1989). The nucleotide change named A-to-I RNA editing has been demonstrated to be catalyzed by adenosine deaminase acting on RNA (ADAR) enzymes (Kim et al., 1994; O'Connell et al., 1997). Inosine is formed by ADAR-catalyzed hydrolytic deamination of adenosine at the *C*6 position in dsRNA (Gerber et al., 1997). In the 1990s, a limited number of editing sites were identified in the coding region of mRNA by Sanger sequencing (Sommer et al., 1991). In the last decade, the emergence of next generation sequencing has enabled the comprehensive identification of 4.5 million editing sites in the noncoding and coding regions of the human transcriptome (Picardi et al., 2017). Since inosine is recognized by the cellular machinery as guanosine, the conversion of nucleotides can affect gene function and expression.

Molecular Basis of ADAR. In mammals, there are three types of ADARs: ADAR1, ADAR2 (ADARB1), and ADAR3 (ADARB2) (Bass et al., 1997). ADARs have a highly conserved deaminase domain in the *C*-terminal region and dsRNA-binding domains in the *N*-terminal region required for A-to-I RNA editing activity (Nishikura, 2016). ADAR1 has two isoforms: ADAR1 p110 (110 kDa protein) and ADAR1 p150 (150 kDa protein), by using alternative promoters. ADAR1 p110 is constitutively expressed in most human tissues and is localized in the nucleus, whereas ADAR1 p150 expression is induced by interferon and is localized in both the nucleus and cytoplasm (Patterson and Samuel, 1995; Desterro et al., 2003). ADAR2, which is also a ubiquitous protein, is highly expressed in the brain and is localized in the nucleus (Melcher et al., 1996a). ADAR1 has a 5′ neighbor preference (A = U > C > G) but no apparent 3′ neighbor preference (Riedmann et al., 2008). ADAR2 has a 5′ neighbor preference (A ≈ U > C = G) that is similar to that of ADAR1 and has a 3′ neighbor preference (U = G > C = A) (Polson and Bass, 1994). Homodimerization is required for ADAR1 p110, ADAR1 p150, and ADAR2 to exert their editing activities (Cho et al., 2003; Poulsen et al., 2006; Valente and Nishikura, 2007). ADAR3 is mainly expressed in the brain, but it does not have editing activity (Melcher et al., 1996b; Herbert et al., 1997; Chen et al., 2000). A recent study has shown that ADAR3 may disturb ADAR2 function by acting as a competitive inhibitor (Oakes et al., 2017).

Functional Roles of A-to-I RNA Editing in Gene Regulation. According to a study by Picardi et al. (2017), 73% of A-to-I RNA editing events (3,399,200) occurred in protein coding genes in humans. Among them, 0.2% (6,786), 97% (3,286,779), 0.2% (5,236), and 3% (100,399) of editing sites were located in coding regions, introns, 5′-untranslated regions (UTRs), and 3′-UTRs, respectively. The functional consequences of this nucleotide conversion depend on the region where the editing events occur (Fig. 1). Of 6,786 editing events in the coding region, the conversion of 4,388 sites resulted in changes of the amino acid sequence. Nonsynonymous A-to-I RNA editing has a great impact on protein function, although such editing events infrequently occur (Chen et al., 2013). The majority of A-to-I changes occur in introns and possibly affect splicing by creating or deleting alternative splice sites (Hsiao et al., 2018; Tang et al., 2020). The identified RNA editing sites have been compiled into databases such as DARNED (https://darned.ucc.ie/, Kiran et al., 2013) and REDIportal (http://srv00.recas.ba.infn.it/atlas/, Picardi et al., 2017), but the functional significance of most of them remains to be clarified.

In recent years, the presence of circular RNAs, ring-shaped stable noncoding RNAs, which are produced during the "back-splicing" of exons and function as miRNA sponges, has been revealed (Hsiao et al., 2017). It is interesting that the A-to-I editing event in circular RNA precursors inhibits ring formation (Rybak-Wolf et al., 2015; Ivanov et al., 2015). A-to-I editing within the 3′-UTR possibly creates or destroys the binding site of miRNAs (Borchert et al., 2009) and can affect the nuclear retention of mRNA (Hundley and Bass, 2010). In the process of miRNA biogenesis, primary transcripts (pri-miRNAs) are processed into precursor miRNAs in the nucleus, and they are then transported into the cytoplasm to undergo further processing into mature miRNAs. The stem-loop structures of pri-miRNAs and precursor miRNAs can be targeted by ADARs (Luciano et al., 2004; Kawahara et al., 2007).

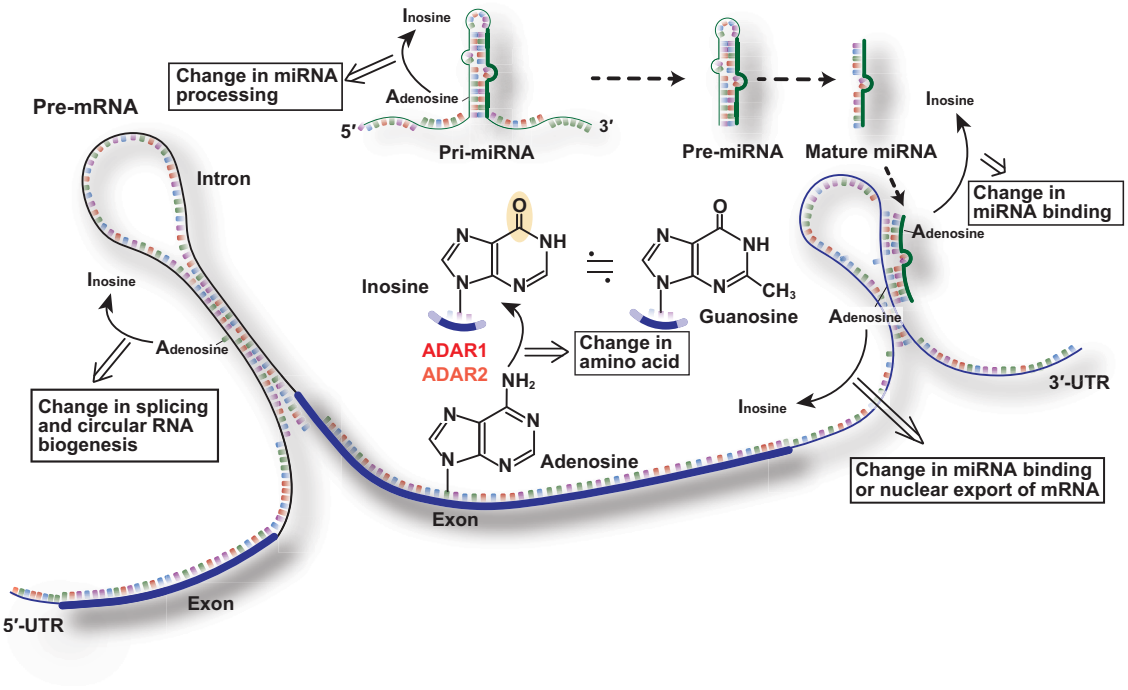

Fig. 1. Functional significance of A-to-I RNA editing. Adenosine deaminase acting on RNA (ADAR) enzymes converts adenosine to inosine by hydrolytic deamination. Inosine is recognized by the cellular machinery as if it were guanosine. Through nucleotide conversion, amino acid sequence, splicing, microRNA (miRNA) processing, and miRNA targeting can change.

The editing events in a miRNA transcript can change its processing, influencing miRNA expression. In other cases, A-to-I conversion of the miRNA seed sequence, which is nucleotides 2–7 at the 5′-end of the miRNA and is critical for recognition of the targets (Lewis et al., 2005), could change its target binding efficiency (Alon et al., 2012; Nakano et al., 2017). In addition, it has been reported that ADAR1 promotes the generation of miRNA in an editing-independent manner by interacting with the microprocessor complex (Ota et al., 2013).

Physiologic and Pathologic Roles of A-to-I RNA Editing. Early studies showed that A-to-I editing plays key roles in the central nervous system (Tariq and Jantsch, 2012). For example, 5-hydroxytryptamine receptor subtype 2C and glutamate receptor subtype A2 are known to undergo A-to-I RNA editing (Sommer et al., 1991; Lomeli et al., 1994; Burns et al., 1997), and the disruption of RNA editing in these RNAs leads to Prader-Willi syndrome and amyotrophic lateral sclerosis, respectively (Kawahara et al., 2004; Morabito et al., 2010). In addition, aberrant A-to-I RNA editing or ADAR expression is associated with other diseases, including cancer, metabolic diseases, neurologic disorders, viral infections, and autoimmune disorders (Song et al., 2016). In particular, it has been elucidated by recent research that ADAR1 is commonly overexpressed in liver, lung, breast, and esophageal cancer as well as in chronic myelogenous leukemia, and it promotes cancer progression (Xu and Öhman, 2018). ADAR1-mediated RNA editing contributes to carcinogenesis by reducing the activity of tumor suppressors, such as bladder cancer-associated protein, or enhancing the activity of oncogenic proteins, such as antizyme inhibitor 1, by changing their amino acid sequences (Chen et al., 2013; Hu et al., 2015). Our research group demonstrated that ADAR1 positively regulates the expression of dihydrofolate reductase, a key enzyme in folate metabolism, by disrupting the miRNA binding sites in the 3′-UTR of dihydrofolate reductase and enhancing the proliferation of breast cancer cells (Nakano et al., 2017). Therefore, ADAR1 is expected to be a novel target for cancer therapy.

A-to-I RNA Editing Modulates Drug Metabolism Potency. In REDIportal, drug metabolism-related genes are registered as mRNAs that are subjected to A-to-I RNA editing (Table 1). Recently, our research group found large (over 200-fold) interindividual variability in ADAR1 expression in human liver samples (Nakano et al., 2016). This finding prompted us to investigate the involvement of ADAR1 in the interindividual differences in hepatic expression of drug metabolizing enzymes (Nakano and Nakajima, 2018b). Recent findings of A-to-I editing-mediated regulation of drug metabolism-related genes are summarized in Table 1 and introduced below in detail.

ADARs Modulate Drug Metabolizing P450 Expression. To examine the role of ADARs in the regulation of drug metabolizing P450 expression in the human liver, we knocked down ADAR1 or ADAR2 in HepaRG cells (Nozaki et al., 2019), which show higher P450 expression than HepG2 cells (Guillouzo et al., 2007). Knockdown of ADAR1 or ADAR2 resulted in a decrease in CYP2C8 mRNA (66% and 54% of control, respectively) and protein expression (77% and 64% of control, respectively) in a promoter region-dependent manner. The CYP2C8-catalyzed amodiaquine *N*-desethylation activity was significantly decreased to 64% and 40% of control by the knockdown of ADAR1 and ADAR2, respectively. We found that the decreased expression of HNF4α protein (63% and 79% of control, respectively) (but not mRNA) by the knockdown of ADAR1 or ADAR2 was the reason for the decreased transactivity of CYP2C8 (30% and 49% of control, respectively) (Ferguson et al., 2005) (Fig. 2). The mRNA levels of other P450 isoforms, such as CYP2A6, 2C9, 2C19, 2D6, and 2E1, which are known to be regulated by HNF4α (Kamiyama et al., 2007; Chen et al., 2018), were also reduced to 20%–81% of control by ADAR1 or ADAR2 knockdown. Exceptionally, the CYP3A4 mRNA level was increased (3-fold) by ADAR1 knockdown, which could be attributed to the increased CAR and PXR expression, as explained below. Thus, ADARs indirectly regulate P450 expression via modulation of the expression of transcription factors or nuclear receptors. In the following three sections, insights into ADAR-mediated regulation of AhR, CAR, and PXR, which are important for the transcription of drug metabolizing enzymes, are introduced.

A-to-I RNA Editing Downregulates AhR Expression by Creating a miRNA Recognition Site. AhR is a ligand-responsive transcription factor that modulates the transcription of xenobiotic-metabolizing enzymes such as CYP1A1, CYP1A2, CYP1B1, UGTs, and glutathione *S*-transferases. In response to ligand binding, AhR forms a heterodimer with aryl hydrocarbon receptor nuclear translocator, and it binds to the upstream region of its target genes (Ramadoss et al., 2005). The 3′-UTR of AhR has an everted Alu repeat, which can form a dsRNA

TABLE 1

Drug metabolism–related genes whose mRNA is subjected to A-to-I RNA editing and expression is regulated by ADARs

Gene	A-to-I RNA EDITED REGION		Effects, ADARs, Cell Line	Reference
	Evaluation by NGS[a]	Evaluation by Sanger Sequencing		
CYP1A2	Intron, 3′-UTR	ND	Upregulation, ADAR2, HepaRG cells	Nozaki et al., 2019
CYP2A6	Intron	ND	Upregulation, ADAR1 and ADAR2, HepaRG cells	Nozaki et al., 2019
CYP2B6	Intron, 3′-UTR	Not edited in 3′-UTR	Upregulation, ADAR1 and ADAR2, HepaRG cells	Nozaki et al., 2019
CYP2C8	5′-UTR, intron	ND	Upregulation, ADAR1 and ADAR2, HepaRG cells	Nozaki et al., 2019
CYP2C9	Intron, 3′-UTR	ND	Upregulation, ADAR1 and ADAR2, HepaRG cells	Nozaki et al., 2019
CYP2C19	3′-UTR	ND	Upregulation, ADAR1 and ADAR2, HepaRG cells	Nozaki et al., 2019
CYP2D6	Coding region	ND	Upregulation, ADAR2, HepaRG cells	Nozaki et al., 2019
CYP2E1	Intron	ND	Upregulation, ADAR1 and ADAR2, HepaRG cells	Nozaki et al., 2019
CYP3A4	Intron, 3′-UTR	Not edited in 3′-UTR	Downregulation, ADAR1, HepaRG and HepG2 cells	Nozaki et al., 2019; Takemoto et al., 2021b
UGT1A	Intron, 3′-UTR	ND	Downregulation, ADAR1, HepG2 cells	Takemoto et al., 2021b
UGT2B4	Intron	ND	ND	
UGT2B15	Intron	ND	ND	
UGT2B17	Intron	ND	ND	
CES1	Intron	ND	ND	
CES2	5′-UTR, intron	ND	ND	
AhR	3′-UTR	Edited in 3′-UTR	Downregulation, ADAR1, Huh-7 cells	Nakano et al., 2016
CAR	Intron	Not edited in intron	Downregulation, ADAR1, HepG2 cells	Nakano et al., 2019
HNF4α	Intron	ND	Upregulation, ADAR1 and ADAR2, HepaRG and Huh-7cells	Nozaki et al., 2019
PXR	Intron	Not edited in 3′-UTR	Downregulation, ADAR1 and ADAR2, HepaRG and HepG2 cells	Takemoto et al., 2021b

ND, no data; NGS, next generation sequencing.
[a]REDIportal (http://srv00.recas.ba.infn.it/atlas/), a database of A-to-I RNA editing sites identified by NGS.

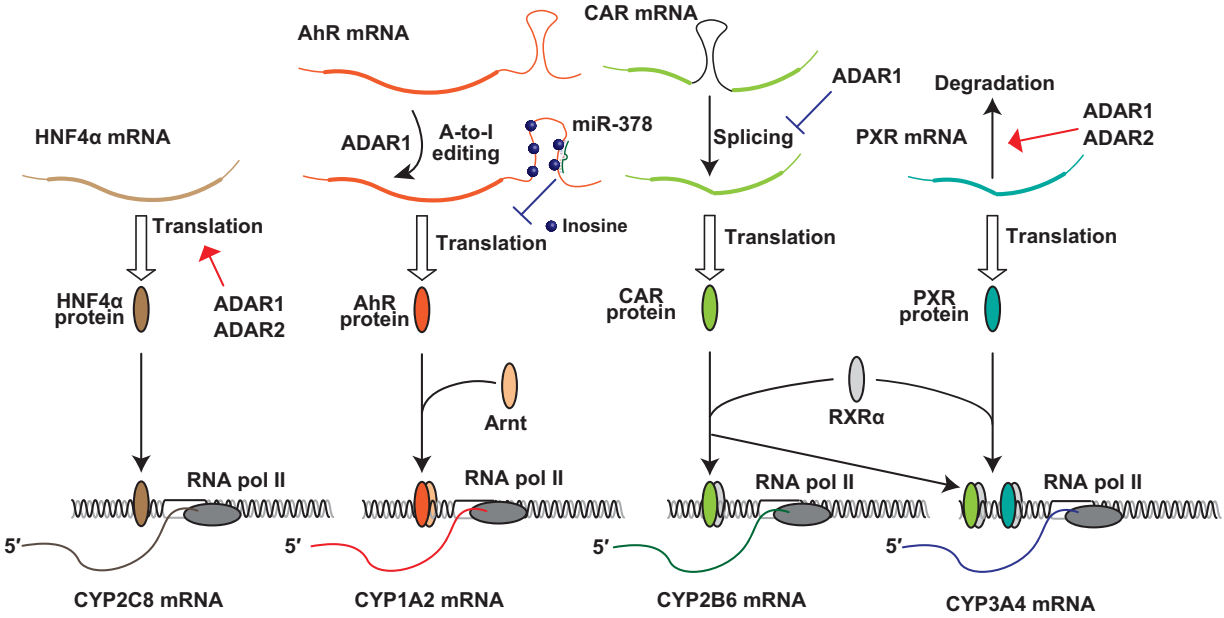

Fig. 2. A-to-I RNA editing modulating drug metabolism-related genes. ADAR1 and ADAR2 positively regulate hepatocyte nuclear factor 4α (HNF4α) by promoting translation, leading to upregulation of CYP2C8 expression. ADAR1 negatively regulates aryl hydrocarbon receptor (AhR) by creating a miRNA binding element, leading to downregulation of CYP1A2 expression. ADAR1 negatively regulates constitutive androstane receptor (CAR) by attenuating splicing, leading to downregulation of CYP2B6 and CYP3A4 expression. ADAR1 and ADAR2 downregulate pregnane X receptor (PXR) by facilitating mRNA degradation, leading to downregulation of CYP3A4 expression. Arnt, aryl hydrocarbon receptor nuclear translocator; RXRα, retinoid X receptor α.

structure, a typical target of ADAR. By Sanger sequence analysis, in which RNA editing sites show a peak of guanosine along with that of adenosine in cDNA but show a single adenosine peak in genomic DNA, we identified 38 ADAR1-mediated A-to-I RNA editing sites in the 3′-UTR of AhR in the human liver. These editing events negatively regulate AhR expression in human hepatoma-derived Huh-7 cells by creating a recognition site of miR-378 in the 3′-UTR of AhR (Nakano et al., 2016) (Fig. 2). The downregulation of AhR attenuated the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-mediated induction of CYP1A. In human liver samples, there is a significant inverse correlation between the miR-378 and AhR protein levels, suggesting that the RNA editing-dependent downregulation of AhR by miR-378 contributes to the variable expression of AhR in human liver. This is the first evidence to show that A-to-I RNA editing regulates xenobiotic metabolism-related genes.

ADAR1 Disrupts Splicing of CAR mRNA. CAR is a member of the nuclear receptor superfamily and it is a crucial regulator of drug metabolism and excretion. CAR is activated by some synthetic compounds, drugs, and natural products (Chang and Waxman, 2006), and it induces the expression of CYP2B6, CYP2C9, CYP3A4, UGTs, sulfotransferases, and drug transporters (Negishi and Honkakoski, 2000; Moore et al., 2006). We found that ADAR1 negatively regulates CAR expression in human hepatoma-derived HepG2 cells, resulting in attenuation of the ligand-dependent induction of CYP2B6 and CYP3A4. As for the mechanism of negative regulation of CAR by ADAR1, it was demonstrated that ADAR1 attenuates the splicing of CAR intron 3, which has two inverted Alu elements but does not have A-to-I RNA editing sites (Nakano et al., 2019) (Fig. 2). ADAR1 may bind to the dsRNA in intron 3 of CAR pre-mRNA, resulting in the attenuation of splicing in an RNA editing activity-independent manner.

ADARs Promote Degradation of PXR mRNA. PXR is a nuclear receptor that regulates the expression of at least 40 types of genes, including P450s, UGTs, sulfotransferases, glutathione *S*-transferases, and drug transporters (Kliewer et al., 2002). In our recent study, it was found that the knockdown of ADAR1 or ADAR2 significantly increased PXR protein levels (1.5–1.9-fold) in HepaRG and HepG2 cells via attenuation of mRNA degradation, resulting in increased CYP3A4 (71-fold) and UGT1A1 (1.9-fold) expression. A luciferase assay demonstrated that the 3′-UTR of PXR mRNA is responsible for the ADAR-mediated posttranscriptional regulation of PXR expression, despite the lack of RNA edited sites in this region (Takemoto et al., 2021b). Although the underlying mechanism of ADAR-mediated downregulation via the 3′-UTR has not been clarified, ADARs may edit the seed sequence of certain miRNAs to create a novel miRNA-PXR mRNA interaction. It has been suggested that the ADAR1-mediated negative regulation of PXR would be a reason for the negative regulation of CYP3A4 by ADAR as described in Section 2.4.1.

Cigarette Smoke Induces Degradation of ADAR1 Protein. Recently, we performed a study to identify extrinsic factors affecting ADAR expression and found that cigarette smoke extract (CSE) treatment decreased ADAR1 protein expression to 45% of control in human lung carcinoma-derived A549 cells (Takizawa et al., 2020). Additionally, exposure to cigarette smoke decreased pulmonary Adar1 protein in mice to 70% of control. The reduction in ADAR1 expression by CSE was demonstrated to be caused by the degradation of ADAR1 protein via the autophagy pathway. Cigarette smoking induces oxidative stress, which is relevant to the pathophysiology of chronic obstructive pulmonary disease (Ahmad et al., 2013). Interestingly, we also demonstrated that the knockdown of ADAR1 resulted in an increase (1.3-fold) in CSE-induced oxidative stress and a decrease in superoxide dismutase activity (88% of control) and heme oxygenase-1 expression (32% of control), indicating that ADAR1 has a role in suppressing oxidative stress. Thus, it was demonstrated that ADAR1 has a role in regulating the biologic response to protect against xenobiotic exposure.

m^{6}A Modification

In addition to A-to-I RNA editing, RNA is subjected to nearly 160 kinds of modifications, such as methylation of adenosine at the N^6 position (m^{6}A modification), methylation of adenosine at the N^1 position (N^1-methyladenosine modification), dimethylation of adenosine at the

N^6 and ribose 2′-*O* positions (N^6,2′-*O*-dimethyladenosine modification), methylation of cytidine at the C^5 position (5-methylcytosine modification), and hydroxylation of 5-methylcytosine (5-hydroxymethylcytosine modification), which do not result in nucleotide conversion. Among them, m^{6}A modification is the most prevalent internal chemical modification of mRNA, which was discovered more than 45 years ago in the rat, mouse, and human transcriptomes (Desrosiers et al., 1974; Adams and Cory, 1975; Wang et al., 2015; Mauer et al., 2017; Furuichi et al., 1975). Due to the lack of a methodo-logy for the site-specific detection of m^{6}A, the cellular function of m^{6}A modification has been unclear over the years (Dominissini et al., 2012). Next generation sequencing techniques combined with RNA immunoprecipitation using an anti-m^{6}A antibody revealed that m^{6}A ubiquitously occurred in the DRACH (D = A, G or U; R = A or G; H = A, C or U) motif mainly located near the stop codon in the last exon and was secondarily located in the 5′-UTR (Dominissini et al., 2012; Meyer et al., 2012), and 480,000 m^{6}A sites were identified in the human transcriptome (Xuan et al., 2018). Elucidation of the physiologic significance of m^{6}A modification has just started.

m^{6}A Writers and Erasers. m^{6}A is deposited by a methyltransferase complex containing methyltransferase-like 3 (METTL3) and METTL14, which are called "m^{6}A writers" (Liu et al., 2014; Ping et al., 2014; Schwartz et al., 2014). METTL3 is catalytically active, whereas METTL14 is an allosteric activator (Śledź and Jinek, 2016; Wang et al., 2016). This METTL3-METTL14 complex catalyzes the majority of m^{6}A modifications on mRNA (Geula et al., 2015). In this reaction, *S*-adenosylmethionine is used as a methyl donor. Additionally, METTL16 has been found to possess potential m^{6}A methyltransferase activity (Warda et al., 2017).

m^{6}A is reversibly demethylated to adenosine by the "m^{6}A eraser". The first identified m^{6}A eraser was fat mass and obesity associated (FTO) (Jia et al., 2011). FTO is a member of the Fe(II)- and α-ketoglutarate-dependent AlkB family (Gerken et al., 2007), and its loss results in reduced body weight and food intake in mice (Fischer et al., 2009; Church et al., 2010). FTO-catalyzed demethylation of m^{6}A involves stepwise oxidation of m^{6}A to N^6-hydroxymethyladenosine and N^6-formyladenosine to adenosine (Fu et al., 2013). FTO is also known to preferentially demethylate N^6,2′-*O*-dimethyladenosine in the 5′ cap of mRNA (Mauer et al., 2017). AlkB homolog 5 (ALKBH5), the second identified m^{6}A eraser, is also a member of the Fe(II) and α-ketoglutarate-dependent AlkB family. Unlike FTO, ALKBH5 directly demethylates m^{6}A to adenosine (Zheng et al., 2013) since the intermediate is unlikely to be detected in the process of demethylation. Thus, m^{6}A is dynamically and reversibly regulated by "m^{6}A writers" and "m^{6}A erasers" (Fig. 3).

Functional Roles of m^{6}A Modification in Gene Regulation. Unlike A-to-I RNA editing, m^{6}A modification does not result in base conversion but instead exerts its functions by recruiting proteins called "m^{6}A readers". The first identified readers were YTH (YT521-B homology) domain-containing proteins (Dominissini et al., 2012). In humans, there are five members: YTHDF1, YTHDF2, YTHDF3, YTHDC1, and YTHDC2. YTHDF1 delivers m^{6}A-containing mRNA to the translation machinery and enhances translation initiation (Wang et al., 2015). YTHDF2 enhances the degradation of m^{6}A-modified mRNA by deadenylation and translocation into the P-body (Wang et al., 2014; Du et al., 2016). YTHDF3 promotes the translation of targeted mRNAs by interacting with YTHDF1 (Shi et al., 2017). YTHDC1 mediates splicing (Xiao et al., 2016) and nuclear export of m^{6}A-modified mRNA (Roundtree et al., 2017). YTHDC2 promotes the translation efficiency of its target m^{6}A-containing mRNAs and promotes mRNA degradation (Hsu et al., 2017; Kretschmer et al., 2018). Thus, various steps of mRNA processing are potentially affected by the binding of readers to m^{6}A.

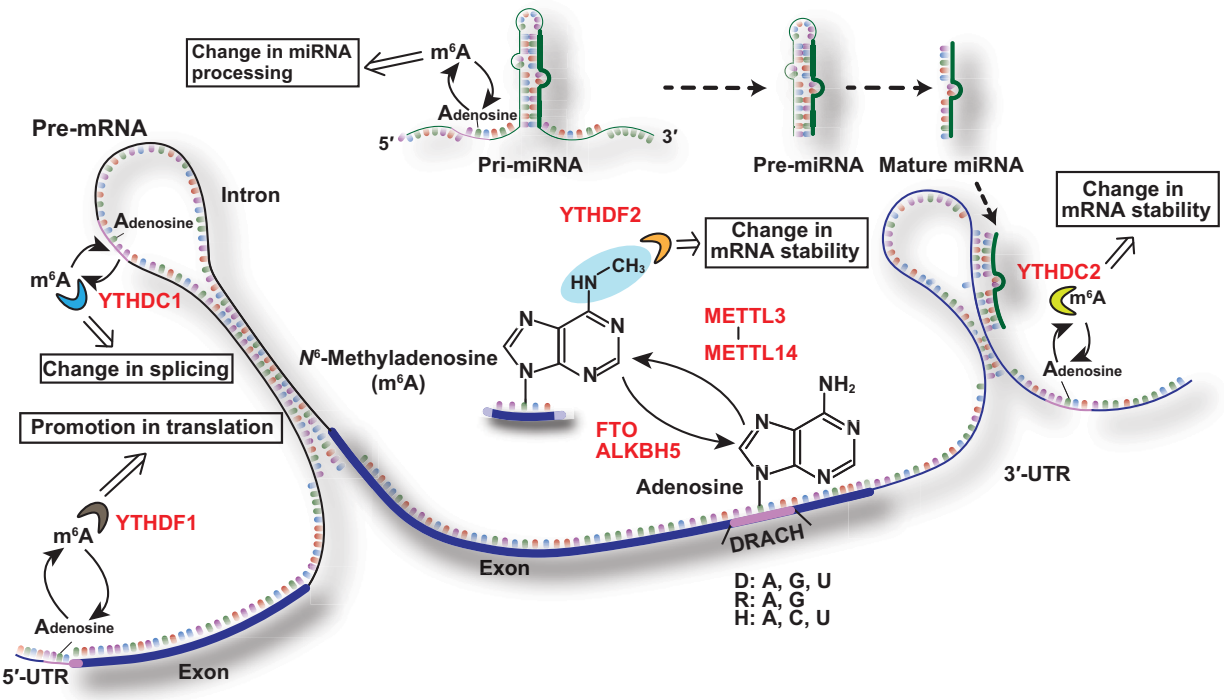

Fig. 3. Functional significance of m^{6}A modification. m^{6}A is installed by a methyltransferase complex such as methyltransferase-like 3 (METTL3) and METTL14, which are called "m^{6}A writers", whereas the methyl group can be removed by RNA demethylases including fat mass and obesity associated (FTO) and AlkB homolog 5 (ALKBH5), which are called "m^{6}A erasers". As an "m^{6}A reader", YTHDF1 promotes the translation efficiency of m^{6}A-modified mRNA. YTHDF2 and YTHDC2 enhance the degradation of m^{6}A-modified mRNA. YTHDC1 mediates splicing of m^{6}A-containg mRNA.

m^{6}A modification can affect miRNA processing (Fig. 3). m^{6}A modification of pri-miRNAs recruits heterogeneous nuclear ribonucleoprotein A2/B1, which interacts with DiGeorge syndrome critical region 8 of pri-let-7e to facilitate miRNA biogenesis (Alarcón et al., 2015). Another example is that nuclear factor-kappa B-activating protein recognizes m^{6}A in pri-miR-25 to recruit DiGeorge syndrome critical region 8, resulting in maturation promotion (Zhang et al., 2019). Since knowledge about the functional roles of m^{6}A modification of miRNA precursors is limited, additional studies are needed for a comprehensive understanding of the roles of m^{6}A modification in miRNA maturation.

Physiologic and Pathologic Roles of m^{6}A Modification. Recently, accumulating evidence suggests that m^{6}A modification plays significant roles in biologic processes such as cell differentiation (Batista et al., 2014), development (Heck and Wilusz, 2019), immune response (O'Connell et al., 2015), and circadian rhythms (Fustin et al., 2013). Thus, disruption of m^{6}A levels and dysregulation of m^{6}A writers, erasers, and readers is associated with various diseases, such as obesity (Jia et al., 2011), neuronal disorders (Maity and Das, 2016), infectious diseases (Gokhale et al., 2016), cancer (Deng et al., 2018), and infertility (Zheng, et al., 2013). Regarding the physiologic role of m^{6}A modification in the liver, it has been reported that METTL3 decreases hepatic insulin sensitivity by upregulating fatty acid synthase (Xie et al., 2019), and it enhances lipid accumulation by downregulating peroxisome proliferator-activator α expression (Zhong et al., 2018). Hepatic METTL3 expression in type 2 diabetes mellitus patients is higher than that in healthy subjects (Xie et al., 2019). Thus, m^{6}A modification plays important roles in hepatic lipid metabolism.

m^{6}A Modification Modulates Drug Metabolism Potency. In RMBase (https://rna.sysu.edu.cn/rmbase/index.php), a database of RNA modification, drug metabolism-related genes are registered as mRNAs that are subjected to m^{6}A modification (Table 2). Our group has revealed that the m^{6}A modification actually has significance in the regulation of drug metabolism. In the following sections, our recent findings that m^{6}A modification regulates P450, UGT, and esterase isoforms are summarized in Table 2 and introduced in detail.

m^{6}A Modification Downregulates CYP2C8 Expression. To examine whether m^{6}A modification affects P450 expression, we evaluated the expression levels of P450 isoforms in HepaRG cells treated with 3-deazaadenosine (DAA), an inhibitor of *S*-adenosylmethionine synthesis (Chiang, 1998; Nakano et al., 2020). CYP1A2 (1.6-fold), 2B6 (2.2-fold), and 2C8 (2.7-fold) expression levels were significantly increased by treatment with DAA. The CYP2C8 expression level and enzymatic activity were increased (1.4–2.0-fold) by silencing of METTL3 and METTL14, but it was reduced to 22%–75% of control by silencing of FTO but not by ALKBH5. By immunoprecipitation of m^{6}A-containing RNA using an anti-m^{6}A antibody, we found that the 5′-UTR and the 3′-UTR of CYP2C8 mRNA undergo m^{6}A modification in HepaRG cells and human liver samples. The m^{6}A modification in the 3′-UTR of CYP2C8 negatively regulates CYP2C8 expression by recruiting YTHDC2, which promotes the degradation of CYP2C8 mRNA (Fig. 4). This is the first study to demonstrate m^{6}A modification-mediated regulation of P450 isoforms.

m^{6}A Modification Downregulates UGT2B7 Expression. To expand the knowledge of m^{6}A modification-mediated regulation of drug metabolism potencies, we investigated whether m^{6}A modification regulates UGT isoforms (Ondo et al., 2021). Treatment of HepaRG cells with DAA significantly increased (1.3–2.6-fold) the UGT1A1, 1A3, 1A4, 1A9, 2B7, 2B10, and 2B15 mRNA levels. Among them, we focused on UGT2B7 because it most highly contributes to glucuronidation of clinically used drugs (Williams et al., 2004). The UGT2B7 expression level in Huh-7 cells was significantly increased (1.5-fold) by double knockdown of METTL3 and METTL14 but was reduced to 70% and 66% of control by knockdown of FTO or ALKBH5, respectively, suggesting that m^{6}A modification negatively regulates UGT2B7 expression. Methylated RNA immunoprecipitation assays revealed that the 5′-UTR and the 3′-UTR of UGT2B7 mRNA have m^{6}A modification sites in HepaRG

TABLE 2

Drug metabolism-related genes whose mRNA is subjected to m^{6}A modification and whose expression is regulated by m^{6}A-related enzymes

Gene	m^{6}A Modified Region		Effects, m^{6}A-Related Enzymes, Cell Line	Reference
	Evaluation by NGS[a]	Evaluation by RIP with Anti-m^{6}A Antibody		
CYP2B6	3′-UTR	ND	ND	
CYP2C8	Coding region, 3′-UTR	Modified in 5′-UTR, coding region, and 3′-UTR	Downregulation, METTL3/14; upregulation, FTO; downregulation, YTHDC2, HepaRG and Huh-7 cells	Nakano et al., 2020
UGT1A	Coding region, intron, 3′-UTR	ND	ND	
UGT2B4	Coding region	ND	ND	
UGT2B7	Not registered	Modified in 5′-UTR, coding region, and 3′-UTR	Downregulation, METTL3/14; upregulation, FTO and ALKBH5, Huh-7 cells	Ondo et al., 2021
UGT2B15	Coding region	ND	ND	
UGT2B17	Intron	ND	ND	
CES1	5′-UTR, coding region	ND	ND	
CES2	5′-UTR, coding region, intron, 3′-UTR	Modified in 5′-UTR, coding region, and 3′-UTR	Downregulation, METTL3/14; upregulation by FTO and ALKBH5; downregulation by YTHDC2, HepaRG and Huh-7 cells	Takemoto et al., 2021
AhR	5′-UTR, coding region, 3′-UTR	ND	ND	
CAR	Coding region, intron, 3′-UTR	ND	ND	
HNF4α	3′-UTR	Modified in 5′-UTR, coding region, and 3′-UTR	Upregulation, FTO, Huh-7 cells	Ondo et al., 2021
PXR	Coding region, 3′-UTR	ND	ND	

ND, no data; NGS, next generation sequencing; RIP, RNA immunoprecipitation.
[a]RMBase (https://rna.sysu.edu.cn/rmbase/index.php), a database of m^{6}A modification sites identified by NGS.

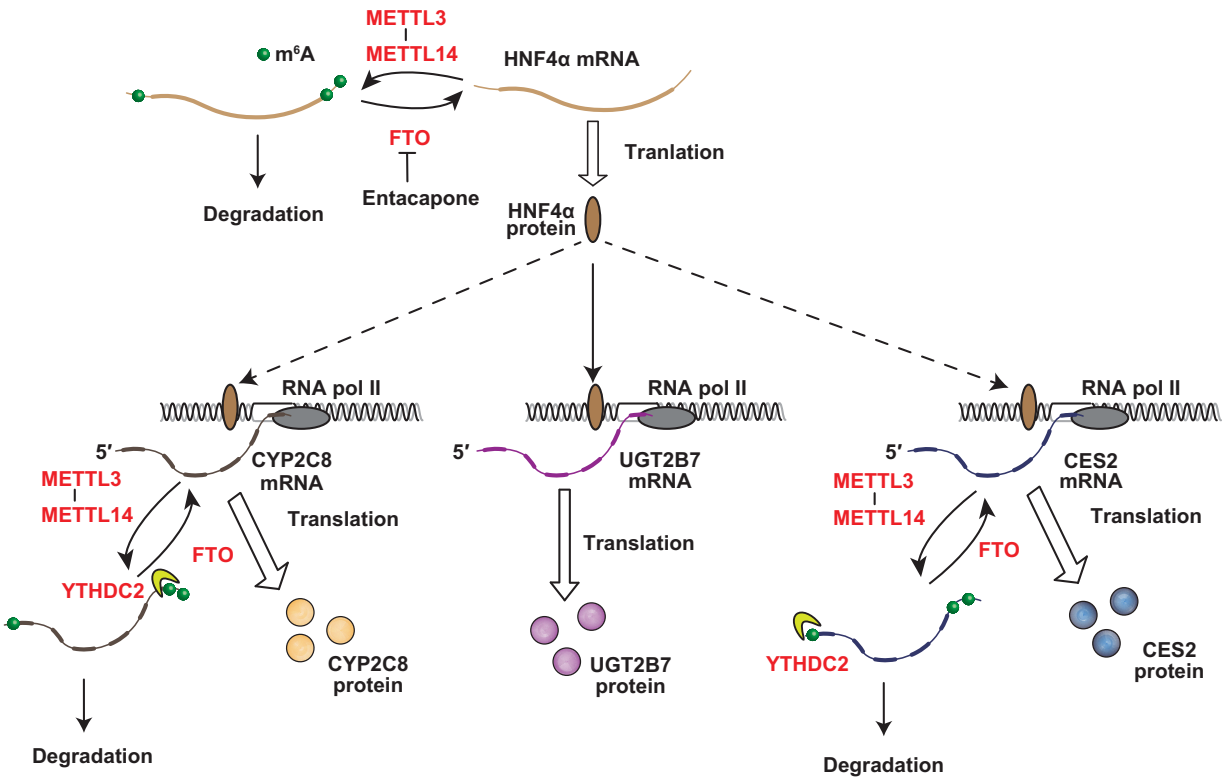

Fig. 4. m^{6}A modification modulating drug metabolism-related genes. CYP2C8 mRNA is subjected to m^{6}A modification, leading to YTHDC2-mediated degradation of its mRNA. m^{6}A modification negatively regulates UGT2B7 expression by downregulating HNF4α. YTHDC2 promotes degradation of CES2 mRNA by recognizing m^{6}A in the 5′-UTR.

cells and human livers. However, these methylation events do not directly affect the UGT2B7 expression level. Reporter assays demonstrated that the promoter region has a key role in m^{6}A modification-mediated regulation of UGT2B7. We found that the expression level of HNF4α, which regulates the transcription of UGT2B7, was significantly reduced to 46% of control by knockdown of FTO, indicating that this would be the underlying mechanism of m^{6}A modification-mediated negative regulation of UGT2B7 transactivation. Since HNF4α also transactivates *CYP2C8* (Ferguson et al., 2005), the negative regulation of HNF4α by m^{6}A modification contributes to the downregulation of CYP2C8 expression by the m^{6}A modification described above. Further study is needed to clarify the effects of m^{6}A modification-mediated repression of HNF4α expression on the other P450 and UGT isoforms. Entacapone, which is used for treating Parkinson's disease, has been reported to inhibit FTO in vitro and in vivo (Peng et al., 2019). Interestingly, treatment with entacapone decreased HNF4α and UGT2B7 expression to 35% and 21%, respectively. Thus, entacapone has the potential to cause drug-drug interactions through the negative regulation of UGT2B7 (Fig. 4). This study provided novel insight into a unique regulatory mechanism for UGT expression.

m^{6}A Modification Downregulates Carboxylesterase 2 Expression. Carboxylesterase 2 (CES2) is a serine esterase responsible for the hydrolysis of various drugs, such as 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin, and endogenous substrates, such as triglycerides and diacylglycerides (Humerickhouse et al., 2000; Ruby et al., 2017). Recently, we revealed that m^{6}A modification affects drug and lipid metabolism by regulating hepatic CES2 expression (Takemoto et al., 2021a) (Fig. 4). The CES2 expression level and its hydrolase activity for 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin in HepaRG and HepG2 cells were significantly increased (1.2–1.4-fold) by double knockdown of METTL3 and METTL14 but were reduced to 25%–81% of control by knockdown of FTO or ALKBH5, suggesting that m^{6}A modification downregulates CES2 expression. Consistently, cellular lipid accumulation was decreased to 82% of control by double knockdown of METTL3 and METTL14, but it was increased (1.2–1.5-fold) by knockdown of FTO or ALKBH5. RNA immunoprecipitation assays using an anti-m^{6}A antibody revealed that the 5′-UTR and the last exon of CES2 are subjected to m^{6}A modification. We found that YTHDC2 recognizes m^{6}A in the 5′-UTR of CES2 and promotes the degradation of CES2 mRNA. Because CES2 is also transcriptionally regulated by HNF4α (Li et al., 2016), m^{6}A may indirectly contribute to the downregulation of CES2 via attenuation of HNF4α expression. Collectively, m^{6}A modification has a strong impact on the regulation of CES2, affecting pharmacokinetics, drug responses, and lipid metabolism.

Conclusion

In this review article, current knowledge about A-to-I RNA editing and m^{6}A modification-dependent regulation of drug metabolism is summarized. The significance of such posttranscriptional regulation in the field of pharmacokinetics research has only recently started to become clear. In addition to drug metabolizing enzymes, some drug transporters have been reported to be subjected to posttranscriptional regulation. Omata et al. (2021) reported that ADAR1 regulates P-glycoprotein in human renal cells by affecting its alternative splicing. Xiao et al. (2021) reported that FTO upregulates multidrug resistance-associated protein 7 expression in nonsmall cell lung cancer to confer gefitinib resistance. Thus, A-to-I RNA editing and m^{6}A modifications could be distinct regulators of pharmacokinetics.

In addition to the regulation of drug metabolizing enzymes and transporters, A-to-I RNA editing and m^{6}A modification have been shown to impact the sensitivity of anticancer drugs. Ishizuka et al. (2019) reported,

by using syngeneic mouse models, that knockout of *Adar1* sensitizes tumors to immune checkpoint blockade. Sun et al. (2020) reported that ADAR1 contributes to resistance to bromodomain and extraterminal domain inhibitors in pancreatic cancer cells. Thus, inhibition of ADAR1 would be a promising approach to overcome cancer resistance. There are currently no clinically used ADAR inhibitors, although adenosine analogs, 8-azaadenosine and 8-chloroadenosine, have been reported to inhibit ADAR1 (Zipeto et al., 2016; Ding et al., 2020; Ramírez-Moya et al., 2020). These compounds would be useful, but a recent study reported that they are not selective inhibitors of ADAR1 (Cottrell et al., 2021). Thus, the development of novel specific inhibitors of ADAR1 is desired. Knowledge about the roles of m^6A modification in the cancer therapy response is more mature than knowledge about A-to-I editing, and it has been recently summarized in a review by Lan et al. (2021). For example, FTO expression levels are increased during the development of resistance to tyrosine kinase inhibitors, and rhein, an inhibitor of FTO, restores sensitivity to tyrosine kinase inhibitors (Yan et al., 2018). m^6A modification would also be an attractive target for cancer therapy. In addition to cancer, knowledge about significance of FTO in energy metabolism has been accumulated. Research using mouse models has shown that ubiquitous overexpression and knockout of *Fto* results in obesity and reduced body weight, respectively (Fischer et al., 2009; Church et al., 2010). Because HNF4α and CES2 are known to regulate lipid and glucose metabolisms (Gonzalez, 2008; Ruby et al., 2017), FTO-mediated upregulation of HNF4α and CES2 (described in *Sections 2.4.1* and *2.4.2*) would be a piece of the functional roles of FTO in energy metabolism. Inhibition of FTO using entacapone and rhein would be a promising strategy for treatment of metabolic disorders such as obesity and diabetes.

Until now, the roles of A-to-I RNA editing and m^6A modification in the regulation of drug metabolism-related genes have been examined separately. It has been reported that A-to-I editing preferentially occurs in m^6A-negative transcripts, indicating that these posttranscriptional regulations may suppressively interfere with each other (Xiang et al., 2018). As described above, A-to-I RNA editing positively regulates CYP2C8 expression (Fig. 2), whereas m^6A modification negatively regulates CYP2C8 expression (Fig. 4). There is a possibility that A-to-I RNA editing upregulates CYP2C8 expression by repressing m^6A modification-mediated negative regulation and vice versa. To deeply understand the importance of these two posttranscriptional regulations in the regulation of drug metabolizing enzymes, it should be noted that one posttranscriptional modification affects the levels of the other modification on the same transcript.

It is now clear that A-to-I RNA editing and m^6A modification are novel regulators of drug metabolism potency. Elucidation of the extent and causes of inter- and intraindividual differences in these posttranscriptional modifications as well as the development of inhibitors and activators for RNA modifications are expected to contribute to the practice of personalized medicine.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Nakano, Nakajima.

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