Abplatin$^{(IV)}$ Inhibited Tumor Growth On A Patient Derived Cancer Model of Hepatocellular Carcinoma And Its Comparative Multi-Omics Study With Cisplatin

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Abstract

Background: Cisplatin is the most common antitumor alkylating agent of platinum(II) (Pt(II)) in clinic, however it had many side effects. It is necessary to develop low toxicity platinum(IV) (Pt(IV)) drugs. Multi-omics was frequently used to help one understand the mechanism of a certain therapy at the molecular level. Little was known about the mechanism of Pt(IV) drugs, which may be benifical for clinical translation.

Methods: We developed a Pt(IV) drug of cisplatin with two hydrophobic aliphatic chains and further encapsulated it with a drug carrier human serum albumin (HSA) to prepare Abplatin$^{(IV)}$. Transcriptomics, metabolomics and lipidomics were performed to clarify the mechanism of Pt(IV) drugs. T-test assay and fold change were used to find the differential substances.

Results: We had further shown Abplatin$^{(IV)}$ had better tumor-targeting performance and greater tumor inhibtion rate than cisplatin. Lipidomics study showed that Abplatin$^{(IV)}$ might induce the changes of BEL-7404 cell membrane, and caused the disorder of glycerophospholipids and sphingolipids. In addition, transcriptomics and metabolomics study showed that Abplatin$^{(IV)}$ mainly disturbed more significant purine metabolism pathway than cisplatin.

Conclusions: This research highlighted the development of Abplatin$^{(IV)}$ and the use of multi-omics to help one understand the mechanism of action of prodrugs and their DDS, which was the key to the clinical translation of them.

Background

Multi omics can help one to understand the interaction between a variety of substances in biological systems, including genomics, epigenomics, transcriptomics, proteomics, metabolomics, microbiomics, etc. These substances jointly affect the phenotypes and traits of biological systems.[1, 2] Taking cancer as an example, the occurrence and development of a certain cancer is a complex process with DNA, epigenetic mutations and abnormal gene expressions.[3, 4] With the popularization of sequencing technology and the improvement in data analysis, single omics can no longer meet the needs of scientific research, and multi omics has gradually become the mainstream in cancer research by integrating genomics and transcriptomics etc., which is of great value in cancer pathogenesis, screening treatment targets, discovery of biomarkers, early diagnosis, drug sensitivity prediction and cancer prognosis.[4-8] Taken together, Multi omics may play an important role in individualized medicine and medication guidance.

Cisplatin is the most common antitumor alkylating agent of platinum(II) (Pt(II)) based anticancer drugs in clinic. It kills cancer cells by inducing apoptosis with substantial changes at the molecular level, including genome, transcriptome, proteome and metabolome.[9, 10] The great obstacle of cisplatin is its side effects and evolution of drug resistance.[11, 12] Therefore, in recent years, numerous so-called

platinum(IV) (Pt(IV)) prodrugs have been prepared.[13] In addition, in order to increase the targeting efficiency of Pt(IV) prodrugs and reduce their toxic and side effects, people have also designed various drug carriers such as polymers, lipids, proteins and peptides to physically encapsulate or chemically conjugate with these Pt(IV) prodrugs, resulting in thousands of Pt(IV)-based drug delivery systems (DDS).[14-17] It is generally believed that after Pt(IV) prodrugs and their DDS enter cancer cells, Pt(IV) prodrugs will be reduced by intracellular reducing agents such gluatione (GSH) and ascorbic acid and then release Pt(II) drugs to bind with DNA for cell killing.[18-21] However, there was different views that Pt(IV) prodrugs themselves can interact with DNA to exert the anticancer effect, making the mechanism of Pt(IV) prodrugs and their DDS more blurred. In a word, up to now, although thousands of Pt(IV) prodrugs and their DDS have been developed, and some of them showed excellent clinical translational prospect and even entered clinical research, a basic scientific problem is still here that the mechanism of action of Pt(IV) prodrugs and their DDS at the molecular level remains unknown, which heavily hampers the further translation of them into clinic.

Herein, starting from cisplatin, we firstly prepared a hydrophobic Pt(IV) prodrug (CisPt(IV), Scheme 1), which is characterized by the presence of two apliphatic chains in the axial position. To further deliver CisPt(IV), HSA was chose to encapsulate this prodrug to form nanoparticles, ie, AbPlatin$^{(IV)}$ **(**Scheme 1**A)**. AbPlatin$^{(IV)}$ was supposed to release Pt(II) after it entered the cancer cells via chemical reduction. AbPlatin$^{(IV)}$ not only had a strong killing ability to cancer cells (even cisplatin-resistant cells), but also had a good tumor targeting and low toxic side effects. We have shown here on a hepatocellular carcinoma patient-derived tumor xenograft model (PDX^{HCC}), AbPlatin$^{(IV)}$ also had a good anti-tumor effect **(**Scheme 1**B)**. To understand the mechanism of action of AbPlatin$^{(IV)}$, we further systematically investigated the effects of AbPlatin$^{(IV)}$ as compared to cisplatin on the transcriptomics, metabolomics and lipidomics. It was found that Abplatin$^{(IV)}$ was in a more significant purine metabolism pathway and ATP was down-regulated, and xanthosine and hypoxanthine were up-regulated in cells treated with Abplatin$^{(IV)}$, which resulted in cell apoptosis **(**Scheme 1**B)**. Besides, it was found that Abplatin$^{(IV)}$ affected more genes expression than cisplatin. ABAT, CLDN6 and other genes were significantly up-regulated (Scheme 1**B**). We found carnitine was down-regulated, and arginine and homoarginine content was up-regulated. This work highlighted the translational research and the possible mechanism of action study of Abplatin(IV).

Results And Disscussion

Synthesis, Preparation and Characterization of Abplatin$^{(IV)}$

CisPt(IV) with two aliphatic chains was synthesized as previously described and characterized (**Figure S1 and Figure S2**). HSA was the most abundant protein in the human bloodstream[22, 23], which is widely used as drug carriers as Abraxane has been the most successful nanodrugs.[24-26] Therefore, Abplatin$^{(IV)}$ was prepared by encapsulation of CisPt(IV). Transsmission electron microscopy (TEM) and dynamic light scattering (DLS) demonstrated that Abplatin$^{(IV)}$ was in spherical structure with a diameter at 174.7 nm at a feed mass ratio of 1/10 (Pt to HSA) (**Figure 1A□1B**). Moreover, Abplatin$^{(IV)}$ had good stability within 7

days with limited size variations (**Figure 1C**). Element mapping mappings of Abplatin$^{(IV)}$ via STEM revealed that the C, N, O, Cl and Pt elements in Abplatin$^{(IV)}$ were uniformly distributed, further confirming that CisPt(IV) was successfully packaged into the nanoparticles by HSA **(Figure 1D)**. Abplatin$^{(IV)}$ was believed then possibly to be reduced by intracellular reducing agents such as gluatione(GSH) and ascorbic acid to release cisplatin for DNA binding.[3, 18, 19] The results showed that within 12 h, 80%, 10% and 8% of Pt drugs were released in sodium ascorbate (NaVc) aqueous solution, acetate buffer solution and PBS , respectively, indicating Pt drugs could be released faster in the reductive environment **(Figure 1E)**.

In vitro Anticancer Efficacy of Abplatin$^{(IV)}$

Abplatin$^{(IV)}$ was supposed to be endocytosed by BEL-7404 cancer cells. To prove this process, Abplatin$^{(IV)}$ was firstly labelled by Cy5.5 (Abplatin$^{(IV)}$@Cy5.5) and observed by confocal laser scanning microscope (CLSM). The results demonstrated that the red fluorescence in the BEL-7404 cells increased with longer incubation time (**Figure 2A**). Further semi-quantification via flow cytometry showed that the uptake of Abplatin$^{(IV)}$@Cy5.5 at 7 h was 2.24 times than that at 1 h (**Figure 2B**). The above results together demonstrated that the Abplatin$^{(IV)}$ could effectively enter into the cells. To futher explore the tumor penetration of Abplatin$^{(IV)}$, a 3D cell sphere was established. The fluorescence distribution of different focal planes after 3D cell sphere treated by Abplatin$^{(IV)}$@Cy5.5 at 12 h showed all focal planes were full of red, indicating that Abplatin$^{(IV)}$ could effectively penetrate into tumor spheriods (**Figure 2E**).

There are numerous Pt atoms in Abplatin$^{(IV)}$, making it possile to quantivatiely study the intracellular uptake of Abplatin$^{(IV)}$ by ICP-MS. Results showed that the Pt uptake by BEL-7404 cells gradually increased over time. Specifically, it was 1303 ng Pt/million cells at 7 h, while this was only 39 ng Pt/million cells and 948 ng Pt/million cells for cisplatin and CisPt(IV), which was 33.4 and 1.4 fold-increase in uptake for nanoparticles (**Figure 2C**). Cisplatin could bind with DNA to form Pt-DNA adducts, thereby affecting DNA replication and killing cancer cells.[27, 28] To investigate whether the increase in Pt uptake of Abplatin$^{(IV)}$ could result in more Pt-DNA adducts, DNA was extreacted from BEL-7404 cells treated with various drugs and further the Pt-DNA adducts were tested by ICP-MS. The results showed that the Pt-DNA addcuts of cells treated with Abplatin(IV) was 3.22 times than those treated with cisplatin (**Figure 2D**). The above results together showed that cancer cells treated by Abplatin$^{(IV)}$ could promote the formation of Pt-DNA adducts, thereby resulting in more DNA damage and inhibiting cell proliferation.[28]

The results showed that Abplatin$^{(IV)}$ were much effective in killing cancer cells than cisplatin and CisPt(IV) on all these cell lines (**Figure 2F)**. Taking A2780 cell (human ovarian cancer cell) for an example, the IC_{50} of Abplatin$^{(IV)}$, CisPt(IV) and cisplatin were 1.36 μM, 1.93 μM and 25.10 μM, respectively. Even to cisplatin-resistant cells, like A2790DDP and BEL-7404DDP, Abplatin$^{(IV)}$ still had the killing capability. Furthermore, the apoptosis test showed that Abplatin$^{(IV)}$ (10 μM) induced an apoptosis

rate of 90.2% on BEL-7404 cells. However, at the same concentration, cisplatin and CisPt(IV) only induced an apoptosis rate of 11.1% and 38.9%, respectively (**Figure S3)**.

To test whether Abplatin$^{(IV)}$ could enter tumors and induce cell death, a 3D cell sphere was cultured to mimic the process. The calcein AM/PI (acetyl methoxy methyl ester/ propidium iodide) cell viability kit was used. Green fluorescence could be detected in living cells while red fluorescence could be detected in the dead cells. The results showed that the BEL-7404 cells in a 3D cell sphere after treated with Abplatin$^{(IV)}$ showed a large amount of red fluorescence(**Figure 2G**), indicating that most of the cells were died.

In vivo Anticancer Efficacy of Abplatin$^{(IV)}$

Further, the therapeutic effect of Abplatin$^{(IV)}$ on a PDX^{HCC} model was evaluated. PDX model is a human xenotransplantation model established by directly transplanting liver tumor tissue from patients to immunodeficient mice (**Figure 3A**). PDX model retains the characteristics of patient tumors at the level of histopathology, molecular biology, and genetics and is currently the preferable model for drug evaluation at this stage. Moreover, PDX model was often used to screen for anticancer drugs.[18, 21]

When the tumors reached approximately 300 mm^3, Abplatin$^{(IV)}$ labelled with Cy7.5 (dose = 1.5 mg Cy7.5/kg and 3.5 mg Pt/kg body weight) was *i.v.* injected into the mice. Imaging of mice showed that Abplatin$^{(IV)}$ were distributed in the whole body at 3 h. Then Abplatin$^{(IV)}$ began to accumulate in the tumor at 6 h, indicating that Abplatin$^{(IV)}$ had good tumor-targeting ability. After 36 h, the mice were killed and all organs of heart, liver, spleen, lung and kidney were taken out. *Ex vivo* imaging showed that Abplatin$^{(IV)}$ mainly accumulated in the liver and tumor regions (**Figure 3B, 3C**).

For tumor inhibition study, Abplatin$^{(IV)}$ was *i.v.* injected into the mice and the tumor volume was measured every two days (**Figure 3A**). The resultant tumor inhibition curve indicated that Abplatin$^{(IV)}$ had the most obvious tumor inhibition effect, followed by cisplatin and CisPt(IV) (**Figure 3D**). The tumor inihibiton rate was further calculated to be 34.3% and 64.8% for cisplatin and Abplatin$^{(IV)}$ respectively. Subsequent hematoxylin and eosin (H&E) staining on the tumor tissues showed the cells in the tumor tissue in mice treated with PBS presented a rich and full state, while the cells in the tumor tissues in mice treated with cisplatin and Abplatin$^{(IV)}$ presented a nuclear shrinkage state. Moreover, the damage caused by Abplatin$^{(IV)}$ was the most serious. Further TUNEL staining also showed that the tumor tissues treated with Abplatin$^{(IV)}$ had the most severe apoptosis than cisplatin **(Figure 3E)**.

The toxic side effect of Abplatin$^{(IV)}$ was further investigated. Fristly, the body weight and blood physiological parameters of the mice were monitored. The results showed that the body weight of healthy BALB/c mice in the Abplatin$^{(IV)}$ treatment group at a dose of 3.5 mg Pt/kg increased within 10 days (**Figure S4**), and their blood physiological and biochemical indexes were normal (**Figure S5**).Subsequently H&E staining demonstrated that the heart, liver, spleen, lung and kidney did not show

morphological damage (**Figure 3F**).In a word, Abplatin$^{(IV)}$ at a dose of 3.5 mg Pt/kg had good enough biosafety.

RNA-seq analysis in BEL-7404 cells treated with Abplatin$^{(IV)}$

The molecular mechanism of cisplatin had been extensively studied. It was found that p53 activated pro-apoptotic genes and resulted in apoptosis when cells were treated by cisplatin.[29] In addition, it was proposed that caspase-6 and -7 genes were the transcriptional targets of p53 in cisplatin injury. [30] However, altought there were numerous reports on Pt(IV) drugs and their DDS, little is known about the molecular mechanism of them. To understand this, RNA-seq analysis was performed on BEL-7404 cells treated with PBS, cisplatin and Abplatin$^{(IV)}$. We found that there are 9227 genes in the transcription detected. Among those genes, 428 genes were specifically transcribed in the cells treated with cisplatin, while 6344 genes were exclusively transcribed in the cells treated with Abplatin$^{(IV)}$ **(Figure 4A)**. Compared with the cells treated with PBS, 179 genes were up-regulated (red dots) and 715 genes were down-regulated (blue dots) in the cells treated with cisplatin (**Figure 4B**). However, there were 980 genes up-regulated and 3963 genes down-regulated in the cells treated with Abplatin$^{(IV)}$ **(Figure 4C)**. Obviously, Abplatin$^{(IV)}$ treatment resulted in more genetic disorders than cisplatin. Thereafter, we only selected 20 genes with significant differences for heat map analysis. Results showed that genes such as HSPA6, ABAT, PNLDC1, TENT5C, CLDN6, TMEM151B, ID2, NACAD, SPANXC, MAFB were significantly up-regulated while SEMA3A, FNDC3B, ATXN1, ZNF609, DAPK1, GLIS3, CDK17, PDE3A, GRB10 and RUNX2 were significantly down-regulated (**Figure 4D, Table S1**). Moreover, it was reported that overexpression of ABAT gene would significantly reduce cell proliferation and migration, and impair the production of lactic acid.[31] We found here, the increased expression of ABAT in the Abplatin$^{(IV)}$-treated cells might be the one of reasons for its better cell killing effect. Claudin 6 (CLDN6), a member of the Claudin (CLDN) family of tight junction proteins, was reported to be able to inhibit the proliferation and induce apoptosis of cancer cells.[32] We found here CLDN6 was also highly expressed in cells treated with Abplatin$^{(IV)}$. Subsequent Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis further demonstrated that purine metabolism, alanine, aspartate and glutamate metabolism, etc., were mainly affected in BEL-7404 cells treated with Abplatin$^{(IV)}$ (**Figure 4E**).

Lipidomics and metabolomics analysis in BEL-7404 cells treated with Abplatin$^{(IV)}$

In order to further illustrate the molecular mechanism of Abplatin$^{(IV)}$, lipidomic and metabolomic studies were performed. The representative total ion chromatograms (TICs) of BEL-7404 lipids and metabolites were shown in **Figure S6** and **Figure S7**. Further, the partial least-squares discriminant analysis (PLS-DA) score plots in positive and negative ion detection mode indicated that cell treated with PBS, cisplatin and Abplatin$^{(IV)}$ showed very distinct separation. There were 40 differential lipids between Abplatin$^{(IV)}$ and PBS treatment groups (**Figure S8 and S9, Figure 5A, Table S2**), while there were 31 differential lipids between cisplatin and PBS treatment groups (**Figure S10A**).Specifically, in the Abplatin$^{(IV)}$ treatment group, twenty phosphatidylcholine (PCs), three phosphatidylglycerols (PGs), four

lysophosphatidylcholines (LPCs), one lysophosphatidylethanolamine (LPE), one sphingomyelin (SM) and two lysosphingomyelins (LSMs) were up-regulated compared with the PBS treatment group. Moreover, we found here there were nine PCs were down-regulated in the Abplatin$^{(IV)}$ treatment group compared with the PBS treatment group (**Figure 5A, Table S2**). Notably, the main differential lipids were glycerophospholipids (LPC, LPE, PC and PG) and a very small amount of spingolipids (SM, LSM) in the cells treated with Abplatin$^{(IV)}$ compared with PBS. However, there were no significant changes in glycerides and sterol lipids for the cells treated with Abplatin$^{(IV)}$ compared with PBS. As the glycerophospholipids and sphingolipids were the main components of cell membrane, the disorder induced by Abplatin$^{(IV)}$ revealed by lipodomics might indicate the changes of BEL-7404 cell membrane.

Further, metabolomics analysis on BEL-7404 treated with Abplatin$^{(IV)}$ and cisplatin was performed. Results showed that there were 25 differential metabolites between Abplatin$^{(IV)}$ and PBS treatment groups (**Figure 5B, Table S4**), while there were 30 differential metabolites between cisplatin and PBS treatment groups (**Figure S10**). Further, we found that among these differential metabolites, there were 14 common differential metabolites, such as ATP, nicotinic acid adenine dinucleotide, glutamate, hypoxanthine, etc **(Table S3).** Moreover, these common differential metabolites had the same trend in change. To be specific, ATP and nicotinic acid adenine dinucleotide were down-regulated and others were up-regulated in the cells treated with Abplatin$^{(IV)}$ and cisplatin. It is believed that Pt(IV) drugs can be reduced to Pt(II) to exert anticancer efficacy, so it makes sence that part of metabolites showed the same change trend in the two treatment groups.

As we described that there were 25 metabolites significantly changed in the cells treated with Abplatin$^{(IV)}$ (**Figure 5B** and **Table S4)**. Further metabolic pathways analysis indicated that purine metabolism, arginine biosynthesis, histidine metabolism and glutathione metabolism were mainly affected (**Figure 5C and Figure 5D**). Compared with cisplatin, Abplatin$^{(IV)}$ more significantly disturbed the purine metabolism pathway (**Figure S11)**. In purine metabolism pathway, ATP was down-regulated and xanthosine and hypoxanthine were up-regulated. As purine was the basic component of nucleotides in cell proliferation, the impaired purine metabolism pathway is related to cancer progression.[33] ATP is the energy source of living cells,[34] and the decreased ATP in the cells treated with Abplatin$^{(IV)}$ indicated there is insufficient cell energy, resulting in cell apoptosis. It was reported that Xanthosine could inhibit cancer cell proliferation and Hypoxanthine could induce apoptosis by regulating the expression of apoptosis-related proteins. [35, 36] Therefore, the increase of hypoxanthine and xantholine in Abplatin$^{(IV)}$ treated cells might also a cause for cell death. Moreover, we found here in arginine biosynthesis pathway, the aspartic acid and arginine in Abplatin$^{(IV)}$ treated cells were significantly up-regulated and the content carnitine were down-regulated in the cells treated with Abplatin$^{(IV)}$ compared with PBS. It was reported that the increase of arginine could also result in cell apoptosis and carnitine could improve energy status, reduce oxidative stress and prevent subsequent cell death.[37],[38] Therefore, the results we found here further indicated the increase of arginine and decrease of carnitine resulted in cell apoptosis.[39] Finally,

homoarginine was found to be up-regulated in the cells treated with Abplatin$^{(IV)}$ compared with PBS, indicating the increase of homoarginine inhibited cell proliferation and thus resulted in cell death.[40]

Conclusion

Due to the importance of Pt(II) based drugs in cancer therapy, Pt(IV) drugs and their DDS are very promising. Although the molecular mechanism of Pt(II) based drugs such as cisplatin has been widely reported in the literature, there is very limited report on the molecular mechanism of Pt(IV) based drugs, especially from the perspective of multi-omics. Therefore, in this paper, CisPt(IV) with two hydrophobic aliphatic chains in the axial position was chosen. Subsequently, the most common drug carrier albumin was adopted to encapsulate it to form Abplatin$^{(IV)}$. Thereafter,we explored the anticancer effect of Abplatin$^{(IV)}$ on cancer cells, 3D cell sphere and on a PDX^{HCC} mice model. *In vitro*, IC_{50} of Abplatin$^{(IV)}$ was 1.36 μM on A2780 cells, much lower than that of cisplatin at 25.10 μM., Abplatin$^{(IV)}$ still had the killing capability even on the cisplatin-resistant cells such as A2780DDP and BEL-7404DDP cells. Moreover, with the help of a 3D cell sphere model of BEL-7404 cells, our study showed that Abplatin$^{(IV)}$ had better anticancer activity on 3D cell spheres, with enhanced Pt-DNA addcuts formation, thereby resulting in greater DNA damage and inhibiting cell proliferation. *In vivo*, we established a PDX^{HCC} model, Abplatin$^{(IV)}$ showed better tumor-targeting performance and tumor inhibition ability than cisplatin. Finally, a multi-omics strategy, including transcriptomics, metabolomics and lipidomics, was carried out on BEL-7404 cells o elucidate the molecular mechanism of Abplatin$^{(IV)}$. We found here that Abplatin$^{(IV)}$ might induce the change of BEL-7404 cell membrane, resulting in the disorder of glycerophospholipids and sphingolipids. More importantly, many genes were affected, and the significant up-regulation of ABAT and CLDN6 genes might prohibit the cancer cells proliferation and induce cell apoptosis. Meanwhile, the transcriptomics and metabolomics study showed Abplatin$^{(IV)}$ mainly disturbed more significant purine metabolism pathway that cisplatin. We further found that in purine metabolism pathway, ATP was down-regulated, and xanthosine and hypoxanthine were up-regulated. In addition, arginine and homoarginine were increased, and carnitine was decreased after Abplatin$^{(IV)}$ treatment, which might result in cell apoptosis as well. This research highlighted the development of Abplatin$^{(IV)}$ and the related multi-omics study for the study of molecular mechanism of action, which might be helpful for clinical translational of Pt(IV) drugs and their DDS in the future.

Methods

Materials and Reagents. N-Octyl isocyanate and hydrogen peroxide (H_2O_2), dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Aladdin (Shanghai, China). Cisplatin (purity 99%) was bought from Kunming Institute of Precious Metals (Yunnan, China). Human serum albumin (HSA) was purchased from Shang Hai Yuan Ye Bio-Technology Co.,Ltd (Shanghai, China). 2-(4-Amidinophenyl)-1H-indole-6-carboxamidine (DAPI), Alexa Fluor 488 (Alex 488), Cy5.5 and Cy7.5 were purchased from Sigma-Aldrich (Shanghai, China). Calcein AM/PI (acetyl methoxy methyl ester/

propidium iodide) cell viability kit, DNA extraction kit and Annexin V-FITC apoptosis detection kit was purchased from Beyotime Institute of Biotechnology (Jiangsu, China). Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) kit were purchased from Roche (Basel, Switzerland). RPMI-1640 medium, DMEM, 0.25% trypsin-EDTA, fetal bovine serum (FBS), and penicillin/streptomycin (P/S) were purchased from Gibco (NY, U.S.A.). Cell culture vessels were purchased from Corning (NY, U.S.A.).

***In vivo* biodistribution imaging.** When the tumors reached approximately 300 mm^3, $Abplatin^{(IV)}$ labelled with Cy7.5 (Cy7.5 dose:1.5 mg Cy7.5/kg, Pt dose: 3.5 mg Pt/kg) was *i.v.* injected into the mice. At designed time points post injection (3h, 6h, 8h, 12h, 24h and 36h), the mice were imaged by an *in vivo* imaging system (IVIS) (PerkinElmer, E_x/E_m=780 nm/810 nm, Waltham, USA) to detect the tumor accumulation. After 36 h of injection, mice were sacrificed and the main organs including heart, liver, lung, kidney, spleen and tumors were isolated and used for *ex vivo* imaging.

Therapeutic effect of $Abplatin^{(IV)}$ on a multidrug resistant liver carcinoma PDX model. PDX^{HCC} model was established as previously described. When the subcutaneous tumors reached approximately 100 mm3, the mice were randomly divided into three groups with 5 mice in each group. The drugs were *i.v.* injected in the day 0, 2 and 4. Treatment groups were: 1) PBS (200 μL), 2) cisplatin (3.5 mg Pt/kg), 3) $Abplatin^{(IV)}$ (3.5 mg Pt/kg). The tumor volume were recorded every other day. The tumor volume was calculated following the equation below: tumor volume = $1/2 \times LW^2$, where “L” refers to the long diameter of the tumor and “W” means the short diameter of the tumor. The relative tumor volume was set as the V_t/V_0. “V_t” refers to the mean tumor volume at a desirable day when tumor volumes were collected. “V_0” refers to total mean value of the initial tumor volume. In order to detect the damage and apoptosis of tumors treated with different drugs, hematoxylin and eosin (H&E staining) assay and TUNEL staining analysis were performed.

***In vivo* toxicity evaluation.** Healthy female KM mice (weighed 18~22 g) were randomly assigned to 2 groups and injected via the tail vein with PBS and $Abplatin^{(IV)}$ at a dose of 3.5 mg Pt/kg body weight (n=3). The mice were weighted every other day and sacrificed 10 days post the first administration. In order to assess the potential toxicity of $Abplatin^{(IV)}$, histology analysis was performed on major organs including heart, spleen, liver, kidney and lung. Tissues from these organs were collected, fixed with 4% paraformaldehyde solution, and sliced for H&E staining assay. Meanwhile, blood biochemistry assays were conducted to quantitatively evaluate the potential kidney, heart and liver function as follows: blood urea nitrogen (BUN) (indicators for kidney function), creatinine (CRE) (indicators for kidney function), minotransferase (AST) (indicators for hepatic function), creatine kinase (CK) (indicators for heart function), aminotransferase (ALT) (indicators for hepatic function), and creatine kinase MB (CKMB) (indicators for heart function).

Statistical Analysis. Student’s t test was used to determine the statistical difference between groups. Differences were considered statistically significant at a level of *p < 0.05; **p < 0.01; ***p <0.001; ****p <0.0001.

List Of Abbreviations

Pt(II) drugs: platinum(II) drugs

Pt(IV) drugs: platinum(IV) drugs

HSA: human serum albumin

CisPt(IV): hydrophobic Pt(IV) prodrug

AbPlatin$^{(IV)}$: CisPt(IV) was encapsulated by HSA

Declarations

·Ethics approval and consent to participate

We provide ethics approval and consent to participate.

·Consent for publication

We provide consent to publish that article.

·Availability of data and material

Not applicable.

·Competing interests

There are no competing interests.

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·Authors' contributions

All authors contributed signifcantly to the preparation of this article and the research work associated with it. All authors read and approved the fnal manuscript.

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Scheme

Schemes 1 is available in the Supplemental Files section

Figures

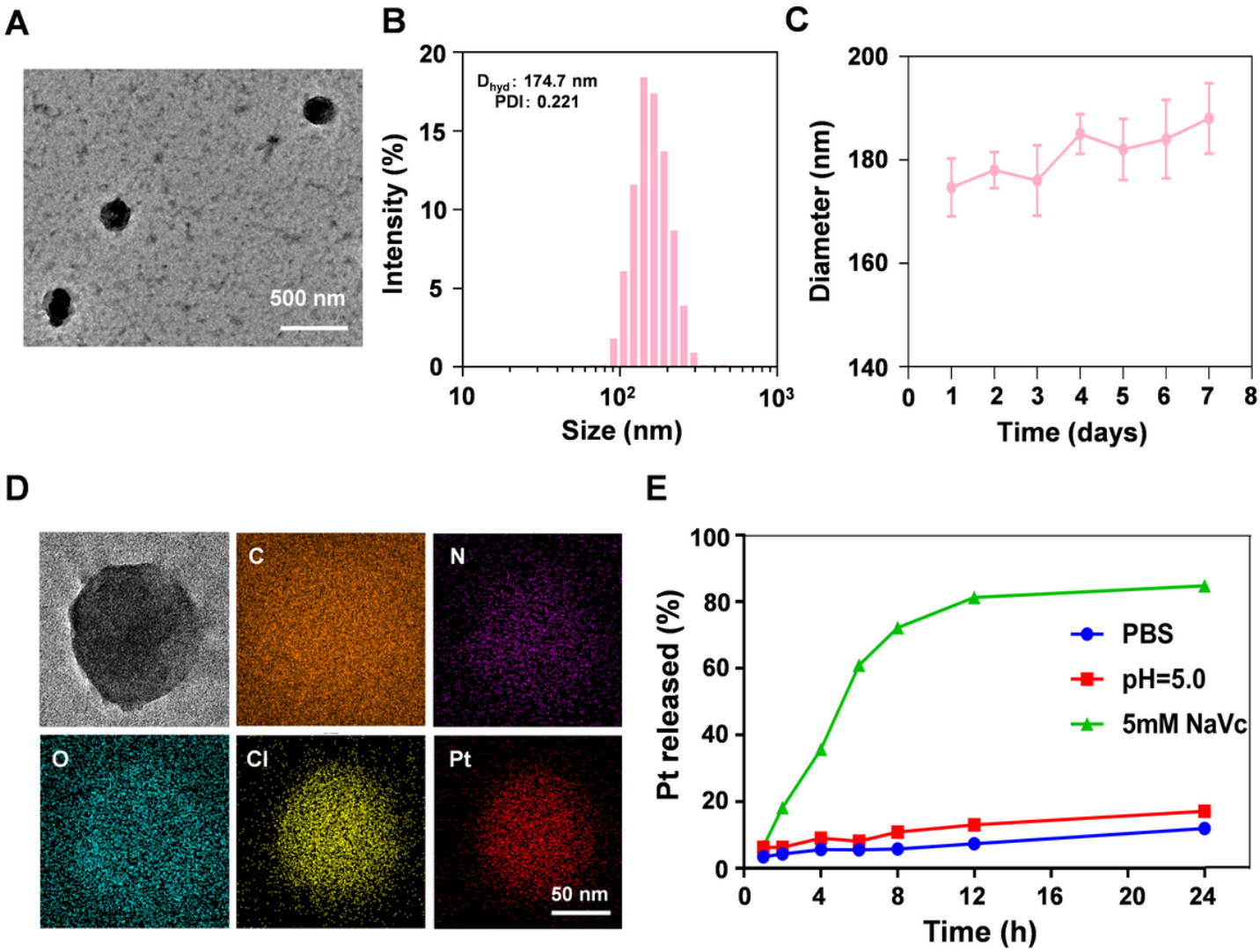

Figure 1

Characterizations of Abplatin$^{(IV)}$. (A) Representative transmission electron microscope (TEM) image of Abplatin$^{(IV)}$. (B) Hydrodynamic diameter of Abplatin$^{(IV)}$ by dynamic light scattering (DLS). (C) a seven-day

stability of Abplatin$^{(IV)}$. (D) Element mapping of Abplatin$^{(IV)}$ by scanning transmission electron microscope (STEM). (E) Representative drug release profile of Abplatin$^{(IV)}$ under various medium.

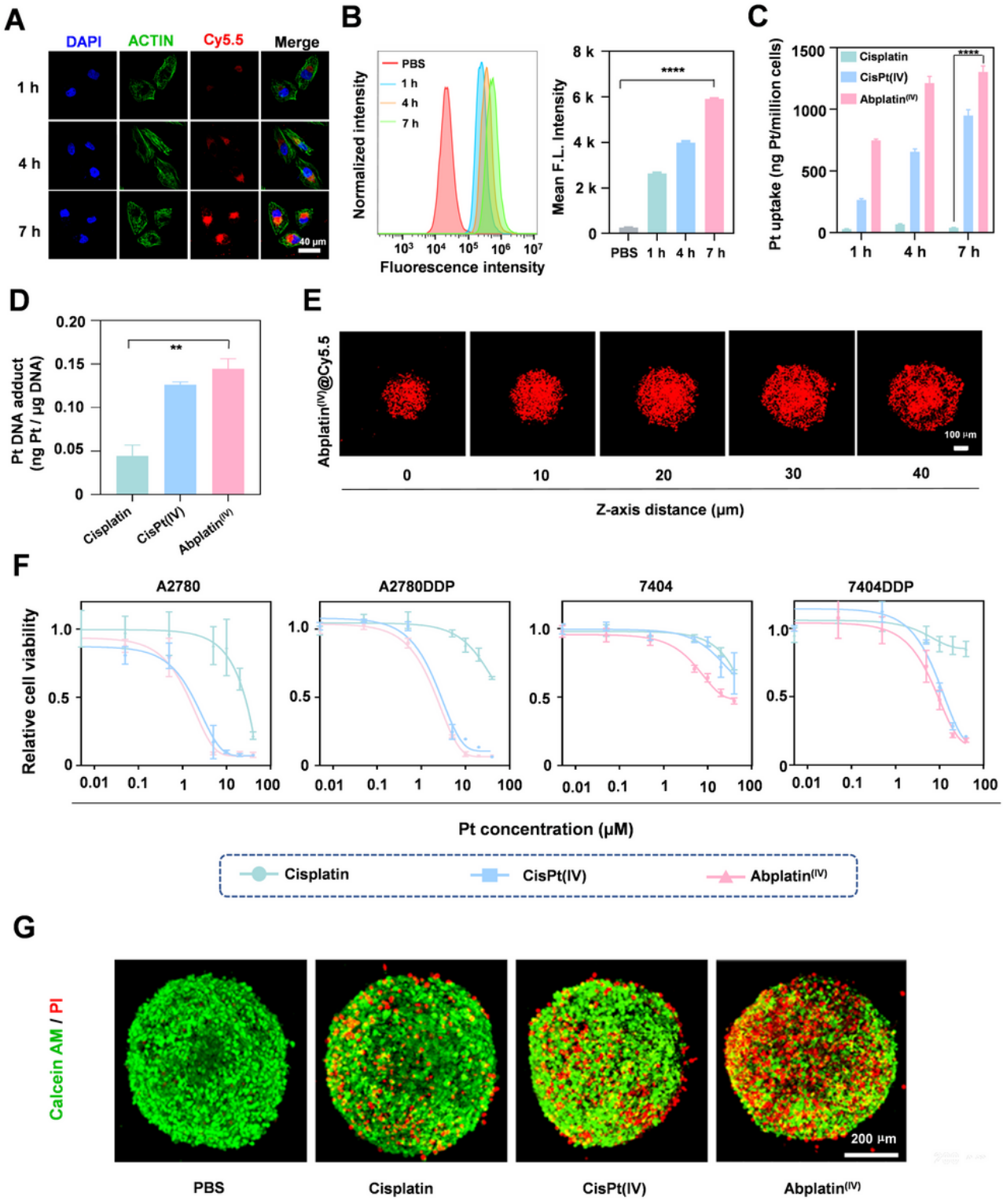

Figure 2

Intracellular uptake and *in vitro* anticancer efficacy of Abplatin$^{(IV)}$. (A) Intracellular uptake of Abplatin$^{(IV)}$ labeled by Cy5.5 by 7404 cells via confocal laser scanning microscope (CLSM). (B) Semi-quantitative

study of the intracellular uptake of Abplatin$^{(IV)}$ labeled by Cy5.5 via flow cytometry. (C) Intracellular Pt uptake of the cells treated with various drugs (10 μM Pt) for 1, 4, 7 h respectively via ICP-MS. (D) Pt-DNA adducts of cells treated with cisplatin, CisPt(IV) and Abplatin$^{(IV)}$. (E) Fluorescence distribution of Abplatin$^{(IV)}$ labeled by Cy5.5 at 12 h in different focal planes of 3D tumor spheres. (F) *In vitro* anticancer activity of cisplatin, CisPt(IV) and Abplatin$^{(IV)}$. (G) Cell apoptosis induced by various drugs (10 μM) in 3D tumor spheres at 24 h.

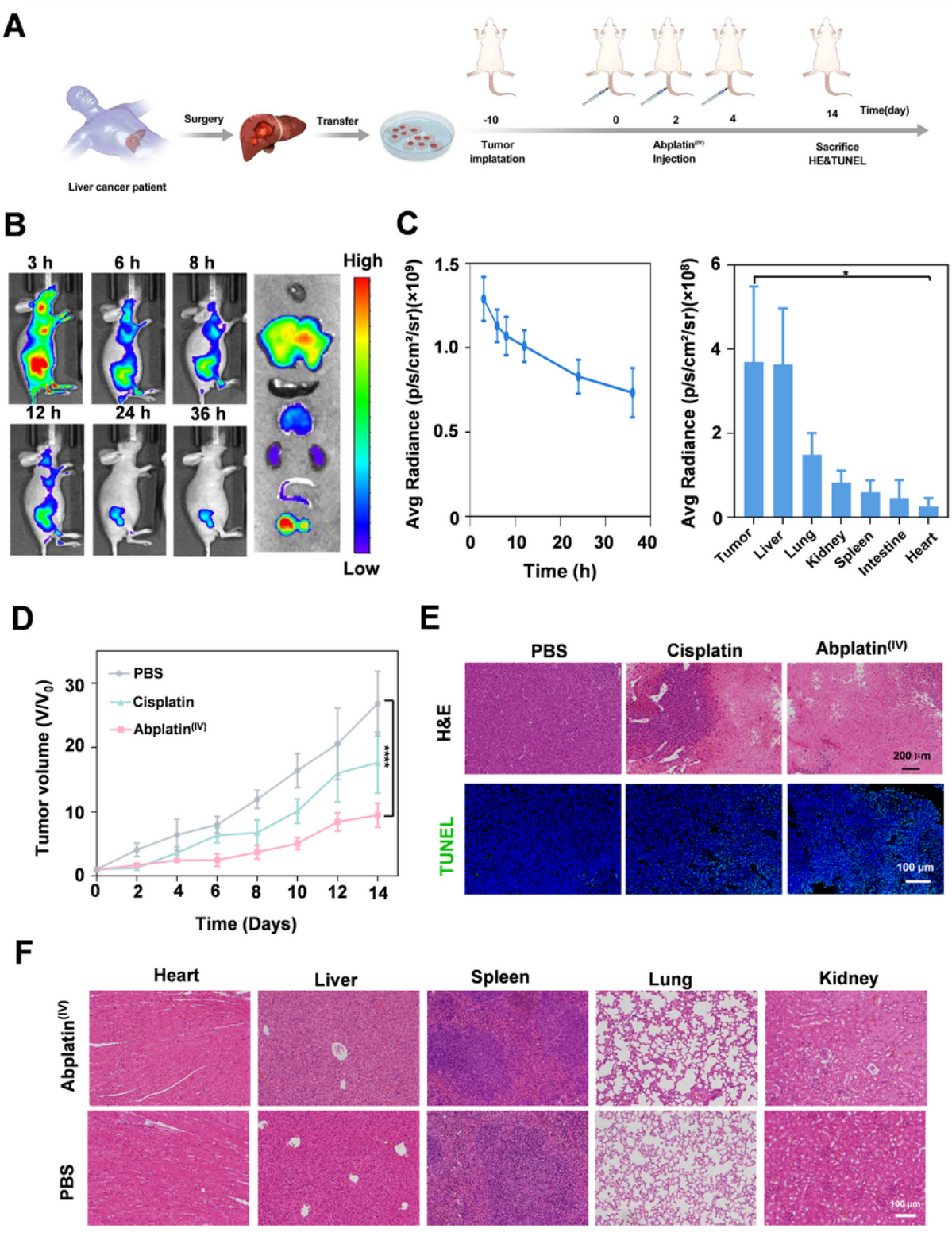

Figure 3

Abplatin$^{(IV)}$ inhibited tumor growth on a PDX^{HCC} model. (A) Establishment of a PDX^{HCC} model for *in vivo* therapeutic studies. (B) *In vivo* biodistribution of Abplatin$^{(IV)}$ labeled with Cy7.5 via *in vivo* imaging and *ex vivo* imaging of major organs at 36 h. (C) *In vivo* imaging for semi-quantification of the biodistribution of Abplatin$^{(IV)}$ over time and *ex vivo* imaging for semi-quantification of biodistribution in major organs after 36 h. (D) Tumor growth inhibition curves of mice treated with cisplatin and Abplatin$^{(IV)}$ at 3.5 mg Pt/kg body weight. (E) H&E staining and TUNEL of representative tumor tissues. (F) H&E staining of heart, liver, spleen, lung and kidney of mice treated by PBS and Abplatin$^{(IV)}$ at 3.5 mg Pt/kg body weight.

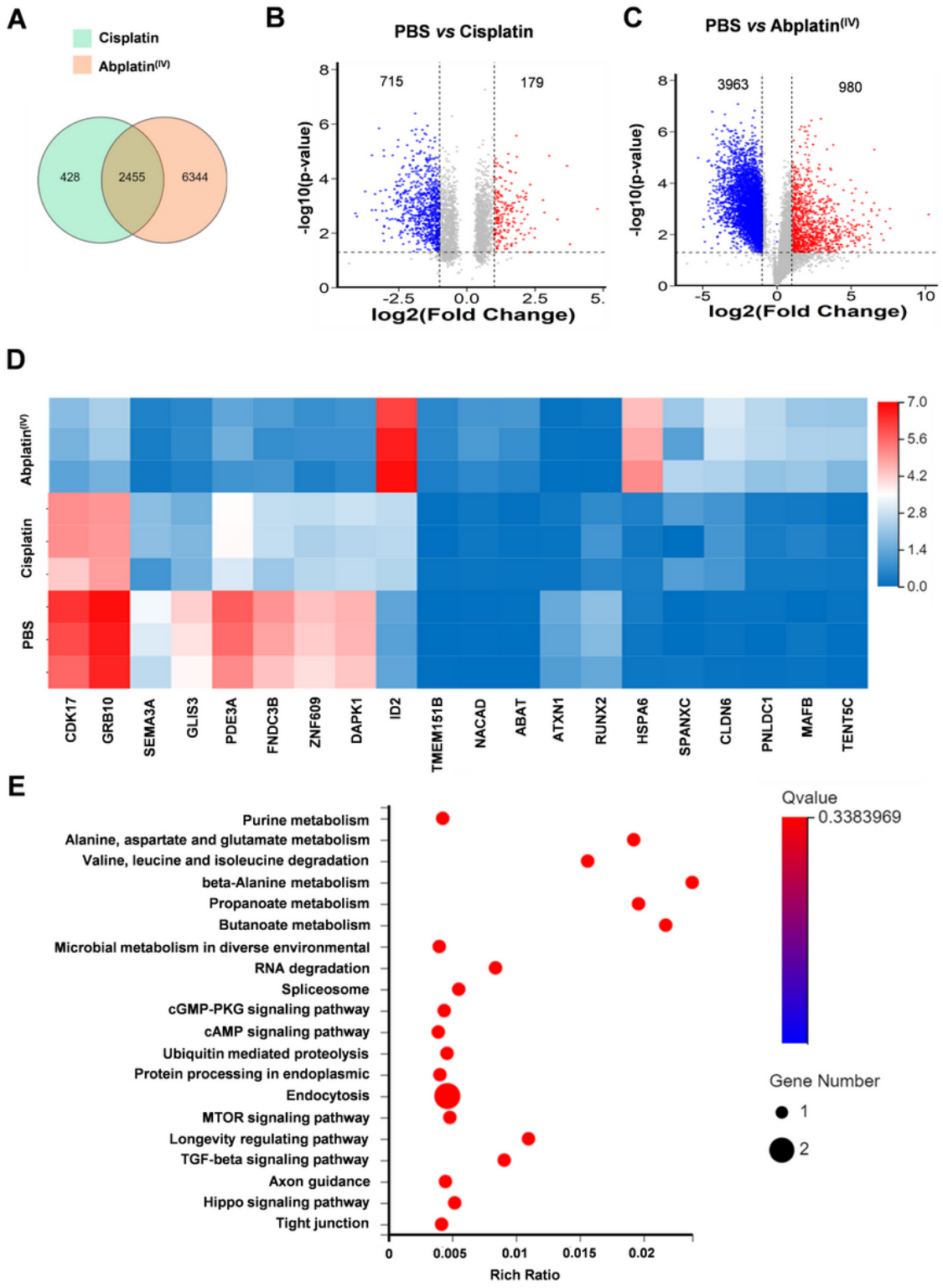

Figure 4

Transcriptomic analysis of BEL-7404 cells treated with cisplatin（CisPt(IV) and Abplatin$^{(IV)}$ by RNA-seq. (A) A Venn diagram revealed the number of genes transcribed in each treatment group. (B-C) Volcano plots displayed the differentially expressed genes. (D) A heat map showed the significant differential transcription genes. (E) Kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment analysis based on differential genes.

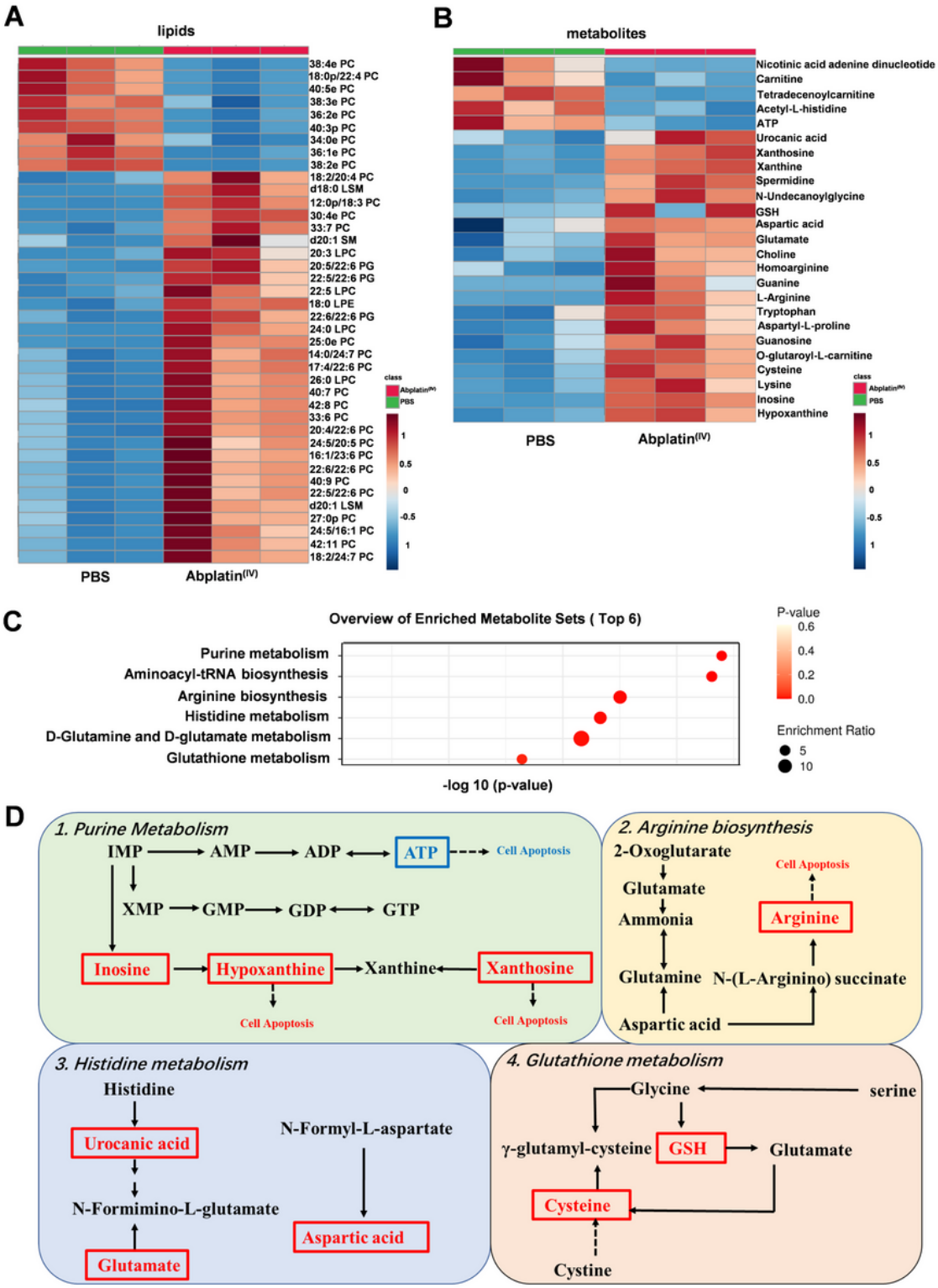

Figure 5

Lipidomic and metabolomic study on BEL-7404 cells treated with Abplatin$^{(IV)}$. (A) Differential lipids between PBS and Abplatin$^{(IV)}$-treated cells. (B) Differential metabolites between PBS and Abplatin$^{(IV)}$-treated cells. (C) Metabolite sets enrichment overview of differential metabolites in the cells treated with Abplatin$^{(IV)}$ and PBS. (D) Abplatin$^{(IV)}$ disturbed the purine metabolism, arginine biosynthesis, histidine

metabolism and glutathione metabolism in BEL-7404 cells. Up-regulated and down-regulated metabolites were represented in red and blue, respectively.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Scheme1.png
- 211227lixingsupplementarymaterialforAbplatinIV.docx