Original Article

Inhibition of MAT2A-Related Methionine Metabolism Enhances The Efficacy of Cisplatin on Cisplatin-Resistant Cells in Lung Cancer

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Abstract

Objective: Tumor drug resistance is a vital obstacle to chemotherapy in lung cancer. Methionine adenosyltransferase 2A has been considered as a potential target for lung cancer treatment because targeting it can disrupt the tumorigenicity of lung tumor-initiating cells. In this study, we primarily observed the role of methionine metabolism in cisplatin-resistant lung cancer cells and the functional mechanism of MAT2A related to cisplatin resistance.

Materials and Methods: In this experimental study, we assessed the half maximal inhibitory concentration (IC_{50}) of cisplatin in different cell lines and cell viability via Cell Counting Kit-8. Western blotting and quantitative real-time polymerase chain reaction (qRT-PCR) was used to determine the expression of relative proteins and genes. Crystal violet staining was used to investigate cell proliferation. Additionally, we explored the transcriptional changes in lung cancer cells via RNA-seq.

Results: We found H460/DDP and PC-9 cells were more resistant to cisplatin than H460, and MAT2A was overexpressed in cisplatin-resistant cells. Interestingly, methionine deficiency enhanced the inhibitory effect of cisplatin on cell activity and the pro-apoptotic effect. Targeting MAT2A not only restrained cell viability and proliferation, but also contributed to sensitivity of H460/DDP to cisplatin. Furthermore, 4283 up-regulated and 5841 down-regulated genes were detected in H460/DDP compared with H460, and 71 signal pathways were significantly enriched. After treating H460/DDP cells with PF9366, 326 genes were up-regulated, 1093 genes were down-regulated, and 13 signaling pathways were significantly enriched. In TNF signaling pathway, CAS7 and CAS8 were decreased in H460/DDP cells, which increased by PF9366 treatment. Finally, the global histone methylation (H3K4me3, H3K9me2, H3K27me3, H3K36me3) was reduced under methionine deficiency conditions, while H3K9me2 and H3K36me3 were decreased specially via PF9366.

Conclusion: Methionine deficiency or MAT2A inhibition may modulate genes expression associated with apoptosis, DNA repair and TNF signaling pathways by regulating histone methylation, thus promoting the sensitivity of lung cancer cells to cisplatin.

Keywords: Cisplatin Resistance, Lung Cancer, Methionine Adenosyltransferase 2A

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Introduction

Lung cancer is the first malignant tumor with morbidity and mortality in the world (1). The treatment of lung cancer includes surgery, radiotherapy and chemotherapy, as well as targeted therapy. Although targeted therapy is a huge advancement in the field of lung cancer treatment, due to the low gene mutation rate and insufficient understanding of molecular typing of lung cancer, the application range of targeted therapy is limited. Therefore, for most patients with lung cancer, chemotherapy is still the preferred strategy for oncology treatment (2). Platinum-based drugs, such as cisplatin, are commonly used in lung cancer chemotherapy regimens. Cisplatin triggers apoptosis by inducing double-strand break damage mainly through DNA cross-linking (3). Although the use of cisplatin for clinical treatment has been a remarkable success, the drug resistance of tumor cells also hinders the effects of cisplatin, leading to chemotherapy failure (4). The study of drug resistance will remain a continuous and prolonged process.

Tumors are often accompanied by metabolic abnormalities in the process of development, including glucose metabolism, amino acid metabolism, lipid metabolism and other processes (5). The amino acids in the human body are divided into two major categories: essential amino acids and non-essential amino acids. The demand for amino acids in tumor cells is altered, and the demand for non-essential amino acids such as glutamine and serine are much higher than that of normal cells (6,

7). Methionine, an essential amino acid, is necessary for maintaining the demands of cell growth (8) and protein translation (9). Abnormal methionine metabolism has been observed in many tumors such as glioma and lung cancer (10, 11). Methionine adenosyltransferase 2A (MAT2A), a critical enzyme in cell life activity, can catalyze the integration of methionine with adenosine triphosphate (ATP) to methylate bio-macromolecules such as DNA, RNA, proteins and lipids via supplying the biosynthesis of S-adenosylmethionine (SAM), the bio-methylation donor. The methylation of histones can regulate chromatin conformation and transcription in response to changes in environment or physiology (12, 13). H3K4 mono-, di-, or tri-methylation (H3K4me1/2/3) and H3K36me3 are activating marks, promoting gene transcription (14). On the contrary, H3K9 and H3K27 methylation (H3K9me2/3 and H3K27me2/3) are commonly related to gene silencing (14, 15).

MAT2A is highly expressed in various tumors such as liver cancer, gastric cancer, kidney cancer and colon cancer (16-19). Studies have reported that the maintenance of lung cancer stem cells depends on methionine metabolism mainly through increasing MAT2A expression, and targeting MAT2A can impede the initiation of lung cancer cells (20). Therefore, MAT2A is considered to be a potential target for lung cancer treatment. However, the role MAT2A plays in platinum-resistant lung cancer is still unclear.

In this study, we elucidated the regulatory mechanism of methionine availability (methionine deficiency or MAT2A inhibition) in reducing the tolerance of cisplatin-resistant lung cancer cells to cisplatin. Therefore, MAT2A may serve as a new target for targeted interventions in platinum-resistant lung cancer, providing a scientific basis for the development of new strategies to overcome lung cancer resistance.

Materials and Methods

Reagents

This experimental study complies with the requirements of the Regulations on the Ethical Review of Biomedical Research Involving Humans issued by the National Health and Family Planning Commission and the Helsinki Declaration issued by the Joint Congress of the World Medical Associations. The ID number of the Ethical Committee is 2020-205-001. RPMI-1640 medium (31800-105) was purchased from Gibco (Grand Island, New York, USA), and fetal bovine serum (11011-8611) was purchased from Every Green (Hangzhou, China). Trypsin (GNM25200) and penicillin-streptomycin solution (GNM15140) were obtained from Genome (Hangzhou, Zhejiang, China), RPMI-1640 w/o Amino acids and Sodium Phosphate (powder) were bought from US Biological (Salem, MA, USA), arginine, methionine and the other 18 amino acids included in the RPMI-1640 medium, as well as dimethyl sulfoxide (D2650) were bought from Sigma (Louis, MO, USA). TRIzol (15596026) was purchased from Ambion (Carlsbad CA, USA) and Cisplatin and PF9366 were obtained from MedChem Express (Shanghai, China). Radio-immunoprecipitation assay lysis buffer (FD009), BCA Protein Assay Kit (FD2001) as well as enhanced chemiluminescence kit (FD800) were purchased from Fdbio Science (Hangzhou, Zhejiang, China). Rabbit Cleaved PARP (#5625T), rabbit anti-H3 (#4499), rabbit anti-H3K4 me3 (#9751), anti-H3K9 me2 (#4658), anti-H3K27 me3 (#9733) and anti-H3K36 me3 (#4909) were purchased from Cell Signaling Technology (Danvers, MA, USA). Rabbit anti-Tubulin (AF0001) (AA128) was obtained from Beyotime Biotechnology (Shanghai, China) and rabbit anti-MAT2a (ab154343) from Abcam (Cambridge, UK).

Preparation of medium with various concentrations of methionine

Methionine-free RPMI-1640 medium was produced by dissolving RPMI-1640 w/o Amino acids, Sodium Phosphate (powder), sodium bicarbonate, and 19 types of amino acids (excluding methionine), in double-distilled water, which was termed as 0XMet, similar to our previous study (21). We defined the methionine content in the RPMI-1640 complete medium (15 μg/mL) as 1XMet. The 100X Met (1,500 μg/mL) solution was prepared in advance, and subsequently added to methionine-free RPMI-1640 medium to generate 1/8 X Met (1.875 μg/mL), 1/4 X Met (3.75 μg/mL), 1/2 X Met (7.5 μg/mL) and 1 X Met (15 μg/mL), which were used in the following experiments.

Cell culture

We purchased human lung cancer H460 and PC-9 cell lines from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). Cells were cultured in RPMI-1640 medium containing 10% newborn bovine serum (NBS), 100 U/ml penicillin, and 100 μg/ml streptomycin (Thermo Scientific, MA, USA). A humidified incubator with 5% CO_2 was employed to incubate cells and the temperature was set to 37℃. The cisplatin-resistant cell line H460/DDP was generated by persistently subjecting the parental cell line H460 to gradient exposure of cisplatin for about 10 months, through increasing cisplatin concentration from 0.1 μg/ml until the cells acquired resistance to 1 μg/ml. Furthermore, the PC-9 cell line is naturally resistant to cisplatin compared with H460.

Cytotoxicity assay

We plated 8×10^3 cells per well onto 96-well plates and treated cells with various concentrations of drugs for about 24 hours. Then, we analyzed the cell viability through Cell Counting Kit-8 (Beyotime Biotechnology) guided by the manufacturer's instructions. The cellular viability was indicated as mean ± SD from at least three independent experiments.

Western blotting analysis

Cells were lysed in whole-cell lysate buffer [50 mM Tris (pH=7.4), 150 mM NaCl, 1% NP-40] containing 1% protease inhibitor cocktail (100×, MCE, Shanghai, China). Lysates containing 30 μg protein were loaded into 10% or 15% sodium dodecyl sulfate-polyacrylamide gels for electrophoresis (SDS-PAGE) and the separated proteins were transferred to poly vinylidene fluoride (PVDF) membranes (Pall, NY, USA). After blocking with 5% fat-free milk for 1 hour in Tris-buffered saline (TBS), the membranes were incubated with the primary antibody overnight at 4˚C and then with the peroxidase labelled secondary antibody for 1 hour on the next day. Proteins were visualized using an enhanced chemiluminescence kit exposed to immunoblotting membranes, developing and fixing solutions and x-ray films and then quantified with Image J, version 1.52 (NIH, Bethesda, MD, USA).

Quantitative real-time polymerase chain reaction

Trizol reagent was employed to extract total RNA according to the manufacturer's instructions. The concentration and purity of extracted RNA were determined by absorbance assay at 260, 230, and 280 nm wavelengths as well as electrophoresis patterns. Reverse transcription was performed with 1μg of total RNA and Quantscript RT kit (Takara, Osaka, Japan). The mRNA expression level was determined by quantitative real-time polymerase chain reaction (PCR) by Roche lightCycler® 480II qPCR system (Roche, Basel, Switzerland). *GAPDH* was used as an internal control of RNA integrity. The following primers were used:

MAT2A (>NM_005911.6)-

F: 5′-ATGAACGGACAGCTCAACGG-3′
R: 5′-CCAGCAAGAAGGATCATTCCAG-3′

GAPDH (>NM_001256799.3)-

F: 5′-GGAGTCAACGGATTTG GT-3′
R: 5′-GTGATGGGATTTCCATTGAT-3′

The gene expression was calculated by $2^{-\Delta\Delta CT}$. The calculation process is as follows, ΔCT (test)=CT (target, test)-CT (ref, test), ΔCT (calibrator)=CT (target, calibrator)-CT (ref, calibrator), ΔΔCT (calibrator)=ΔCT (test)-ΔCT (calibrator), $2^{-\Delta\Delta CT}$=gene expression.

Crystal violet staining experiment

The density of H460/DDP cells in the logarithmic growth phase was adjusted to 1×10^5 cells per well, the suspension was mixed well and 1 mL was added per well and the cells were incubated in a 24-well plate for 24 hours. The cells were treated with 10 μM of PF9366 and a control group was also established. The original culture solution (control group and PF9366 group) in both wells was discarded every 24 hours, washed with PBS, fixed with a formaldehyde solution for 30 minutes, and then crystal violet staining solution was added for 15 minutes. After the dyeing was finished, the excess crystal violet dye was washed away with phosphate buffer solution (PBS, Beyotime, China) and dried naturally.

RNA-sequencing

TRIzol was used to isolate the total RNA from three independent samples of H460/DDP and H460 cells either without or with 10 μM PF9366 for 24 hours. Illumina TruSeq RNA sample preparation kit (RS-122-2001) and Illumina high-seq 2000 with a read length of 50 bp with pair ends were used to produce and sequence RNA-seq libraries. We mapped RNA-seq reads to the human genome (hg19) through TopHat (22). Next, we just analyzed those reads mapped to unique genomic locations and with <5% mismatches. FPKM (23) was used to test gene transcripts, and DEGSeq (24) was employed to identify genes expressed differentially. The differentially expressed genes were counted and annotated with NCBI, Uniprot, GO and KEGG databases to obtain detailed description information.

Statistical analysis

Data are shown as the mean ± standard deviation. The parametric unpaired Student's t test was used to calculate the statistical significance of the differences between the cell lines by Graph Pad Prism 6.02 for Mac (San Diego, CA, USA). $P<0.05$ indicates a significant difference.

Results

The deficiency of methionine promotes lung cancer cells sensitive to cisplatin

We have established the cisplatin-resistant lung cancer cell (H460/DDP) by the concentration gradient method, as published previously, and conducted relevant research (25). Here, we first reconfirmed the drug resistance of H460/DDP by CCK-8 cytotoxicity assay. The half maximal inhibitory concentration (IC_{50}) value of cisplatin in the parental cell H460 was 0.4384 μg/mL, while the IC_{50} value of the drug-resistant cell H460/DDP was 3.915 μg/mL, and the drug resistance index was 8.93, meanwhile, the IC_{50} value of cisplatin in PC-9 cells was also detected to be 1.755 μg/mL (Fig.1A). Evidence has shown that depleting dietary methionine could lead cisplatin-resistant xenograft tumors to become sensitive to cytotoxic agents (26). To explore the impact of methionine on sensitivity to cisplatin in lung cancer cells, we detected the viability of H460 with the treatment of different concentrations of cisplatin under the medium with or without methionine and H460/DDP with cisplatin under 1 X Met, 1/2 X Met, 1/8 X Met and 0 X Met. We found H460 was more vulnerable to cisplatin under the methionine-deprived medium and the sensitivity of H460/DDP to cisplatin was enhanced under methionine deficiency (Fig.1B, C). When explored further, it was found that the Cleaved-PARP was up-regulated under the treatment of cisplatin accompanied with methionine deficiency in H460, H460/DDP and PC-8 cells (Fig.1D-F), while the Cleaved-PARP was not detected when exposed to methionine-deficient medium only (Fig.1E, F). These results confirm that depleting the methionine could induce sensitivity to cytotoxic agents and apoptosis in cisplatin-resistant lung cancer cells such as H460/DDP and PC-9.

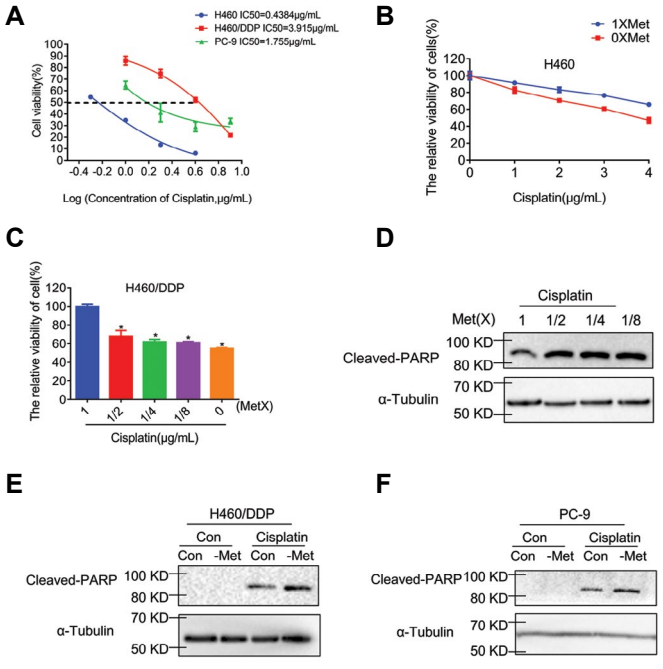

Fig.1: The deficiency of methionine promotes lung cancer cell sensitivity to cisplatin. **A.** The viability of H460, H460/DDP and PC-9 cells with the treatment of various concentrations of cisplatin for 24 hours analyzed by CCK-8 assay. **B.** The activity of H460 cells with different concentrations of cisplatin with or without methionine for 24 hours detected by CCK-8 assay. **C.** The activity of H460/DDP cells with 5 μg/mL cisplatin under various concentrations of methionine for 24 hours analyzed by CCK-8 assay. Error bars show SD (n=3). **D.** Western blotting of Cleaved-PARP in H460 cells with 1 μg/mL cisplatin under different concentrations of methionine. The concentrations of 1 X Met, 1/2 X Met, 1/4 X Met and 1/8 X Met are 15 μg/mL, 7.5 μg/mL, 3.75 μg/mL and 1.875 μg/mL, respectively. The Cleaved-PARP levels were calculated against α-tubulin. **E,** and **F.** Western blotting of *Cleaved-PARP* in H460/DDP cells treated with 5 μg/mL cisplatin and PC-9 cells treated with 2 μg/mL cisplatin cultured in complete medium or medium without methionine. The Cleaved-PARP levels were quantified against α-tubulin. *; P<0.05.

Targeting MAT2A suppresses proliferation and induces apoptosis of cisplatin-resistant lung cancer cells

Methionine adenosyltransferase 2A (MAT2A), an essential enzyme in catalyzing methionine cycle, was located to influence aberrant cell growth and apoptosis via SAM regulation (Fig.2A) and deregulated in several cancer types (27). We found the expression of MAT2A was upregulated in H460/DDP and PC-9 cells compared to H460 (Fig.2B, C). Next, we treated cells with PF-9366, an inhibitor of MAT2A (28). Results showed that the viability of H460/DDP and PC-9 was reduced by PF9366 treatment, which has a positive relationship to the dose (Fig.2D). The proliferation of H460/DDP was significantly inhibited by continuous culture with 10 μM PF9366 (Fig.2E, F). Cleaved-PARP, an apoptosis marker was increased under the joint treatment of cisplatin and PF9366 against to that with cisplatin alone (Fig.2G). These experimental results indicate that MAT2A plays a vital role in the resistance of lung cancer to cisplatin and targeting MAT2A inhibits proliferation and promotes apoptosis of cisplatin-resistant lung cancer cells.

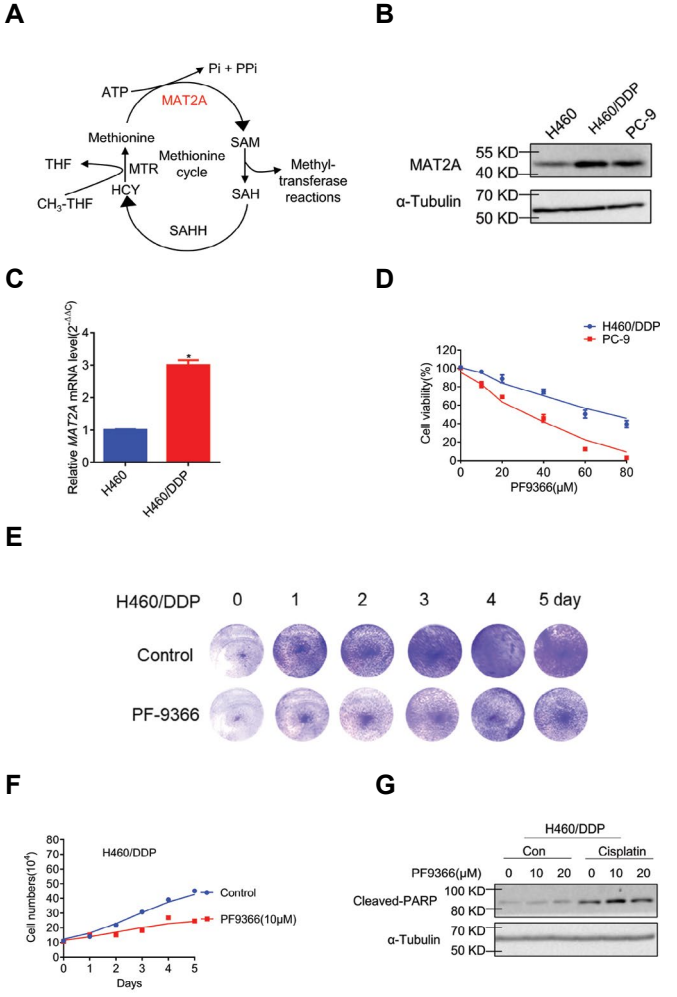

Fig.2: Targeting *MAT2A* inhibits proliferation and enhances sensitivity to cisplatin in lung cancer cells. **A.** The methionine cycle. S-adenosylmethionine synthase, also known as methionine adenosyltransferases (MAT), converts methionine into S-adenosylmethionine (SAM) depending on ATP availability. Subsequently, SAM-dependent methyltransferases transfer the methyl from SAM through the reaction of methylation, producing S-adenosylhomocysteine (SAH), which is then converted into homocysteine (HCY) by S-adenosylhomocysteine hydrolase (SAHH). Methionine synthase (MTR) converts homocysteine back into methionine with a methyl donation from methyl-tetrahydrofolate (CH_3-THF). **B.** Western blotting of *MAT2A* in H460, H460/DDP and PC-9 cells. The *MAT2A* levels were calculated against α-tubulin. **C.** RT-qPCR determined the expression of *MAT2A* mRNA in H460 and H460/DDP cells as indicated. Error bars represent SD (n=3). **D.** The cell viability of H460/DDP and PC-9 cells with different concentrations of PF9366 (0 μM, 10 μM, 20 μM, 40 μM, 60 μM and 80 μM) for 24 hours was analyzed via CCK-8. Error bars show SD (n=3). **E, F.** The proliferation of H460/DDP cells treated with 10 μM PF9366 is shown by crystal violet staining. **G.** Western blotting of *Cleaved-PARP* in H460/DDP cells with 5 μg/mL cisplatin treated with PF9366 (0 μM,10 μM and 20 μM) for 24 hours. The *Cleaved-PARP* levels were quantified against α-tubulin. *; P<0.05.

The transcriptional changes in lung cancer cells after inhibiting MAT2A

Here, we performed RNA-seq of H460 and H460/DDP to gain insight into the mechanism contributing to H460/DDP resistance to cisplatin. Comparative transcriptional expression profiling shown in the heat map (Fig.3A) revealed that there were 10124 gene expression changes in H460/DDP cells compared with H460, involving 4283 up-regulated genes including MAT2A (P=6.61e-08, log2 FC=1.96) and 5841

down-regulated genes including CASP8, CASP7, CASP3, CASP9, CASP2 and CASP10 (Fig.3B, Table S1, See Supplementary Online Information at www.celljournal.org). Seventy one signaling pathways involved in these differential genes were enriched by Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis (Fig.3C, Table S2, See Supplementary Online Information at www.celljournal.org), including TNF signaling pathway and Nucleotide Excision Repair pathways, which was motivated. In order to understand the mechanistic functions of increased MAT2A in cisplatin-resistant cells, we quantified differential genes between H460/DDP and H460/DDP treated with PF9366. We observed 326 up-regulated genes and 1093 down-regulated genes compared with H460/DDP cells (Fig.3D, E). Furthermore, KEGG analysis revealed that 13 signaling pathways were significantly enriched (Fig.3F, Table S3, See Supplementary Online Information at www.celljournal.org). The above 71 signal pathways and 13 signal pathways were analyzed, then 6 common signaling pathways were found, namely, cell adhesion molecules, Fanconi anemia pathway, Ether lipid metabolism, Endocytosis, Endocrine resistance, and TNF signaling pathway (Fig.3G, H). Among them, TNF signaling pathway attracted our attention. The expression levels of major apoptosis genes CASP7 and CASP8 in TNF signaling pathway are significantly downregulated in H460/DDP cells. When treated with PF9366, the expression levels of CASP7 and CASP8 in H460/DDP cells were significantly upregulated (Fig.3I). These observations demonstrate that targeting MAT2A contributes to apoptosis in lung cancer-resistant cells by enhancing the TNF signaling pathway.

Next, we conducted RNA sequencing on H460 cells with PF9366 to assess how MAT2A affects the parent cell H460 (Table S4, See Supplementary Online Information at www.celljournal.org). 1821 expressed mRNAs in H460 cells presented differential expression between control and PF9366 culture conditions (Fig. S1A, See Supplementary Online Information at www.celljournal.org), 1074 down-regulated genes and 747 up-regulated genes were contained (Fig.S1B, See Supplementary Online Information at www.celljournal.org), and cell-cycle regulation and DNA replication pathway were enriched (Fig.S1C, See Supplementary Online Information at www.celljournal.org). Among the genes sensitive to targeting MAT2A we focused on genes with regards to cell apoptosis (Fig.S1B-a, See Supplementary Online Information at www.celljournal.org) and DNA repair (Fig.S1B-b, See Supplementary Online Information at www.celljournal.org), noticing that most of the genes, for example, MAPK8, ATM, BRCA2, TOpBP1, XRCC2, etc., were decreased. These data suggest that inhibiting the activity of MAT2A may impede proliferation via preventing DNA replication and the cell cycle, and accelerate apoptosis through disturbing DNA repair in H460 cells.

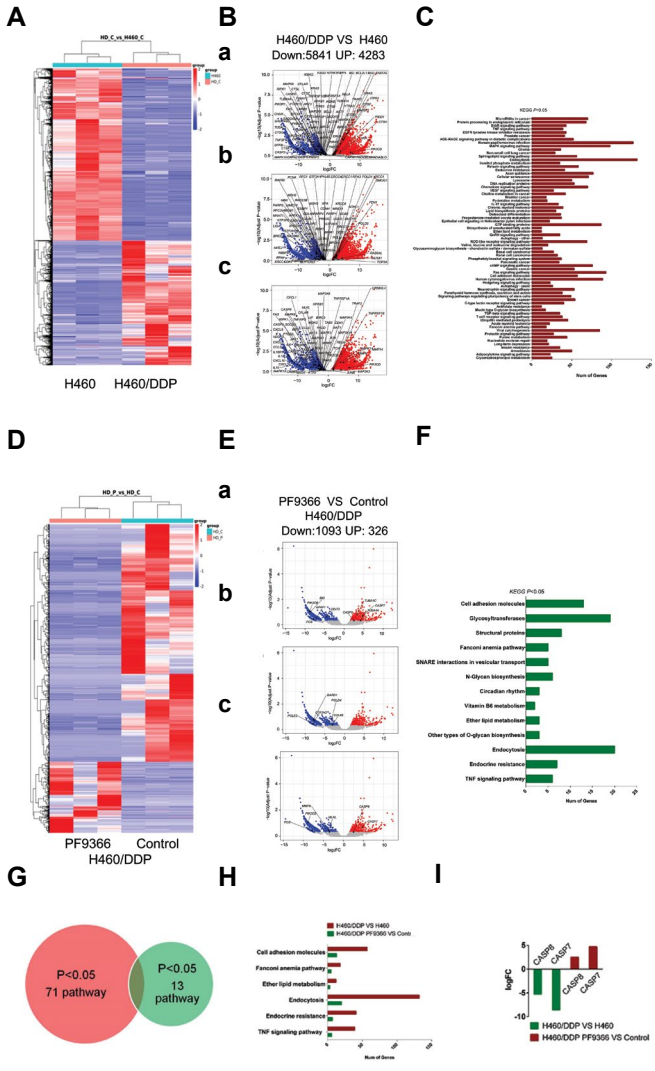

Fig.3: Transcriptional changes in lung cancer cells after inhibiting *MAT2A*. **A, B.** Heatmap and volcano plot displaying global transcriptional changes in H460/DDP cells compared with H460 cells. **B.** Each dot represents a gene, and the red dots represent up-regulated genes (4283), while the blue dots represent down-regulated genes (5841) (adjusted P<0.05) in H460/DDP cells. Differentially expressed genes of apoptosis, DNA repair and *TNF* signaling pathways are showing in a-b, respectively. **C.** KEGG analysis of signaling pathways involved in differentially expressed genes in H460/DDP cells or H460 cells. Each bar graph represents a pathway, and the length of the bar shows differential gene numbers in that pathway. **D, E.** Heatmap and volcano map showing all differentially expressed genes between control and PF9366 (10 μM)-treated H460/DDP cells. **E.** Each dot delegates a gene, and the red dots represent up-regulated genes (326), while the blue dots represent down-regulated genes (1093) (adjusted P<0.05) in H460/DDP cells with the treatment of PF9366. Differentially expressed genes of apoptosis, DNA repair and *TNF* signaling pathways are shown in a-c, respectively. **F.** KEGG analysis of signaling pathways for differentially expressed genes in H460/DDP cells under control and PF9366 (10 μM) treatment. Shown in the bar graph is pathway analysis of differentially expressed genes. Bar length represents the number of genes. **G, H.** Venn diagram showing the overlapping of differential signaling pathways in H460/DDP cells vs H460 (Red), and H460/DDP cells treated with PF9366 vs. control (Green). Bar length represents differentially expressed gene numbers. **I.** The seq-data showing the expression of *CASP7* and *CASP8* in each group as indicated.

Methionine availability maintains histone methylation in lung cancer cells

Methionine availability is predominantly necessary for DNA and RNA methylation (29), as well as histone methylation for regulating gene expression (30, 31), since SAM, generated by consuming methionine and ATP with the

help of MAT2A, is the universal methyl donor for cellular methylation reactions (Fig.4A). We assessed the impact of methionine deficiency on histone methylation in lung cancer cells (H460, H460/DDP, PC-9), and found that H3K4me3, H3K9me2, H3K27me3 and H3K36me3 levels were all reduced (Fig.4B-D). Furthermore, reduced H3K9me2 and H3K36me3 levels and non-significant change H3K4me3 and H3K27me3 levels were shown in H460/DDP and PC-9 cells under PF9366 treatment (Fig.4E, F). These experimental results proove that methionine deficiency or targeting MAT2A affect histone methylation and the latter possesses specificity.

H3K4me3 and H3K36me3 are activating marks involved in gene expression. On the contrary, H3K9me2 and H3K27me3 are related to gene silencing. Combining these different changes of histone methylation with differentially expressed genes, we speculate that methionine deficiency or MAT2A inhibition may modulate genes expression associated with apoptosis, DNA repair and TNF signaling pathways through regulating histone methylation in lung cancer cells.

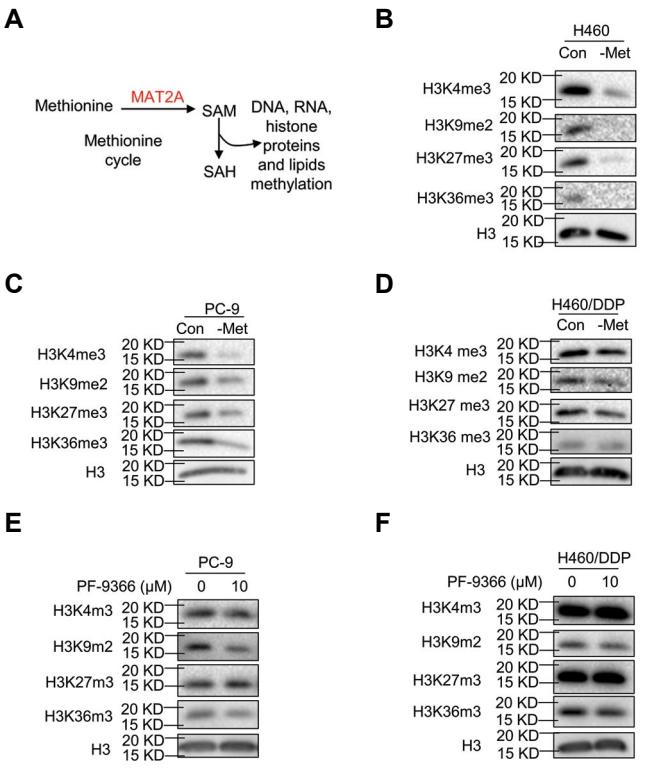

Fig.4: Methionine deficiency or targeting *MAT2A* influences histone methylation in lung cancer cells. **A.** The methionine cycle produces S-adenosylmethionine (SAM), which furnishes the omnipresent methyl group that is used for the methylation of DNA, RNA, histone, proteins and lipids via a large family of SAM-dependent methyltransferases. **B-D.** Western blotting of H3K4me3, H3K9me2, H3K27me3 and H3K36me3 levels in H460, PC-9 and H460/DDP cells under control (Con) and methionine deficiency(-Met) conditions. Total *H3* shown as control. **E** and **F.** Western blotting *of* H3K4me3, H3K9me2, H3K27me3 and H3K36me3 levels in PC-9 and H460/DDP cells with or without PF9366(10 μM) for 24 *hours. Total H3* shown as control.

Discussion

Lung cancer is the malignant tumor with the highest incidence and mortality rate. Chemotherapy is the main strategy for most lung cancer treatments. Platinum plays an important role in lung cancer chemotherapy. However, the resistance of tumor cells to platinum causes failure of chemotherapy. The mechanism of tumor resistance is complex, including enhanced expression of drug efflux protein, enhanced DNA repair ability, inhibition of apoptotic signaling pathways, etc. (32). We performed transcriptome sequencing analysis on lung cancer resistant- (H460/DDP) and -sensitive cells (H460) and enriched the differentially expressed genes. A total of 71 signaling pathways were found to be involved, including resistance-related signaling pathways such as the ErbB signaling pathway, TNF signaling pathway, MAPK signaling pathway, DNA replication proteins and cellular senescence. What's more, differentially expressed genes involved in cell apoptosis (CASP8, CASP7, CASP3, etc..), DNA repair (XRCC5, XRCC6, NHEJ1, etc..) and TNF signaling pathway (CASP8, CASP7, TNFRSF1A, etc..) were shown in figure 3B a-b. These altered pathways and genes suggest that the mechanism by which lung cancer cells are resistant to cisplatin covers multiple aspects and is worthy of further research.

Metabolic reprogramming of tumors, including amino acid metabolism reprogramming, is an important factor leading to tumorigenesis. The amino acids required by the human body include two main types: essential amino acids and non-essential amino acids, of which methionine is an important essential amino acid. Methionine is widely involved in physiological activities such as protein synthesis, amino acid metabolism, and oxidative stress (8). The methionine cycle refers to the action of methionine and ATP under the catalysis of methionine adenosyltransferase to form S-adenosylmethionine (SAM). The methyl group in SAM can be transferred to another substance such as DNA, RNA, protein and lipid, etc. Under the catalysis of methyltransferase, SAM is converted into S-adenosine homocysteine (SAH), from which the removal of adenosine, produces homocysteine (HCY). Integration of homocysteine with the methyl group from N_5-methyltetrahydrofolate can generate methionine. The methionine cycle is essential for the methylation modification of biomacromolecules and is widely involved in DNA replication, transcription, translation, post-translational modifications, etc. (33, 34). Methionine adenosyltransferase (MAT) is a key enzyme in the methionine cycle. There are two types of MAT, including MAT1A and MAT2A in the body (35). MAT1A is expressed only in the liver, while MAT2A is expressed in various organs of the body and participates in the development of plentiful tumors (36). Studies have shown that cultured lung cancer stem cells have a stronger methionine cycle than non-stem cells, as well as methylation processes driven by MAT2A (20). In this study, we explored the inhibitory effect of targeting MAT2A on platinum-resistant lung cancer cells and found that targeting MAT2A can inhibit the proliferation of drug-resistant lung cancer and induce apoptosis. Other studies have also found that targeting MAT2A can inhibit

stem cell proliferation, migration, invasion and drug resistance of tumor cells (36, 37). We have also revealed that inhibition of MAT2A blocked the cell cycle and DNA replication in H460 cells by RNA-seq. These results indicate that MAT2A is a potential target for anti-tumor therapy.

To investigate how targeted MAT2A promotes apoptosis in platinum-resistant lung cancer cells, we performed transcriptome sequencing analysis of cells treated with MAT2A inhibitor PF9366 and combined analysis with previous transcriptome sequencing results of drug-resistant cells. A common enriched signaling pathway, including the TNF signaling pathway was identified. Downstream signaling pathways for TNF activation mainly include caspase family-mediated apoptosis, adaptor protein TRAF-mediated transcription factor NF-κB and activation of JNK protein kinase (38). By sequencing, it was found that the important pro-apoptotic genes CASP7 and CASP8 in the TNF pathway were down-regulated in cisplatin-resistant cells, significantly increased after MAT2A was inhibited. Changes of differential genes associated with apoptosis, DNA repair and TNF signaling pathway were exhibited in our results. These results indicate that cisplatin-resistant cells inhibit the apoptosis process by down-regulating CASP7 and CASP8 genes, while targeting MAT2A reactivates CASP7 and CASP8 to complete the apoptotic process. How CASP7 and CASP8 expression are regulated by MAT2A remains to be further studied.

The lysine methylation modification of histones can remodel the chromatin spatial structure and play an important role in DNA damage repair and transcriptional regulation (39). H3K4 mono-, di-, or tri-methylation (H3K4me1/2/3) and H3K36me3 was a modification that promotes transcription (14, 40), while H3K9 and H3K27 methylation (H3K9me2/3 and H3K27me2/3) restrains gene expression (15, 31). We found a significant decrease in H3K4me3, H3K9me2, H3K27me3, and H3K36me3 levels under methionine deficiency conditions in H460, H460/DDP and PC-9 cells, and a significant reduction of H3K9me2 and H3K36me3 in H460/DDP and PC-9 cells treated with PF9366. We speculate that MAT2A inhibition led to the reduction of H3K9me2 by disturbing the methionine cycle, as a result of which CASP7 and CASP8 were upregulated. The other changed genes involved in cell apoptosis, DNA repair and TNF signaling pathway may also be influenced by the changes of histone methylation level. Subsequent research is needed to further study the dynamic epigenetic regulation mechanism of these genes.

Conclusion

The present study discovered that inhibiting methionine availability enhanced the inhibitory effect of cisplatin on cell activity and the pro-apoptotic effect. Targeting MAT2A can promote sensitivity of cisplatin- resistant lung cancer cells to cisplatin by regulating the expression of apoptosis-related genes. This founding provides a scientific basis for the development of new strategies to overcome lung cancer resistance.

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Authors' Contributions

All authors participated generally in study conception and design, data acquisition, data analysis and interpretation. X.Z., L.W., J.F., W.X., D.Z.; Contributed to write the manuscript or revise it critically. All authors read and approved the final manuscript.

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