Pharmacoepitranscriptomic landscape revealing m6A modification could be a drug-effect biomarker for cancer treatment

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RNA chemical modifications are a new but rapidly developing field. They can directly affect RNA splicing, transport, stability, and translation. Consequently, they are involved in the occurrence and development of diseases that have been studied extensively in recent years. However, few studies have focused on the correlation between chemical modifications and drug effects. Here, we provide a landscape of six RNA modifications in pharmacogene RNA (pharmacoepitranscriptomics) to fully clarify the correlation between chemical modifications and drugs. We performed systematic and comprehensive analyses on pharmacoepitranscriptomics, including basic characteristics of RNA modification and modification-associated mutations and drugs affected by them. Our results show that chemical modifications are common in pharmacogenes, especially N6-methyladenosine (m6A) modification. In addition, we found a very close relationship between chemical modifications and anti-tumor drugs. More interestingly, the results demonstrate the importance of m6A modification for anti-tumor drugs, especially for drugs in triple-negative breast cancer (TNBC), ovarian cancer, and acute myelocytic leukemia (AML). These results indicate that pharmacoepitranscriptomics could be a new source of drug-effect biomarkers, especially for m6A and anti-tumor drugs.

INTRODUCTION

Currently, a total of 163 modifications have been identified in RNA molecules.[1] Some of them were found to be present in the mRNA, such as N^7-methylguanosine (m7G), $N^{6,2}$-O-dimethyladenosine (m6Am), N^1-methyladenosine (m1A), 5-methylcytosine (m5C), N^6-methyladenosine (m6A), and pseudouridine (φ).[2,3] These modifications could affect RNA splicing, transport, stability, and translation. Thus, they could be involved in pathological and physiological processes by ultimately affecting gene expression. Recently, a large num-

ber of studies have focused on the correlation between RNA chemical modification and disease occurrence and development.[4,5] And a few studies found that they were closely related to drug effects. For example, several m6A regulators could affect drug sensitivity, especially for cancer chemotherapy and immunotherapy.[6,7] These studies proposed the new area of pharmacoepitranscriptomics, which investigates the influence of RNA modifications on drug effects. However, this area largely remains unknown.

"Pharmacogenes" refers to genes involved in pharmacokinetics and pharmacodynamics processes. They mainly include drug-metabolizing enzymes, transporters, receptors, and targets. The function and expression of pharmacogenes could directly affect drug efficacy and safety. Therefore, modifications on pharmacogenes' mRNA could potentially alter their expression and consequently affect drug effects. This was supported by the fact that altered m6A levels on the RNA of pharmacogenes ultimately affected the expression levels of cytochromes P450 (CYP450s) and solute carrier family 1 member 5 (SLC1A5).[8] Thus, the RNA modification of pharmacogenes is important for pharmacoepitranscriptomics investigation. It could be critical for our understanding of this new area. However, the RNA modifications of pharmacogenes and their potential role in drug effects are unknown.

In this study, we attempted to provide a pharmacogene RNA modification landscape and found that m6A modification could be drug-effect biomarker for cancer treatment.
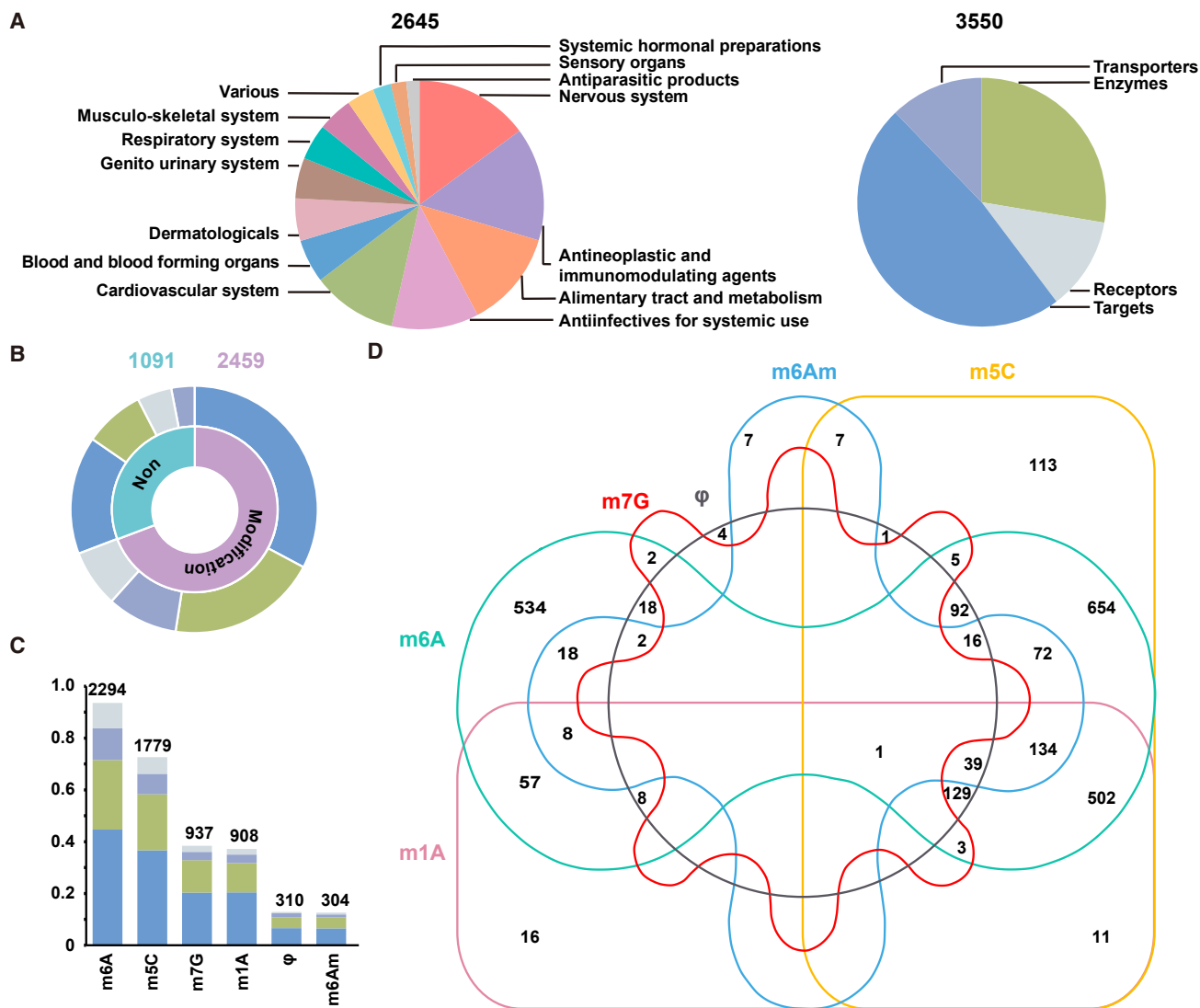
Figure 1. Overview of pharmacogene RNA modification

(A) The pie charts show all analyzed drugs (left), which were divided into 14 categories according to the ATC system, and pharmacogenes (right), which were divided into the four categories of enzyme (green), receptor (light gray), target (blue), and receptor (purple). (B) The inner circle of the sunburst chart shows two types of pharmacogenes: 2,459 pharmacogenes with modified RNAs (rose red) and 1,091 pharmacogenes without modified RNAs (brilliant blue). The outer circle of the chart indicates four types of genes: enzyme, receptor, target, and receptor. (C) The proportion of pharmacogenes with each type of modified RNA. The composition of the four types of genes for each modification is indicated with different colors (consistent with A and B). (D) Overlap analysis of various modified pharmacogene RNAs.

RESULTS

Overview of RNA modifications in pharmacogenes

In this study, a total of 2,645 drugs and their 3,550 corresponding pharmacogenes were analyzed. The data processing flow is shown in Figure S1. The details of their classification are shown in Figure 1A. All of pharmacogenes could be classified into two groups: with or without RNA chemical modification (Figure 1B). Of these, 2,459 pharmacogenes' RNA was modified with m6Am, m1A, m5C, m6A, m7G, or φ, while 1,091 pharmacogenes did not have any of the six modifications. These two groups accounted for 70.28% and 30.73%

of all pharmacogenes, respectively. In both groups, drug targets accounted for the largest proportion, followed by enzymes. Among pharmacogene RNAs with modification, transporters accounted for a slightly larger proportion than receptors. This was reversed in pharmacogene RNAs without modification. The distribution of pharmacogene types was slightly different in the two groups.

We next analyzed the pharmacogenes with RNA chemical modification in detail. m6A modification could be found in RNAs of 2,294 pharmacogenes, accounting for 93.29%, while the percentages for

m5C, m7G, m1A, φ, and m6Am were 72.35%, 38.10%, 36.93%, 12.61%, and 12.36%, respectively (Figure 1C). The category distribution of pharmacogenes for each type of modification was consistent with above result. Based on our overlap analysis, we found that 685 pharmacogenes' RNA had only one type of chemical modification, m6A accounting for the largest proportion (Figure 1D). Most RNAs with other modifications could also be modified with m6A. This result unveiled the major role of m6A modification in pharmacogenes. It was interesting to note that only one gene, ATP citrate lyase (ACLY), had all six types of modifications. This is the main enzyme involved in the synthesis of cytoplasmic acetyl-CoA in many tissues. The gene was more complicated for RNA chemical modification.

In summary, these results indicated that more than two-thirds of pharmacogenes' RNA could be modified, and almost all of them were m6A modifications.

Localization of chemical modifications on pharmacogene RNA

Modifications at different positions on RNA play different functions, so we assessed the regional distribution of all six modifications (Figure 2A). The highest numbers of modifications were in the coding sequence (CDS) regions of the pharmacogene RNA, except for m6Am. The highest number of m6Am sites was in the 5′ untranslated regions (5′ UTRs), followed by intron, CDS, and 3′ untranslated region (3′ UTR). In addition to the CDS region, m6A and φ were mainly distributed in the 3′ UTR, followed by intron and 5′ UTR; m1A and m7G were mainly distributed in the 3′ UTR, followed by 5′ UTR and intron; while m5C was distributed in introns, followed by 3′ UTR and 5′ UTR. In addition to the overall distribution, the mean number of modification sites on pharmacogene RNA for the six types of modifications was also different. The mean number of m6A sites on pharmacogene RNA was the largest, followed by m7G, m5C, m1A, φ, and m6Am (Figure 2B).

To investigate whether the distribution patterns of these modifications were specific for pharmacogenes, we compared them with non-pharmacogenes. The results showed that there was no difference in the chemical modification distribution and the mean number of modification sites between pharmacogenes and non-pharmacogenes (Figures 2A and 2B). To further verify that there was no difference in regional distribution of the six modifications in the pharmacogene and non-pharmacogene RNA, we calculated the ratio of the number of modification sites in each RNA region to the total number of modification sites of the gene. The results showed that there were no statistically significant differences in modification distribution between pharmacogene and non-pharmacogene in the four RNA regions (Figure 2C).

m6A is one of the most fully studied modifications. And several studies showed that conserved m6A sites may have greater effects on biological function. Therefore, we further analyzed these conserved m6A sites. The results showed that the mean number of conserved sites on pharmacogene RNA was 5.02 (Figure 2D). And these conserved sites were mainly distributed in CDS, 3′ UTR, and

5′ UTR (Figure 2E). This was slightly different from the distribution of all m6A modifications on RNA regions. In pharmacogene RNA, all m6A modifications were distributed more in introns than in the 5′ UTR. Therefore, the distribution of conserved m6A sites in these two regions is opposite that of all m6A sites. In addition, we also compared the mean number and distribution of conserved m6A modifications in pharmacogene and non-pharmacogene RNA (Figures 2D–2F). The results showed that the average number of conserved sites in pharmacogenes (5.02) was significantly lower than that in non-pharmacogenes (5.45). However, there was no significant difference in the overall and regional distributions of conservative m6A sites between the two groups. These results reveal the importance of the m6A sites in CDS, 3′ UTR, and 5′ UTR.

These results indicate that most RNA modifications of pharmacogenes are located in the CDS region, and m6Am showed a distinctive distribution profile.

Modification-associated mutation in pharmacogenes

It was reported that some mutations could affect RNA chemical modifications, which might be associated with certain drug effects. Therefore, we systematically analyzed modification-associated mutations in pharmacogenes. First, we calculated the ratio of the number of pharmacogenes with and without modification-associated mutations. The results showed that 12.25% of pharmacogenes in which RNA could be modified with m5C contained m5C-associated mutations. m6A, φ, m1A, m6Am, and m7G accounted for 12.25%, 8.39%, 5.40%, 5.26%, and 5.02% of the total, respectively (Figure 3A). In addition, we counted the number of modification-associated mutations on each gene. The results showed that m6A had the largest mean number of mutations, followed by φ and m5C (Figure 3B). Statistical analysis showed that there was a significant difference in the mean number of modification-associated mutations between groups of two different modifications (including m1A and m5C, m1A and m6A, etc.)

These modification-associated mutations were divided into two categories based on their effects on modification (loss or gain). We counted the percentages of these two types of mutations for the six modifications (Figure 3C). The results showed that the proportions of gain mutation and loss mutation were equal for all m1A-associated mutations. Loss mutation accounted for more than 50% of all corresponding modification-associated mutations in m5C and m6A. However, loss mutations accounted for less than 50% of all the corresponding modification-associated mutations in m6Am, m7G, and φ. We further analyzed the distribution of these modification-associated mutations in RNA regions (Figure 3D). The results revealed that, except for m6Am-associated mutations, the mutations were mostly distributed in CDS regions, followed by 3′ UTR, 5′ UTR, and intron. The numbers of m6Am-associated mutations in CDS and 5′ UTR were equal, followed by 3′ UTR and intron. In addition, modification-associated mutations in the CDS region may affect the function of the protein. Therefore, they were classified as stop-gain, synonymous, and non-synonymous according to their effects on protein. Among all of the six types of modifications, non-synonymous
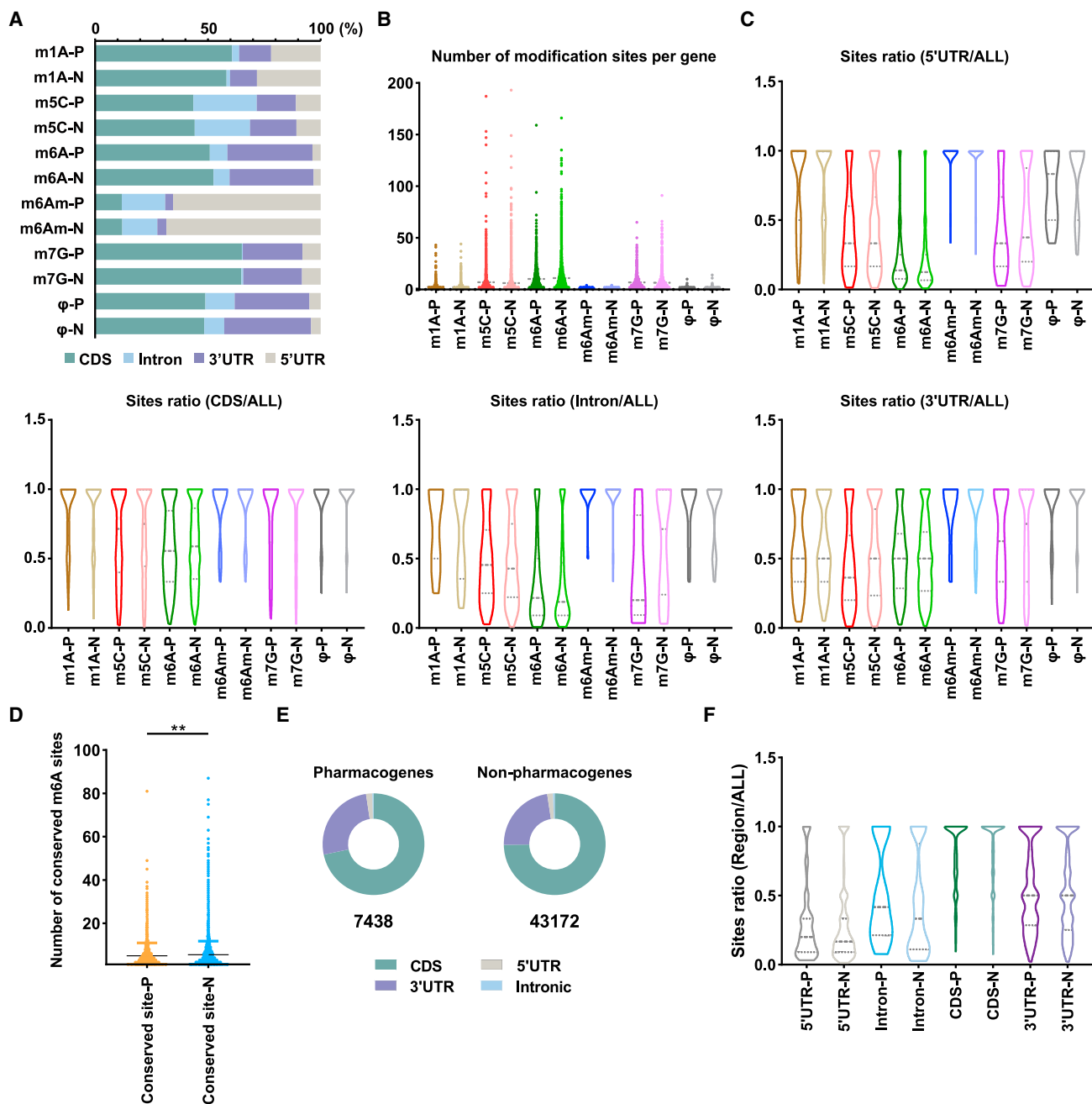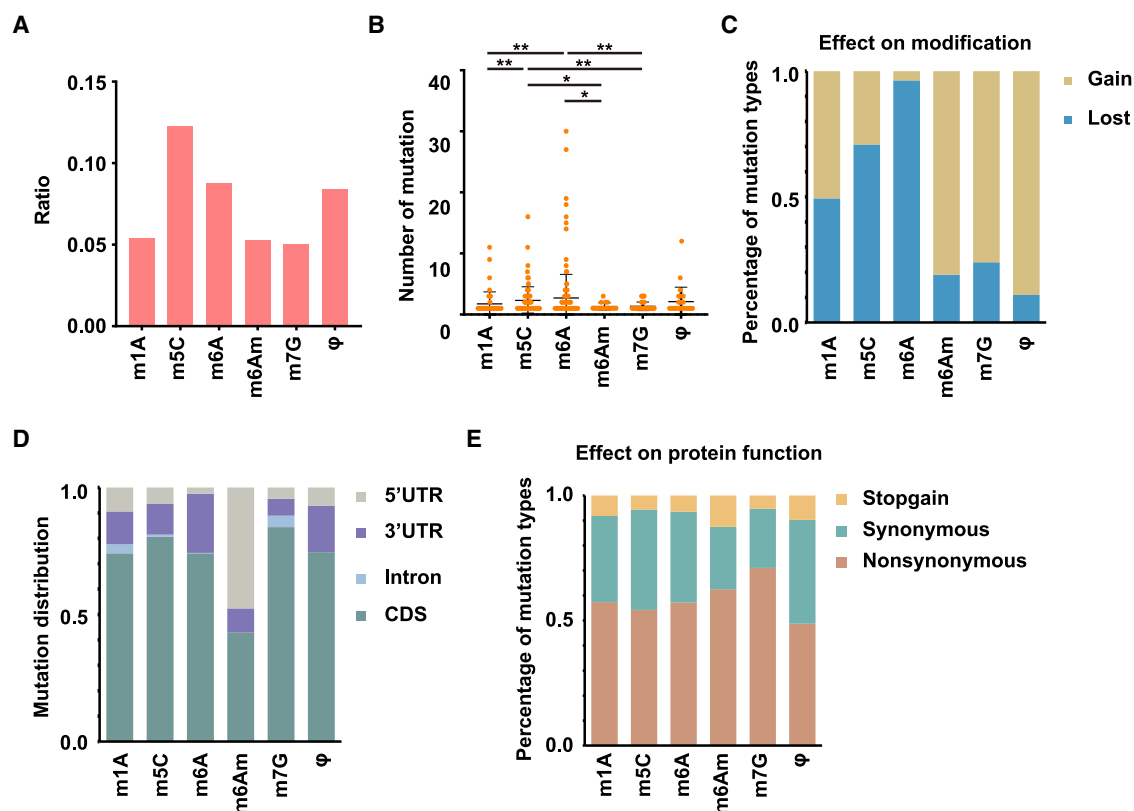
Figure 2. Distribution of modifications on pharmacogenes RNA

(A) The distribution of m1A, m5C, m6A, m6Am, m7G, and φ modifications on pharmacogene and non-pharmacogene RNA regions. (B) The number of modification sites per gene RNA. (C) The ratio of the number of modification sites in a region to all of the sites in the gene. (D) The number of conserved m6A sites per gene RNA. (E) The distribution of conserved m6A sites on pharmacogene and non-pharmacogene RNA regions. The number represents the total number of conserved m6A sites in the two groups of genes. (F) The ratio of the number of conserved m6A sites in a region to all of the sites in the gene. "-P" and "-N" represent pharmacogenes and non-pharmacogenes, respectively.

accounted for the largest proportion of all the modification-associated mutations in the CDS region, followed by synonymous and stop-gain (Figure 3E). These results suggested that modifications on a small number of pharmacogenes' RNA could be affected by disease-associated mutations. However, they could potentially have a large impact on expression and function of pharmacogenes.

Figure 3. Modification-associated mutations in pharmacogenes

(A) The ratio of the number of pharmacogenes with modification-associated mutations to total number of genes with corresponding modifications on their RNA. (B) The number of modification-related mutations in each drug-related gene. *$p < 0.05$, **$p < 0.01$. (C) The percentage of mutations that resulted in modification gain or loss to all modification-associated mutations. (D) The distribution of m1A, m5C, m6A, m6Am, m7G, and φ modification-associated mutations in gene regions. (E) The percentage of three types of mutations to all modification-related mutations in CDS region.

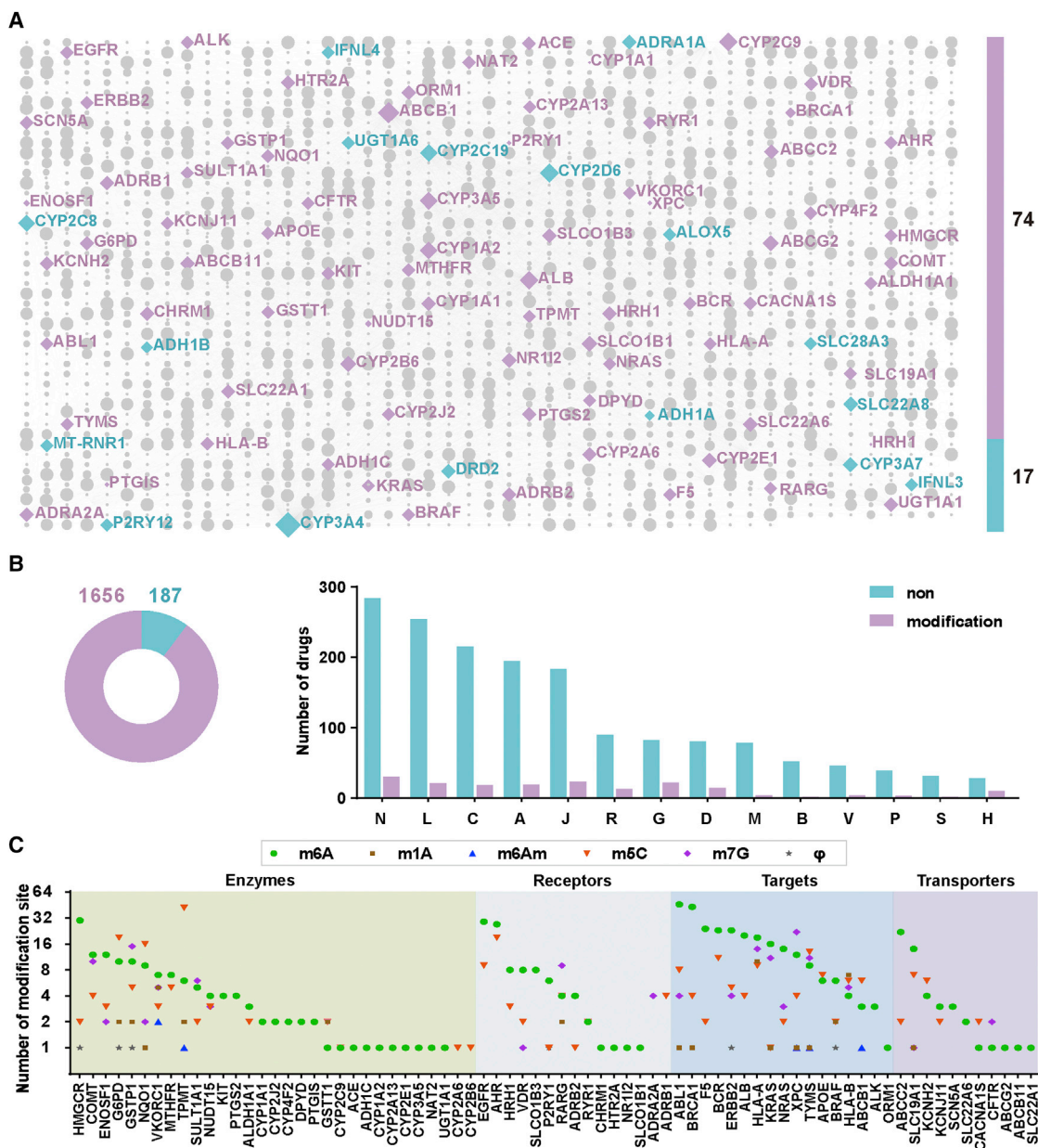## Core pharmacogenes' RNA chemical modification

Some pharmacogenes play core roles in drug efficacy and safety. For example, CYP3A4 metabolizes 29.91% of clinical medications. Therefore, these genes should be paid more attention. We identified 91 core pharmacogenes that are involved in the pharmacokinetics (PK) or pharmacokinetics (PD) process of 1,842 clinical drugs. Their RNA modifications were analyzed in detail. Seventy-four core pharmacogenes had at least one of the six types of modifications, while 17 had no modification (Figure 4A). Compared with the above results, core pharmacogenes could be more affected by RNA modifications. For all drugs, 89.85% (1,656/1,843) would be potentially affected by their pharmacogenes' RNA chemical modification, while 10.15% (187/1,843) could not be affected by any types of chemical modification (Figure 4B). Nervous system drugs were most affected by core pharmacogenes (no matter if their RNA is with or without chemical modifications) (Figure 4B). These results indicated that most of the drugs affected by core pharmacogenes (89.85%) might be affected by chemical modification.

Next, the 74 genes were analyzed. As indicated in Figure 4C, 70, 45, 22, 17, 6, and 5 genes' RNA could be modified by m6A, m5C,

m7G, m1A, φ, and m6Am, respectively. As for the number of modification sites, m6A had far more than the others. The average number of m6A, m5C, m7G, m1A, φ, and m6Am modification sites on these genes' RNA was 8.11, 5.75, 6.82, 2.47, 1.00, and 1.2, respectively. There was no significant difference in the modification sites among drug-metabolic enzymes, transporters, receptors, and targets. The number of drugs affected by each of the 74 pharmacogenes are indicated in Figure S2A. The top ranked genes are ATP binding cassette subfamily B member 1 (ABCB1), albumin (ALB), CYP2C9, CYP3A5, and CYP1A2, which all affect more than 200 drugs. On the other hand, 17 genes without any modifications are indicated in Figure S2B. It is interesting to note that some very important drug-metabolic enzymes and transporters did not show any correlation with RNA chemical modification. They include CYP3A4, CYP2D6, CYP2C19, and CYP2C8, which metabolize a large number of drugs.

Taken together, these results indicate that most RNAs of the core pharmacogenes could be modified by m6A, which affects more than 60% (1,602/2,645) of clinical drugs. Meanwhile, some very important drug-metabolic enzymes and transporters did not have any RNA chemical modifications.
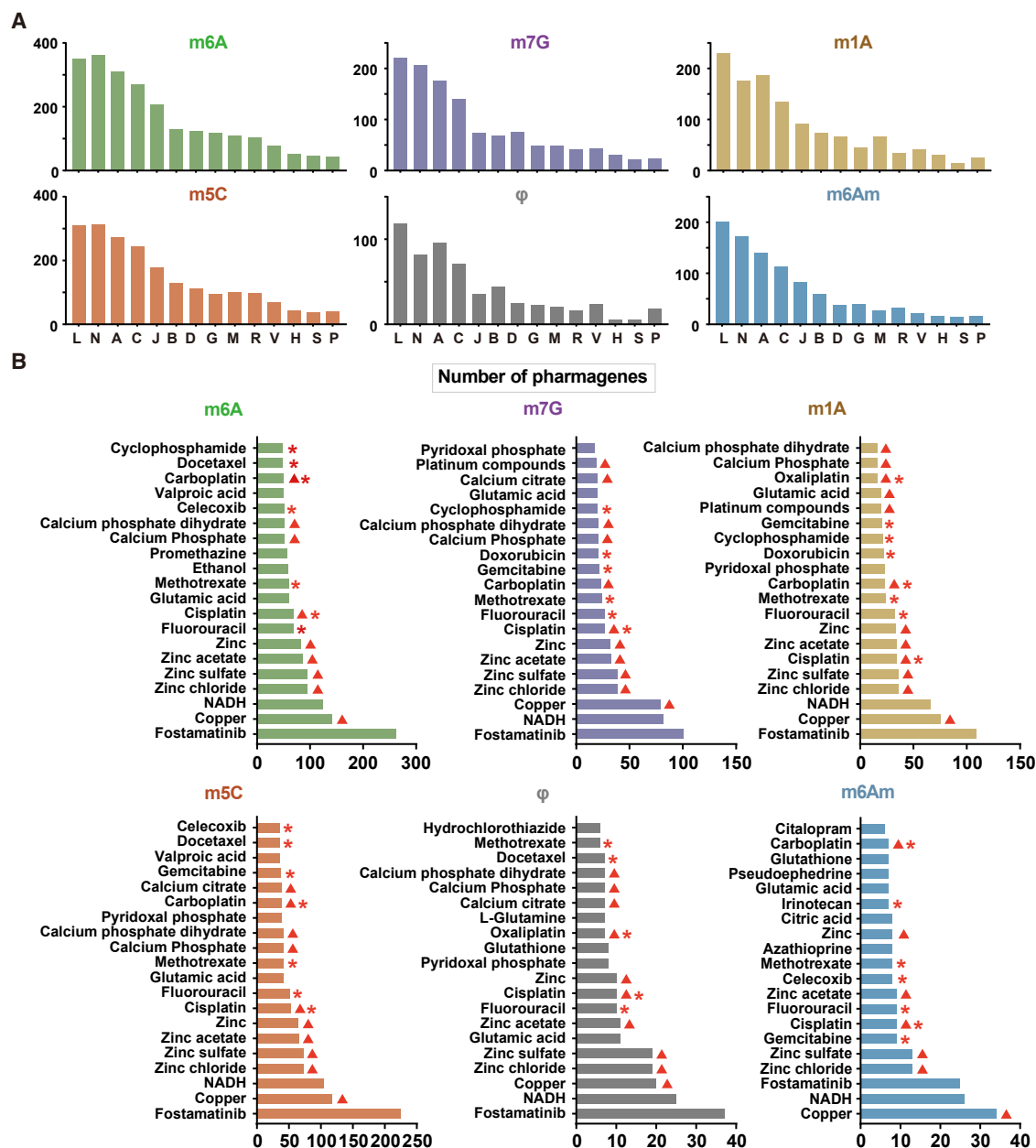
Figure 4. Modification on core pharmacogenes' RNA

(A) The connections of core pharmacogenes and their correlated drugs. Rose red represents 74 core pharmacogenes with modified RNA, brilliant blue represents 17 core pharmacogenes without modified RNA, and gray dots represent drugs. The node size is related to the number of connected drugs or genes. (B) Drugs affected by core pharmacogenes were divided into two categories (left): 1,656 drugs affected by modification (rose red) and 187 drugs not affected by modification (brilliant blue). The bar chart shows the number of drugs classified according to the ATC system (right): alimentary tract and metabolism (A); blood and blood-forming organs (B); cardiovascular system (C); dermatologicals (D); genitourinary system and sex hormones (G); systemic hormonal preparations, excluding sex hormones and insulins (H); anti-infectives for systemic use (J); anti-neoplastic and immunomodulating agents (L); musculoskeletal system (M); nervous system (N); anti-parasitic products, insecticides, and repellents (P); respiratory system (R); sensory organs (S); and various (V). (C) The number of each modification on different types of core pharmacogenes' RNA; enzyme (green), receptor (bright gray), target (blue), and receptor (purple).

Drugs affected by RNA chemical modification

To learn the profiles of all the drugs potentially affected by RNA chemical modifications, they were further analyzed. The results showed that m6A modification affected the greatest number of drugs (2,312), followed by m5C (2,037), m7G (1,224), m1A (1,220), m6Am (975), and φ (585) (Figure 5A). For all modifications, anti-neoplastic

Figure 5. Drugs affected by chemical modification

(A) The number of drugs affected by the six modifications. (B) Ranking of drugs affected by pharmacogene RNA with chemical modification. The abscissa represents the number of pharmacogenes' RNA with five modifications. Asterisk and triangle indicate that the drug is an anti-tumor drug or metal complex, respectively.

and immunomodulating agents accounted for the largest proportion. Overlap analysis showed that there were 227 drugs affected by all modifications (Figure S3A). The main category was alimentary tract and metabolism, followed by anti-neoplastic and immunomodulating agents (Figure S3B). These results emphasize the important and specific influence of m6A on drugs and reveal that anti-tumor drugs are largely susceptible to RNA chemical modification.

We next focused on the drugs that could potentially be most affected by the six types of modifications. Drugs were sorted according to the number of pharmacogenes' modified RNA in Figure 5B. It was interesting to observe that metal complexes and anti-cancer drugs accounted for more than half of them. Taking m6A, for example, 9 of the top 20 drugs were metal complexes. In addition, 7 of the top 20 drugs were anti-tumor drugs, including cyclophosphamide,

docetaxel, carboplatin, celecoxib, methotrexate, cisplatin, and fluorouracil. These results indicate that metal complexes and anti-tumor drugs are more likely to be affected by RNA modifications.

In summary, these results highlight the importance of chemical modifications on pharmacogene RNA in affecting anti-tumor drug effects.

m6A modification for anti-tumor drugs

Next, we focused on m6A and anti-tumor drugs. Based on the available data, a total of 10 cancers were analyzed, including triple-negative breast cancer (TNBC), ovarian cancer, acute myelocytic leukemia (AML), osteosarcoma, glioblastoma, cervical cancer, non-small cell lung cancer (NSCLC), hepatic carcinoma, renal carcinoma, and acute lymphoblastoma leukemia (ALL). Drugs for the treatment of various tumors and their respective original number of pharmacogenes are indicated in Figure S4. Although both the pharmacogenes and the drugs affected by m6A modification are largely different for various tumors, their distribution trends are similar (Figure 6A). It was reasonable to observe that the more modified pharmacogene RNAs in the tumor, the more drugs they would affect. We calculated the percentage of drugs that could be affected by m6A of all drugs of corresponding tumors (with cancer-specific m6A site data). The results showed that the values ranged from 50% (ALL) to 100% (TNBC). Based on this result, TNBC, ovarian cancer, and AML drug treatment would be more affected by the m6A modification, because m6A modification might affect drug response by affecting the mRNA expression level. We further show the correlation between mRNA expression levels of pharmacogenes (whose RNA could be modified with m6A) and drug response in these three tumors in Figures S5A and S5C. As expected, the mRNA expression levels of these genes was associated with drug response. In addition, we also analyzed all 316 pharmacogenes with modified RNA in 10 tumors in detail. We found that 169 (53.48%) of them appeared in only one tumor and 160 (50.63%) of them affected only one drug (Figure 6B). This result indicated that m6A modification could be tumor and drug specific during cancer treatment.

The drugs affected by m6A modification in each cancer are further analyzed in Figure 7. All drugs could be categorized as either a chemotherapy or a targeted drug. There was a total of 74 drugs that could be affected by m6A modification, of which 36 were targeted and 38 were chemotherapy drugs (Figure 7A). The distribution of drug types in each tumor was remarkably different (Figure 7B). Drugs affected in all tumors, except renal carcinoma, were mainly chemotherapy drugs (Figures 7C–7L). Drugs targeting DNA damage and repair were the largest group. Most drugs could be affected by several pharmacogenes' modified RNA, indicating that potential pharmacoepitranscriptomics biomarkers for each drug were selectable.

These results together indicate that m6A modification could be a candidate pharmacoepitranscriptomics biomarker for cancer drug treatment, especially for TNBC, ovarian cancer, and AML.

m6A modification as a potential pharmacoepitranscriptomics biomarker for olaparib sensitivity

Based on our results, chemical modifications on the pharmacogene RNA could be used as biomarkers for drug effects. To test this, we explored the potential pharmacoepitranscriptomics biomarkers for olaparib sensitivity based on the available data of ovarian cancer cells. The m6A modification of olaparib pharmacogenes was analyzed in both resistant and parent sensitive cells. Modifications of five pharmacogenes' RNA were found to be significantly changed in the resistant cell lines. All these genes were involved in homologous recombination repair (HR), which is a key pathway in the olaparib PD process (Figure 8A). Compared with the parent cells, the modification level and expression level of five mRNAs (DDB2, γH2AX, PARP1, DDB2, and FZD10) were all increased in resistant strains (Figures 8B and S6). It is interesting to note that their alteration was specific. Except for DDB2, there was only one changed modification level site on the genes' RNA. The modification sites of γH2AX and PARP2 were located at the 5′ UTR (chr11: 118,964,600–118,965,200) and 3′ UTR (chr1: 226,595,500–226,596,000), respectively, while the modification sites of 53BP1 (chr15: 43,724,400–43,724,600) and FZD10 (chr12: 130,648,800–130,649,200) were located in the CDS region. The two modification sites of DDB2 were located in the first exon (chr11: 47,236,600–47,236,800) and 3′ UTR (chr12: 47,260,400–47,260,600). In addition, the results showed that the mRNA expression of these genes did differ between the parent and the drug-resistant strains. These results indicated that the m6A modification of olaparib pharmacogenes was increased in the resistant cells. These modification sites of five pharmacogenes' RNAs could be used as potential pharmacoepitranscriptomics markers of olaparib drug effects.

DISCUSSION

In this study, we provided a pharmacoepitranscriptomics landscape, including basic characteristics of six types of modifications, modification-associated mutations, and the drugs affected by them. Furthermore, we focused on the effect of m6A modification on anti-tumor drugs. The results showed that RNA chemical modification was specific for both drugs and cancers. m6A modification could be a potential pharmacoepitranscriptomics biomarker for anti-tumor drugs, especially for TNBC, ovarian cancer, and AML.

Individual differences in drug effects are a common clinical condition, which needs to be solved urgently.[9] Precision treatment is especially important for cancer patients. However, a number of drugs still lack personalized treatment biomarkers. Although genetic variations were thought to be the main factor in inducing this process, they have limitations in explaining differences in drug effects.[10–12] With the development of multi-omics, drug efficacy and safety could be further explained by biomarkers from other sources, including epigenetics, epitranscriptomics, metabolomics, microbiome, etc.[13] Epitranscriptomics is one of the rapidly developing fields of recent decades. Its huge potential to be developed to find biomarkers for predicting drug effects will attract a lot of attention. Our results show that more than two-thirds of pharmacogenes' RNAs have chemical modifications, with m6A in particular
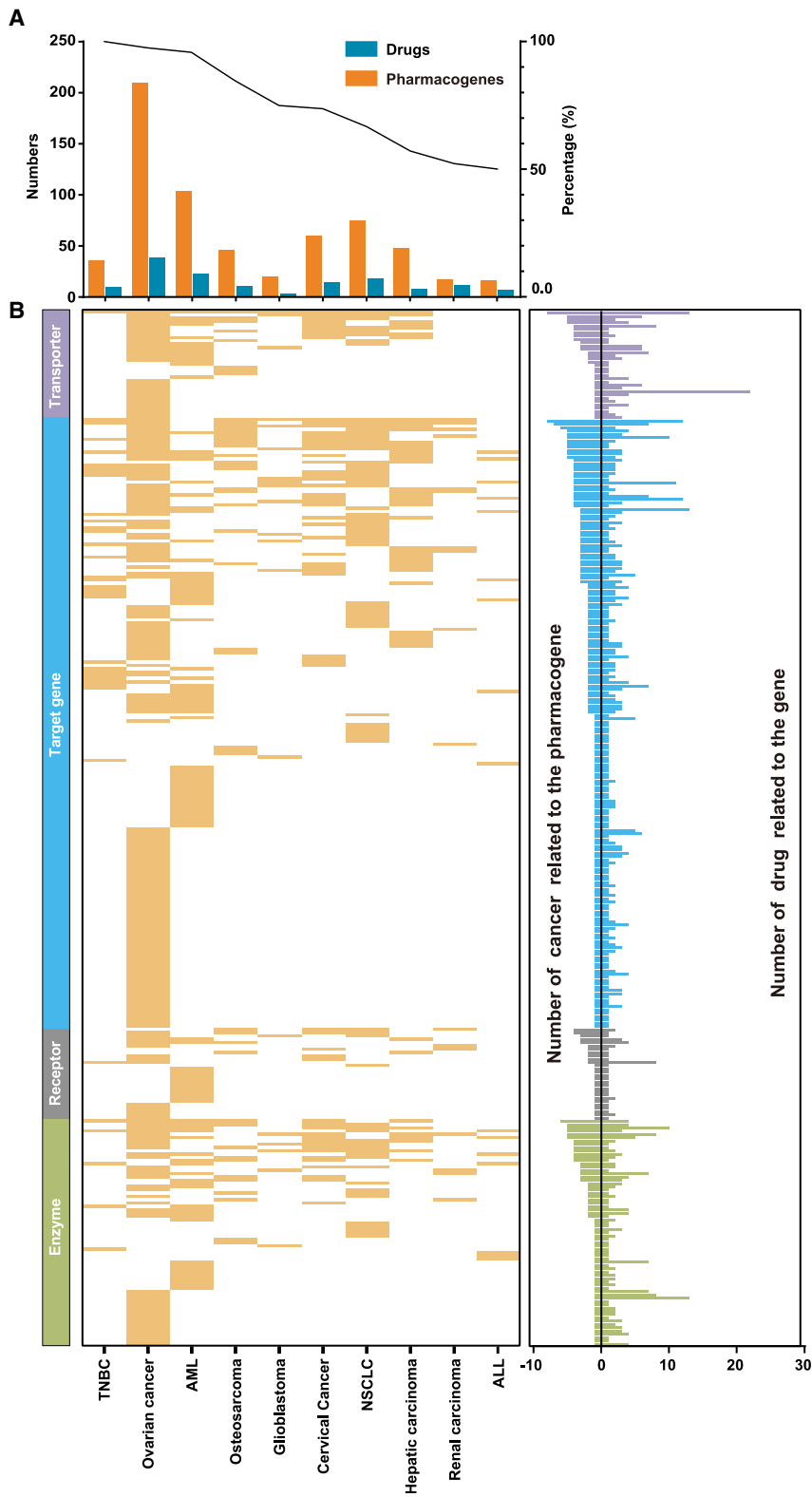
Figure 6. m6A modifications on pharmacogene RNAs in cancers

(A) The bar graph shows the numbers of pharmacogenes with m6A-modified RNA in each tumor (orange) and the numbers of drugs (blue) affected by them. The line chart shows the proportion of drugs affected by m6A modification of all drugs used in each tumor. The left ordinate corresponds to the bar graph and the right ordinate corresponds to the line chart. (B) The heatmap shows the pharmacogenes with m6A-modified RNA in each tumor. Each row represents a pharmacogene, and those with modified RNA in a tumor are shown in gold. These pharmacogenes are arranged according to gene classification (left): enzyme, receptor, target (blue), and receptor. The histogram shows the numbers of tumors (left) and drugs (right) correlated with each pharmacogene with m6A-modified RNA.
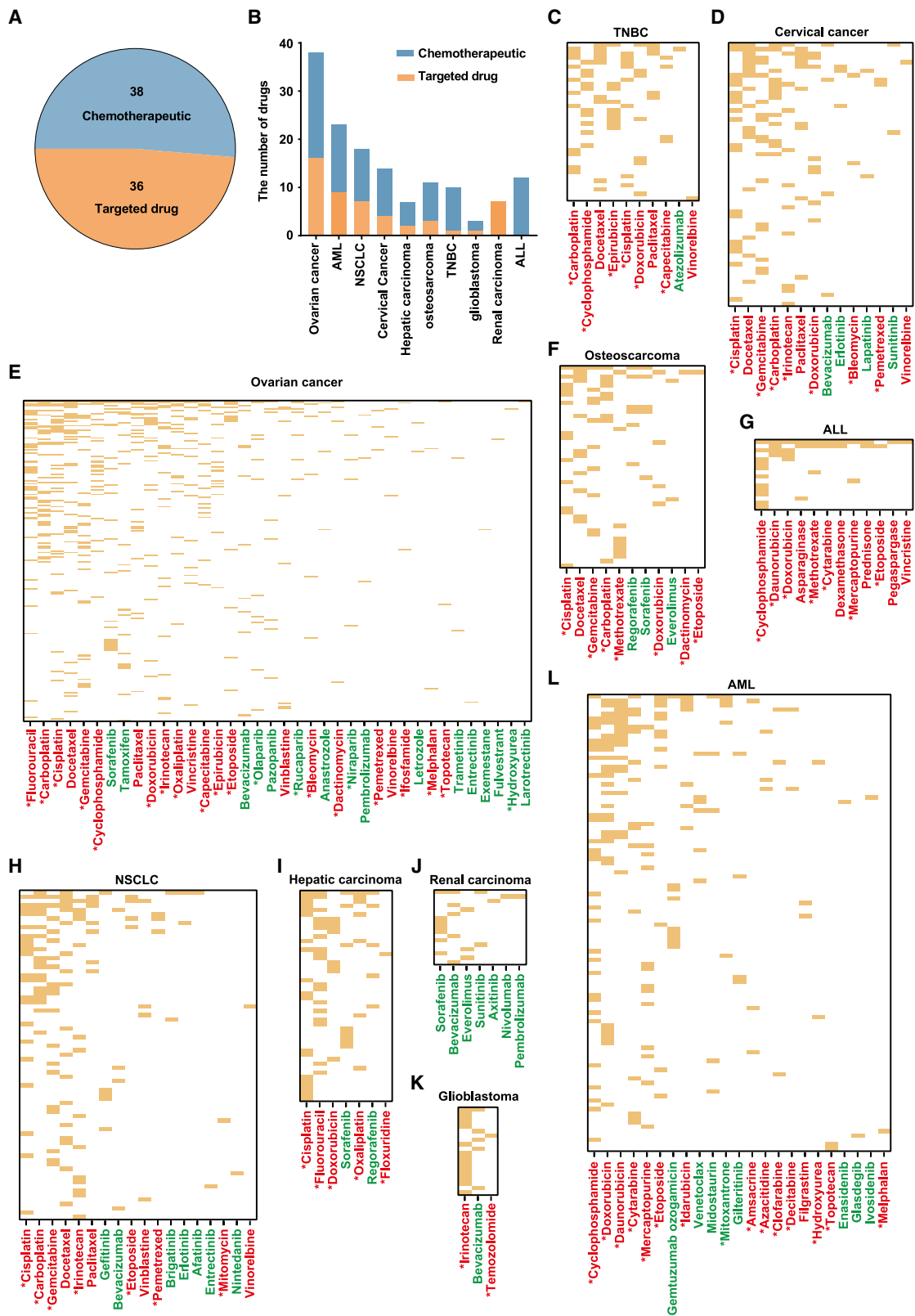
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prevalent among them. Interestingly, anti-tumor drugs seemed to be most affected by chemical modifications. More importantly, m6A is both tumor and drug specific, indicating that it is a potential biomarker for anti-tumor drugs. The effect of m6A modification on the response of anti-tumor drugs for TNBC, ovarian cancer, and AML should be studied first, since these drugs are more likely to be affected by m6A. In addition, our results were further supported by other investigations. For example, a recent study found that tyrosine kinase inhibitor (TKI) treatment resistance in leukemia was attributed to the overexpression of FTO, which led to a decrease in the levels of m6A on some genes' RNA and increased their expression in resistant strains.[14] Another research found that m6A on FZD10 mRNA promoted its stability and activated the Wnt/β-catenin pathway, which ultimately led to resistance of ovarian cancer cells to PARP inhibitors (PARPi).[15]

Our study revealed that m6A on pharmacogene RNA is a potential biomarker for anti-tumor drugs. This suggested that even chemical modifications may be a potential new source of biomarkers for drug effects. They might affect drug efficacy and safety by regulating pharmacogene expression. It represents one of the molecular mechanisms of how pharmacoepitranscriptomic biomarkers affect drug effects. This hypothesis was proven by the data for ovarian cancer that we used. We found that m6A levels on five pharmacogenes' RNA were differentially expressed in drug-resistant strains relative to sensitive cells. On the other hand, m6A modification is a dynamic process. It is regulated by methyltransferase (writer) and demethylase (eraser) and relies on m6A binding protein (reader) for its function. Therefore, in addition to m6A on pharmacogene RNA, m6A regulators (including m6A writers, erasers, and readers) are another type of pharmacoepitranscriptomics biomarker. Our results suggested that m6A regulators are correlated with anti-tumor drug responses, and this correlation is tumor specific (Figure S5D). They may influence gene expression by binding to m6A sites and regulating m6A levels as pharmacoepitranscriptomic biomarkers for drug effects. For example, activated METTL3 (m6A writer) induced cisplatin resistance in lung cancer cell lines by increasing m6A levels on YAP RNA and promoting its translation through YTHDF1/3 (m6A readers).[16] This reminds us that m6A regulators could also be correlated with drug effects and represent another type of pharmacoepitranscriptomic biomarkers. However, all the above proposals still need to be verified by further investigations.

There are several limitations of this study. First, although we showed that pharmacoepitranscriptomic biomarkers could possibly be used to predict drug effects, their development still needs further *in vitro* and *in vivo* studies. Chemical modification is a dynamic process, which can alter gene expression by influencing RNA metabolism (including RNA synthesis, maturation, degradation, etc.).

In this study, available data were used to exploring correlations between chemical modifications and pharmacogenes. The results could reveal how modification levels ultimately affect gene expression, but not how they affect RNA metabolism. Therefore, the mechanism by which RNA modifications affect drug effects needs to be further explored by experimental studies. On the other hand, m6A as a potential biomarker of drug effects is still in its infancy. The location and quantification of m6A modification are equally important for drug effects. However, few data are available for such analysis at present. Therefore, more drug effects experiments with available highly accurate m6A site and quantitative data need to be performed. Second, currently available high-throughput epitranscriptomics sequencing data are limited, especially from clinical samples. This limits the clinical use of chemical modifications as biomarkers for drug effects. In summary, this study could be improved in the future. For example, more cancers and drugs could be analyzed.

In summary, we provided a pharmacogene RNA modification landscape and indicated that pharmacoepitranscriptomics could be considered as a new source of drug effects biomarkers in future studies, especially for anti-tumor drugs.

MATERIALS AND METHODS
Data resources
Drugs and pharmacogenes included in our research were collected from the US Food and Drug Administration (FDA: https://www.fda.gov/drugs), Drugbank,[17] and PharmGKB.[18] The drugs were then classified according to the ATC system (ATC system: https://www.who.int/medicines/regulation/medicines-safety/toolkit_atc/en/), including alimentary tract and metabolism (A); blood and blood-forming organs (B); cardiovascular system (C); dermatologicals (D); genital urinary system and sex hormones (G); systemic hormonal preparations, excluding sex hormones and insulins (H); anti-infectives for systemic use (J); anti-neoplastic and immunomodulating agents (L); musculoskeletal system (M); nervous system (N); anti-parasitic products, insecticides, and repellents (P); respiratory system (R); sensory organs (S); and various (V).

Five high-throughput modification (m1A, m5C, m6A, m6Am, and φ) site sequencing data were collected from m6A-Atlas.[19] And m7G site data were derived from m7GHub.[20] Conserved m6A sites data were derived from ConsRM.[21] All of the six types of modification-associated mutations were collected from RMDisease.[22] Cancer-specific m6A sites were derived from the REPIC and CVm6A databases.[23,24] The data used to calculate the correlation between drug response and the mRNA expression levels of pharmacogenes and associated m6A regulators (Figure S5) were based on the GDSC database.[25] The modification information of mRNA and pre mRNA collected

Figure 7. Drugs affected by m6A modification in each tumor
(A) Drugs affected by m6A modification were classified into two categories: 38 chemotherapeutic and 36 targeted drugs. (B) The numbers of chemotherapeutics and targeted drugs affected by the modification in each tumor. (C–L) Drugs affected by pharmacogenes with modified RNA in 10 tumors. Drugs marked in red represent chemotherapy drugs, and drug marked in green represent targeted drugs. Asterisks indicate drugs targeting DNA damage and repair.
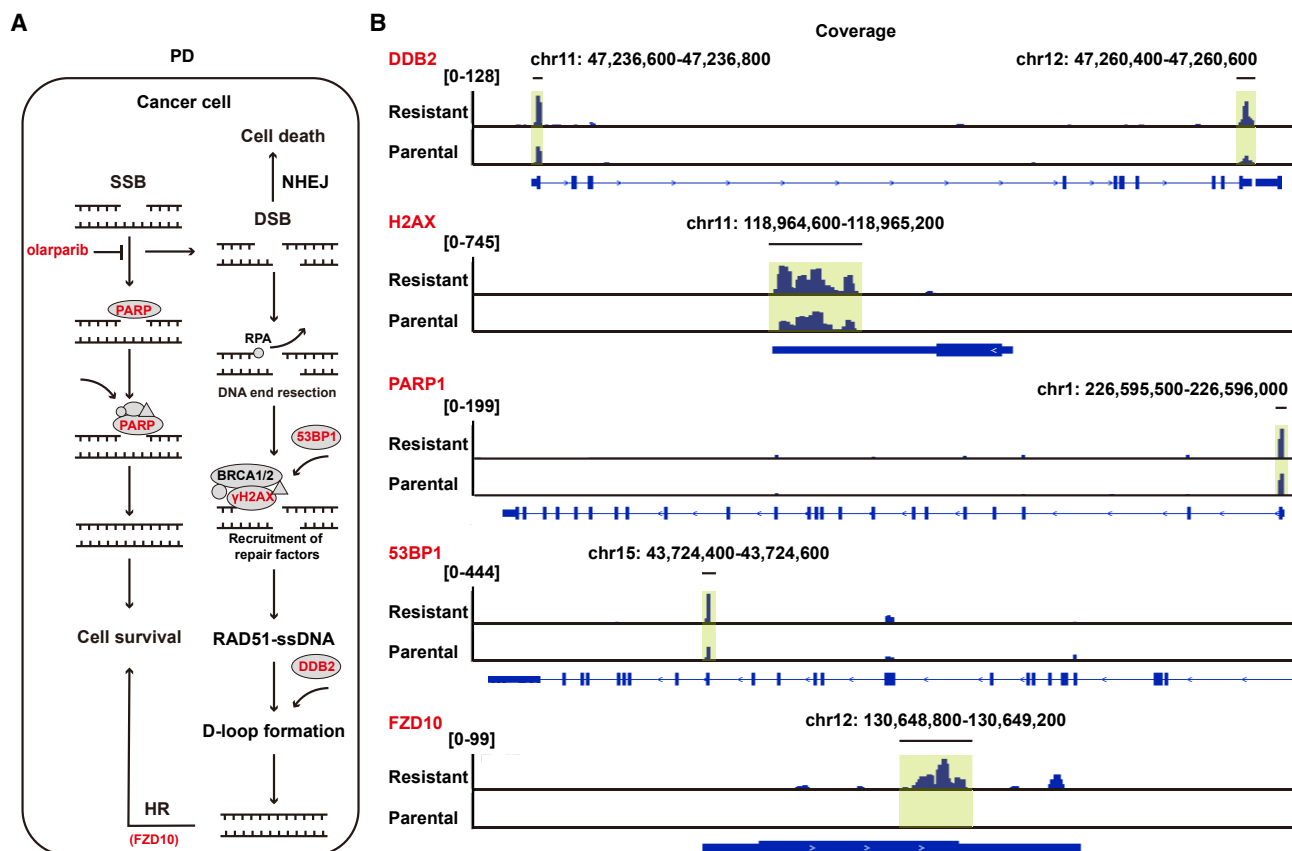
Figure 8. m6A modification as potential pharmacoepitranscriptomics biomarkers for olaparib sensitivity

(A) Diagram showing the PD process of olaparib. (B) The IGV map showing the abundance of m6A modifications on the five gene mRNAs in the olaparib resistance and parental strains. The abscissa indicates the location of the gene, and the ordinate indicates the coverage. The area marked in green indicates the peak position of m6A, and the corresponding chromosome positions are indicated above the area. SSB, single-strand breaks; DSB, double-strand breaks; HR, homologous recombination repair; NHEJ, non-homologous end-joining repair; PARP, poly(ADP-ribose) polymerase; RPA, replication protein A; BRCA1/2, BRCA1/2 DNA repair associated; 53BP1, p53-binding protein 1; H2AX, H2A.X variant histone; DDB2, DNA damage binding protein 2; FZD10, frizzled class receptor 10.

from these databases had been further analyzed and is collectively referred to as RNA.

The core pharmacogenes were defined according to one of the following criteria: (1) very important pharmacogenes (VIPs) in the pharmGKB database, (2) pharmacogenes with an evidence score of 2A or more in the pharmGKB database, (3) pharmacogenes included in three major clinical pharmacogenetics guidelines (Clinical Pharmacogenetics Implementation Consortium [CPIC], the Royal Dutch Association for the Advancement of Pharmacy-Pharmacogenetics Working Group [DPWG], and the Canadian Pharmacogenomics Network for Drug Safety [CPNDS]), and (4) the top 30 pharmacogenes with most linked drugs.

Bioinformatics analysis
Venn diagrams were drawn by TBtools. Heatmaps in Figures 6 and 7 were drawn by the R package of pheatmap (version 1.0.12). The network of pharmacogenes and drugs was drawn by Cytoscape (version 3.7.1). IGV software (version 2.9.3) was used to draw the IGV map.

Statistical method
The t test was used to compare the numbers and regional distributions of chemical modifications on pharmacogene and non-pharmacogene RNA. In addition, it was used to determine whether there were intergroup differences in the numbers of modification-related mutations. R corrplot package was used to calculate Spearman correlation coefficients between drug response parameter (IC_{50}) and pharmacogene or m6A regulator mRNA. A p value <0.05 was considered statistically significant; * represents $0.01 < p < 0.05$, ** represents $p < 0.01$.

AUTHOR CONTRIBUTIONS

K.L., W.W., and J.Y. designed the study. K.L., O.Y., Y.Z., and H.Y. analyzed the data. K.L. drew the figures. K.L. wrote the manuscript. B.X., L.M., R.L., W.W., and J.Y. edited the manuscript. All authors read and approved the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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