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| 氏　　名 | 中野　正隆 |
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| 論文審査委員 | 主査　中島　美紀<br>副査　玉井　郁巳<br>副査　加藤　将夫<br>副査　中西　猛夫<br>副査　深見　達基 |

**Dissertation abstract**

# Regulation of human aryl hydrocarbon receptor and dihydrofolate reductase expression by RNA editing

**Masataka Nakano**

**Drug Metabolism and Toxicology**
**Division of Pharmaceutical Sciences**
**Graduate School of Medical Sciences**
**Kanazawa University**

**Summary**

Adenosine-to-inosine (A-to-I) RNA editing, which is catalyzed by adenosine deaminase acting on RNA (ADAR) enzymes, is the most frequent type of RNA editing in mammals. The conversion of nucleotides possibly affects amino acid sequence, splicing and microRNA (miRNA) targeting. The purpose of this study is to clarify the impact of RNA editing on expression of pharmacokinetics- and pharmacodynamics-related genes, focusing on human aryl hydrocarbon receptor (AhR) and dihydrofolate reductase (DHFR). First, it was revealed that ADAR1-mediated RNA editing negatively regulates AhR expression by creating miR-378 binding site in the 3'-untranslated region (3'-UTR) in human hepatocellular carcinoma-derived Huh-7 cells. The down-regulation of AhR attenuated 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-mediated induction of CYP1A1, a downstream gene of AhR. In human liver samples, high editing levels in the 3'-UTR of AhR and a significant inverse association between miR-378 and AhR protein levels were observed, suggesting the RNA editing-dependent down-regulation of AhR by miR-378 contributes to the variability in the hepatic AhR expression. Second, it was revealed that ADAR1-mediated RNA editing positively regulates DHFR expression in human breast cancer-derived MCF-7 cells by destroying miR-25-3p and miR-125a-3p binding sites in the 3'-UTR. 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 the hyper-editing of DHFR transcript would be responsible for the increased DHFR expression. Taken together, this study demonstrated the biological significance of RNA editing in drug response.

**Background**

RNA editing is a post-transcriptional process that alters nucleotide sequence of RNA transcripts. In mammals, the most common type of RNA editing is A-to-I editing, which is catalyzed by ADAR enzymes. They convert adenosine in double-stranded RNA structures into inosine by hydrolytic deamination. Since much of the cellular machinery treats inosine as guanine, the conversion of nucleotides possibly changes amino acid sequence, splicing,

miRNA targeting or miRNA maturation. In vertebrates, two functional members of the ADAR family, ADAR1 and ADAR2, are ubiquitously expressed. It became apparent that disrupted RNA editing or abnormal ADAR expression are associated with several diseases, including cancer, neurological disorders, metabolic diseases, viral infections, and autoimmune disorders. The purpose of this study is to clarify the biological significance of RNA editing in pharmacokinetics and pharmacodynamics-associated genes, focusing on AhR and DHFR, because RADAR, a database of RNA editing sites, indicates that the 3'-UTRs of these gene transcripts are subjected to RNA editing. In general, 3'-UTR includes binding sites for miRNA, which silences gene expression via translational repression or mRNA degradation. Accordingly, a possibility that RNA editing regulates expression of AhR and DHFR by altering miRNA binding site was examined.

**Down-regulation of human aryl hydrocarbon receptor expression by RNA editing in human liver**

AhR is a ligand-activated transcription factor that regulates the expression of xenobiotic-metabolizing enzymes, including CYP1A1, CYP1A2 and CYP1B1. By direct sequencing of AhR cDNA and genomic DNA, 38 RNA editing sites were identified in the 3'-UTR of AhR in human hepatocellular carcinoma-derived Huh-7 cells. Knockdown of ADAR1 resulted in decrease in the RNA editing levels and increase in AhR protein levels, but not mRNA levels, indicating that ADAR1 negatively regulates AhR expression in a post-transcriptional manner. TCDD-mediated induction of CYP1A1, a downstream gene of AhR, was augmented by ADAR1 knockdown, suggesting that RNA editing affected the expression of P450 isoform. It was surmised that A-to-I RNA editing created miRNA targeting sites in AhR mRNA. By in silico analysis, miR-378 was predicted as a candidate miRNA whose binding affinity to the edited AhR 3'-UTR would be higher than that to the unedited 3'-UTR of AhR. Interestingly, overexpression of miR-378 decreased AhR protein expression levels, and the miR-378-dependent downregulation of AhR was abolished by knockdown of ADAR1. The results indicated that the mechanism of the ADAR1-mediated downregulation of AhR would be

attributed to the creation of the miR-378 recognition site in the 3'-UTR of AhR. The interindividual differences in the RNA editing levels within the 3'-UTR of AhR in a panel of 32 human liver samples were relatively small, whereas the differences in ADAR1 expression were large (220-fold). 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 expression of AhR in human liver. Taken together, it was found that A-to-I RNA editing regulates the expression of AhR by creating miR-378 binding sites. The mechanism contributes to the interindividual variability in AhR expression in human livers. This study is the first to prove that RNA editing modulates the potency of xenobiotic metabolism in the human liver.

**Up-regulation of dihydroforate reductase in human breast cancer by RNA editing**

DHFR plays a key role in folate metabolism, and is a target of methotrexate. In human breast adenocarcinoma-derived MCF-7 cells, 26 RNA editing sites were identified in the 3'-UTR of DHFR. Knockdown of ADAR1 decreased the RNA editing levels in the 3'-UTR of DHFR in MCF-7 cells, and resulted in the decrease of DHFR mRNA and protein levels. By using α-amanitin, a transcriptional inhibitor, it was revealed that the edited DHFR mRNA was more slowly degraded than the non-edited mRNA, suggesting that DHFR mRNA are stabilized by RNA editing. Thus, it was found that ADAR1 up-regulates DHFR expression by stabilization of mRNA. miR-25-3p and miR-125a-3p were predicted, by a computational analysis, to bind to the non-edited 3'-UTR of DHFR but not to the edited sequence. The decrease in DHFR expression by the knockdown of ADAR1 was restored by inhibition of these miRNAs, suggesting that RNA editing-mediated up-regulation of DHFR requires the function of these miRNAs. Interestingly, the knockdown of ADAR1 decreased cell viability and increased the sensitivity to methotrexate of MCF-7 cells. ADAR1 expression levels and the RNA editing levels in the 3'-UTR of DHFR in breast cancer tissues were higher than those in adjacent normal tissues. Collectively, ADAR1 positively regulates the expression of

DHFR through RNA editing by disrupting the binding of miR-25-3p and miR-125a-3p to the 3'-UTR of DHFR, enhancing cellular proliferation and resistance to methotrexate. This study could provide new insights into the regulatory mechanism of DHFR expression and the role of RNA editing in cancer progression and methotrexate response. ADAR1 may be a potential anti-tumor target for use with anti-folate compounds including methotrexate.

## Conclusion

In this dissertation, it was clarified that RNA editing modulates AhR and DHFR expression by creating and destroying miRNA recognition sites in the 3'-UTRs, respectively. This study is the first to demonstrate the biological significance of RNA editing in pharmacokinetics- and pharmacodynamics-related genes.

## Reference

Nakano M, Fukami T, Gotoh S, Takamiya M, Aoki Y and 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, Fukami T, Gotoh S and Nakajima M A-to-I RNA editing up-regulates human dihydrofolate reductase in breast cancer. *J Biol Chem*, in press.

# 審査結果の要旨

RNA 編集の一種である A-to-I RNA editing は遺伝子の発現および機能に影響を与え得る転写後調節であり、adenosine deaminase acting on RNA (ADAR) により触媒される。本研究は、薬物応答性の個人差や個人内変動における RNA 編集の意義を明らかにすることを目的として、芳香族炭化水素受容体 (AhR) およびジヒドロ葉酸還元酵素 (DHFR) に着目して検討したものである。リガンド依存的に薬物代謝酵素の発現を制御する転写因子である AhR が、ヒト肝臓中において ADAR1 によって RNA 編集を受けることで、microRNA による発現抑制を受けることを見出した。この発現制御は AhR 下流遺伝子である CYP 分子種の発現にも影響を及ぼした。葉酸代謝の鍵分子であり、癌化学療法剤メトトレキサートの標的である DHFR が、ヒト乳がん細胞において ADAR1 によって RNA 編集を受けることで、microRNA による発現抑制を免れ、発現量増加につながることを示した。この発現制御は乳がん細胞の細胞増殖やメトトレキサートに対する感受性に影響を及ぼした。

以上、本研究成果は RNA 編集が薬物応答性に影響を与えていることを初めて示したものであり、博士 (創薬科学) に値すると判定した。