Cell J
Cell Journal(Yakhteh)
Homepage: https://www.celljournal.org/
Original Article
Vol 25, No 2, February 2023, Pages: 118-125

High Expression of G9a Induces Cisplatin Resistance in Hepatocellular Carcinoma

Junhao Fu, M.Sc.[1#], Min Yu, M.Sc.[2#], Wenxia Xu, Ph.D.[1], Shian Yu, M.D.[2*]

1. Central Laboratory, Affiliated Jinhua Hospital, Zhejiang University School of Medicine, Jinhua, Zhejiang Province, China
2. Department of Hepatobiliary and Pancreatic Surgery, Affiliated Jinhua Hospital, Zhejiang University School of Medicine, Jinhua, Zhejiang Province, China

Abstract

Objective: Chemotherapeutic drug resistance is the main obstacle that affects the efficacy of current therapies of hepatocellular carcinoma (HCC), which needs to be addressed urgently. High expression of histone methyltransferase G9a was reported to play a pivotal role in the progression of HCC. Regulatory mechanism of aberrant activation of G9a in HCC and the association with subsequent cisplatin (DDP) resistance still remains ambiguous. This study strived to investigate mechanism of G9a overexpression and its impact on cisplatin resistance in HCC cells.

Materials and Methods: In this experimental study, we investigated effects of different concentrations of cisplatin in combination with BIX-01294 or PR-619 on viability and apoptosis of HuH7 and SNU387 cells via CCK-8 kit and flow cytometric analysis, respectively. Colony formation capacity was applied to evaluate effect of cisplatin with or without BIX-01294 on cell proliferation, and western blotting was used to verify expression level of the related proteins. Global mRNA expression profile analysis was adopted to identify differentially expressed genes associated with overexpression of G9a.

Results: We observed that overexpression of G9a admittedly promoted cisplatin resistance in HCC cells. Global mRNA expression profile analysis after G9a inhibition showed that DNA repair and cell cycle progression were down-regulated. Moreover, we identified that deubiquitination enzymes (DUBs) stabilized high expression of G9a in HCC through deubiquitination. Additionally, cisplatin could significantly inhibit proliferation of DUBs-deficient HCC cells, while promoting their apoptosis.

Conclusion: Collectively, our data indicated that DUBs stabilize G9a through deubiquitination, thereby participating in the cisplatin resistance of HCC cells. The elucidation of this mechanism contributes to propose a potential alternative intervention strategy for the treatment of HCC patients harboring high G9a levels.

Keywords: Cisplatin, Deubiquitinating Enzymes, G9a, Hepatocellular Carcinoma, Resistance

Citation: **Fu J, Yu M, Xu WX, Yu Sh. High expression of G9a induces cisplatin resistance in hepatocellular carcinoma. Cell J. 2023; 25(2): 118-125. doi: 10.22074/cellj.2022.557564.1077.**

Introduction

Cancer, as the second leading cause of death in the world, is a serious threat to human health (1). Globally, liver cancer has leaped to the third place among deaths caused by cancer (2). Hepatocellular carcinoma (HCC), which accounts for the vast majority of primary liver cancer, has usual risk factors including hepatitis B/C virus (HBV/HCV) epidemic, alcohol abuse, obesity, non-alcoholic fatty liver disease (NAFLD) and so on (3, 4). For patients with early-stage HCC, surgery, radiofrequency ablation, radiotherapy and percutaneous ethanol injection can be applied for treatment (5, 6). Unfortunately, most of the patients have progressed to the intermediate or advanced stages of HCC when diagnosed, so that the optimal window for radical surgical resection is missed. These patients need to receive non-surgical treatment dominated by chemotherapy to shrink tumors or slow-down growth of tumors, thereby improving their survival time (7). Notwithstanding, a majority of patients with HCC will develop therapy resistance after receiving systemic treatment, resulting in their 5-years survival rate as low as 18% (8-11). Consequently, comprehensive understandings of the mechanism(s) of HCC therapy resistance will aid formulate alternative HCC therapeutic strategies to improve quality of life of the patients.

In recent years, epigenetic modification, which refers to the reversible regulation of gene expression while preserving the DNA sequence unchanged, has played an increasingly prominent role in cancer (12-15). Epigenetic changes involve multiple approaches, including histone modification, DNA methylation, chromatin remodeling,

Received: 06/December/2021, Revised: 09/November/2022, Accepted: 09/November/2022
 # These authors contributed equally to this work.
***Corresponding Address: Department of Hepatobiliary and Pancreatic Surgery, Affiliated Jinhua Hospital, Zhejiang University School of Medicine, Jinhua, Zhejiang Province, China**
Email: ysa513@163.com

Royan Institute
Cell Journal (Yakhteh)

etc (16). Among them, although histone methylation has received relatively late attention, it has gradually become a research hotspot in the field of epigenetics at the moment. Histone methylation modification is a process in which the methyl group in S-adenosylmethionine (SAM) is transferred to the lysine or arginine residues of histones. This process is mediated by histone methyltransferases (HMTs) (17). G9a, also known as histone-lysine N-methyltransferase 2 (EHMT2), is a notable HMT belonging to the members of the Suv39h subgroup, containing the Su(var)3-9-Enhancer of zeste-Trithorax (SET) domain (18). G9a was reported to be overexpressed in a variety of cancers, including HCC (19, 20). Overexpression of G9a is considered to be related to the inferior outcomes of patients with HCC (21). Nonetheless, the mechanism by which G9a is aberrantly expressed in HCC is vague.

Ubiquitination is one of the most extensive post-translational modifications (PTMs), which plays a significant role in regulating stability, activity and localization of proteins (22, 23). Ubiquitination of the target protein is reversed by deubiquitinating enzymes (DUBs), which stabilizes the protein by removing the ubiquitin chain in the protein, thereby preventing it from degradation by proteasome (24). Accordingly, it is reasonable to presume that DUBs may stabilize expression of *G9a* through deubiquitination, although the mechanism remains unclear.

Moreover, G9a plays an important role in the chemotherapy resistance of cancer. For instance, overexpression of G9a can promote the chemotherapy resistance of head and neck squamous cell carcinoma and glioblastoma (25, 26). Yet, little regard has been paid to the impact of G9a on chemotherapy resistance to HCC. Therefore, diverse mechanisms still need to be probed to overcome the obstacles of chemotherapy resistance in HCC patients.

In this study, we showed that overexpression of G9a promotes cisplatin (DDP) resistance in HCC. Consistent with these observations, global mRNA expression profile analysis revealed that inhibiting the activity of G9a can significantly affect enrichment of resistance-related pathways. Furthermore, we found that deubiquitinating enzymes (DUBs) are responsible for overexpression of G9a in HCC. In general, our results suggested that de-stabilizing G9a expression, by targeting deubiquitinating enzymes, may be a potential therapeutic strategy for HCC.

Materials and Methods

All experimental protocols were approved by the Medical Ethics Committee of Jinhua Hospital of Zhejiang University, Jinhua, China (2021-ethical review-319).

Cell culture

Human liver cancer cell lines HuH7 and SNU387 were purchased from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). HuH7 and SNU387 cells were maintained in DMEM (12100-061; Thermofisher, USA) supplemented with 10% fetal bovine serum (11011-8611; Every Green, China), 100 μg/ml streptomycin (GNM15140; Genome, China) and 100 U/ml penicillin (GNM15140; Genome, China). All cells were maintained at 37˚C in a humidified incubator with 5% CO_2, to perform the experiments.

Cell viability assay

For Cell Viability assay, HuH7 and SNU387 cells (5×10^3) were seeded onto 96-well plates. After 24 hours, the cells were treated with DDP (MCE, China) at the indicated concentrations with or without BIX-01294 (HY-10587; MCE) or PR-619 (HY-13814; MCE) and cultured for another 24 hours. After discarding supernatant, the cell viability was detected by the CCK-8 kit (C0042; Beyotime Biotechnology, China) according to the manual. Optical density values, which reflect the viability of cells, were measured at 450 nm, using a Microplate Reader (Synergy HTZX-22; Bio-Tek Instruments, USA).

Cell apoptosis assay

HuH7 and SNU387 cells were seeded onto 6-well plates at a density of 70-80% and incubated for 24 hours. Then the cells were treated with different combinations of drugs (DDP with or without BIX-01294 and DDP with or without PR-619) at the indicated concentrations for another 48 hours. The harvested floating cells were mixed with trypsinized adherent cells and washed twice with PBS after centrifugation. Subsequently, the reaction solution was added following the manufacturer's protocol for the Annexin V-FITC Apoptosis Detection Kit (C1062L; Beyotime Biotechnology, China), and allowed to react at room temperature for 15 minutes (protected from light). Apoptosis rate was measured using a Flow cytometer (EasyCell 204A1/206A1; Wellgrow, China) according to the operating manual.

Colony formation assay

HuH7 and SNU387 cells were grown in 6-well plates with 600 cells per well. After 24 hours, the cells were treated with DDP, BIX-01294 or combination of DDP and BIX-01294 for 14 days. Subsequently, the cells were washed with PBS and fixed with 4% paraformaldehyde for 20 minutes. They were washed again with PBS, and stained with crystal violet for 20 minutes. The whole process was performed at room temperature. Finally, after washing with PBS, the well plates were air-dried and analyzed for colony formation.

Westerns blot analysis

After treatment, the cells were lysed with RIPA lysis buffer containing 1% PMSF at the indicated time, and total cell protein was extracted. The lysates were then separated via sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels (SDS-PAGE) at the appropriate concentration and transferred to poly vinylidene fluoride

(PDVF) membranes. After blocking with 5% (m/v) skim milk in TBST (tris-buffered saline containing 0.1% Tween-20) for 1-2 hours at room temperature, the membranes were flushed with TBST and subsequently incubated with primary antibodies at 4˚C overnight. The next day, the utilized membranes in the previous step were incubated with the corresponding peroxidase-labeled secondary antibodies for 1-2 hour(s). Immunoreactivity was performed using enhanced chemiluminescence (ECL) reagents (FD800; Fdbio Science, China) and visualized on Bio-rad Chemi Doc XRS (USA). The primary antibodies used are as follows: Rabbit Cleaved PARP (#5625T), rabbit anti-H3 (#4499) and anti-H3K9me2 (#4658) was provided from Cell Signaling Technology (USA). Mouse anti-GAPDH (AG019) antibody was obtained from Beyotime Biotechnology (Shanghai, China) and Mouse anti-G9a (66689-1-Ig) antibody was purchased from proteintech. GAPDH was used as the internal control.

RNA-sequencing analysis

Total RNA was extracted by TRIZOL Reagent (15596026, Ambion, USA) and RNA sequencing was performed by KAITAI-BIO (Hangzhou, China). Briefly, HuH7 cells were divided into two groups: normal control group (NC) and BIX-01294-treated group (BIX). After 24 hours of treatment, the cells were harvested and subjected to RNA sequencing. Quality control qualified RNA samples were applied to construct RNA-seq libraries through Illumina TruSeq RNA Sample Preparation Kit (RS-122-2001; Illumina, USA), sequenced and mapped to human genome assembly, version hg19 (GRCh37.75), followed by grabbing reads with <5% mismatches to further analysis, as previously described (27). We used FPKM to measure gene transcripts, followed by counting and annotating differentially expressed genes, which were identified by DEGSeq, for their detailed descriptions, as previously discussed (28, 29).

Statistical analysis

Each experiment was performed at least three times. All data were expressed as mean ± SD. Differences were determined by the parametric unpaired Student's t test between two groups via GraphPad Prism software (GraphPad Software, USA) and statistically significant difference was defined as $P<0.05$.

Results

Inhibition of G9a promotes sensitivity to cisplatin in HCC cells

In order to prove association between G9a and HCC, we started to analyze public tumor databases. The analysis results of the two tumor databases of UALCAN and GEPIA2 showed that with the continuous progress of HCC, expression level of G9a was gradually increased, and high expression of G9a was correlated with poor survival, which echoed previous conclusions (Fig.S1, See Supplementary Online Information at www.celljournal.org) (20, 21, 30). Subsequently, we treated HuH7 and SNU387 liver cancer cells with BIX-01294, as an inhibitor of G9a, combined with different concentrations of cisplatin, to verify effect of G9a on cisplatin resistance in HCC. Cell viability results showed that inhibition of G9a increased sensitivity of liver cancer cells to cisplatin (Fig.1A, B). The results of Western blot analysis utilized to confirm inhibition of G9a showed that treatment of HuH7 and SNU387 cells with BIX-01294 effectively reduced H3K9me2 level without affecting G9a (Fig.S2, See Supplementary Online Information at www.celljournal.org). Cell proliferation assessed by colony formation assay showed similar results. So that cisplatin exerted a stronger inhibitory effect on cell proliferation in those BIX-01294-treated cells (Fig.1C, D). Next, we examined apoptotic response of HCC cells to cisplatin with/without BIX-01294 treatment. The results showed that cisplatin significantly promoted cell apoptosis with BIX-01294 treatment (Fig. 1E, F). In general, the above results reminded us that G9a may be involved in the cisplatin resistance process of HCC, and inhibition of G9a antagonizes the cisplatin resistance of HCC cells.

Analysis of the global mRNA expression profiles after inhibiting G9a to promote HCC cisplatin sensitivity

Then, we performed an analysis of the global mRNA expression profile to comprehensively understand potential molecular mechanism of G9a in promoting HCC cisplatin resistance. Briefly, HuH7 cells were divided into two groups: normal control group (NC) and BIX-01294-treated group (BIX). At 24 hours post-treatment, the cells were collected and subjected to RNA sequencing.

Initially, heat maps were utilized to visually portray the global expression changes and clustering relationships of multiple genes between the two groups (NC and BIX) through hierarchical clustering analysis (Fig.2A). Upon succeeding, the volcano plot was adopted to visualize the integral gene expression alterations. The results revealed that there were a total of 426 gene expression alterations in the BIX group, involving 339 up-regulated and 87 down-regulated genes, compared to the NC group (Fig.2B). Due to the cascade amplification effect of intracellular signals, pathway enrichment analysis frequently fails to capture slightly changed signal molecules, which can be well addressed by Gene Set Enrichment Analysis (GSEA). Consequently, we discovered that processes such as DNA replication, cell cycle, mismatch repair, nucleotide excision repair and base excision repair were inhibited in the BIX group by GSEA analysis (Fig.2C-G). The above results inferred that inhibition of proliferation of G9a-deficient cells by cisplatin may be succeeded by preventing cell cycle progression and promotion of apoptosis may be performed by arresting the DNA repair signaling pathway.

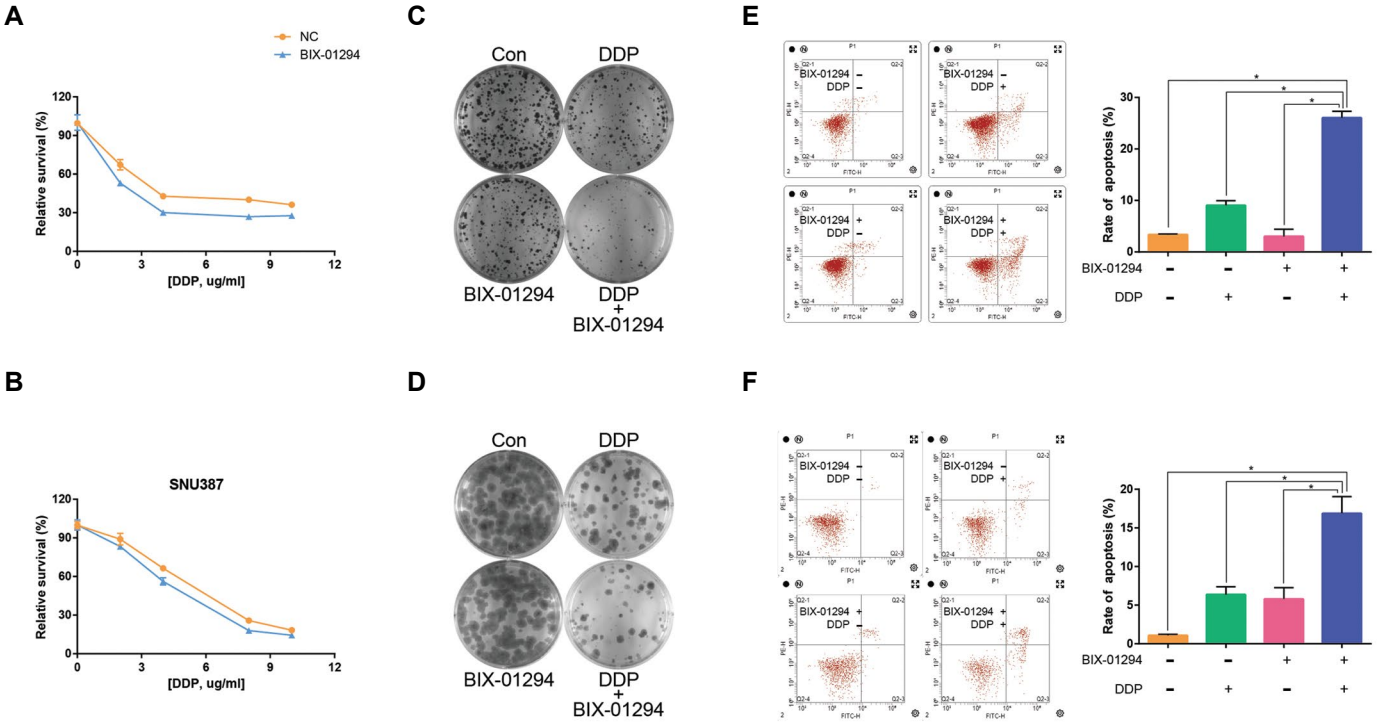

Fig.1: Inhibition of G9a promotes sensitivity to cisplatin in HCC cells. **A.** HuH7 cells were treated with different concentrations of cisplatin with or without BIX-01294 and cell viability was measured at 24 hours post-treatment with the CCK-8 kit. **B.** SNU387 cells were treated with different concentrations of cisplatin with or without BIX-01294 and cell viability was measured at 24 hours post-treatment with the CCK-8 kit. **C.** Colony formation capacity were conducted with HuH7 cells treated with DDP, BIX-01294 or combination of DDP and BIX-01294 for 14 days. **D.** Colony formation capacity were conducted with SNU387 cells treated with DDP, BIX-01294 or combination of DDP and BIX-01294 for 14 days. **E.** Flow cytometry was performed to assess rate of apoptosis in HuH7 cells with DDP (6 µg/ml), BIX-01294 (2 µM) or combination of DDP and BIX-01294 for 48 hours. Error bars represent SD (n=3). *; P<0.05. Specifically, P values from left to right are 6.68×10^{-006}, 4.83×10^{-005}, and 3.08×10^{-005}. **F.** Flow cytometry was performed to assess rate of apoptosis in SNU387 cells with DDP (6 µg/ml), BIX-01294 (2 µM) or combination of DDP and BIX-01294 for 48 hours. Error bars represent SD (n=3). *; P<0.05. Specifically, P values from left to right are 2.33×10^{-004}, 1.63×10^{-003}, and 1.87×10^{-003}. HCC; Hepatocellular carcinoma and DDP; Cisplatin.

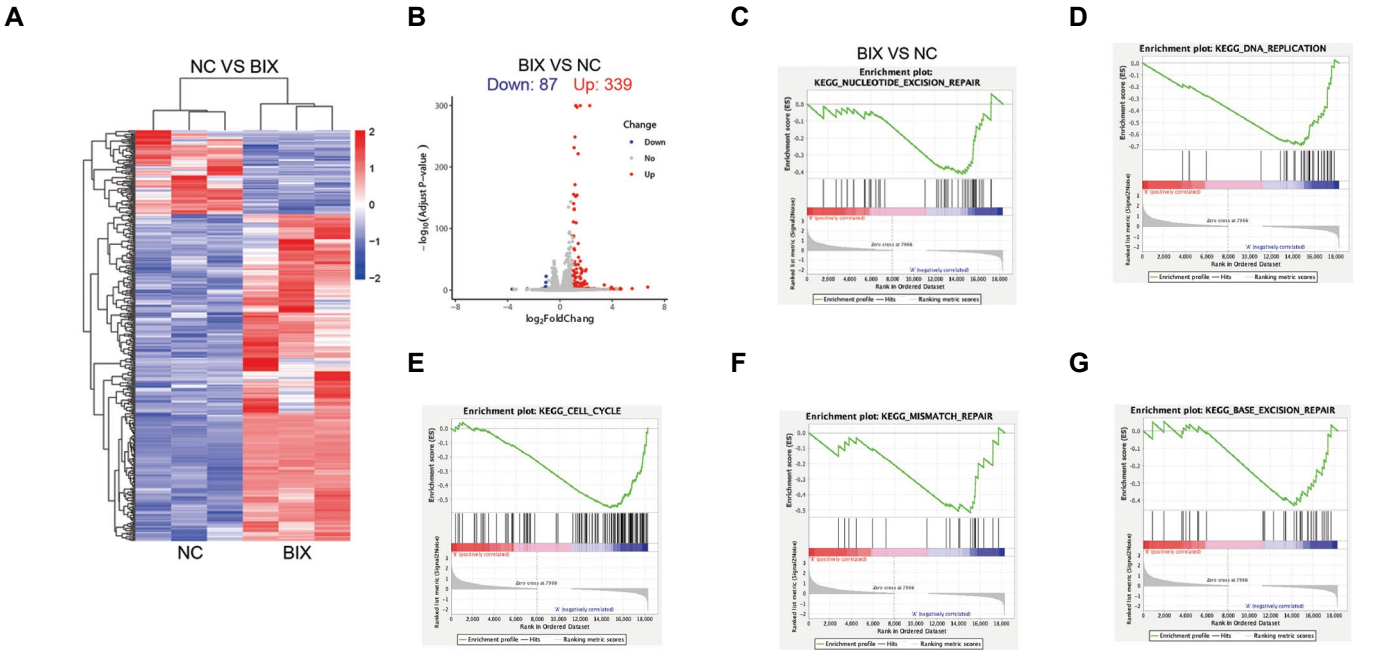

Fig.2: Analysis of the global mRNA expression profiles of inhibiting G9a to promote HCC cisplatin sensitivity. **A.** Heat map representation of global transcription profile variations in the BIX group, compared to the NC group. **B.** Volcano plot display of gene expression alterations in the BIX group, compared to the NC group. Up-regulated genes are indicated by red dots, and down-regulated genes are indicated by green dots. P<0.05 and |log FC|>1 are applied as criteria to characterize differentially expressed genes. **C-G.** GSEA analysis of differential genes between the NC and BIX groups to identify relevant signal pathways. HCC; Hepatocellular carcinoma and NC; Normal control group.

DUBs stabilizes G9a through deubiquitinase activity

Since overexpression of G9a is involved in the cisplatin resistance of HCC, what is the potential mechanism of overexpression of G9a in HCC? DUBs, which can stabilize protein expression, were suggested as a possible mechanism.

To verify our hypothesis, we treated HuH7 cells with PR-619 (broad-spectrum deubiquitinase inhibitor) and Degrasyn (selective deubiquitinase inhibitor, HY-13264; MCE), followed by observing expression of G9a (Fig.S3A, B, See Supplementary Online Information at www.celljournal.org). As expected, inhibition of DUBs with PR-619, but not Degrasyn, could significantly inhibit expression of G9a indicating that DUBs positively regulate stability of G9a (Fig.S3C, See Supplementary Online Information at www.celljournal.org). This finding was further corroborated by treating two liver cancer cell lines with PR-619 at different concentration gradients and time gradients: PR-619 reduced protein abundance of G9a in a dose-dependent and time-dependent manner (Fig.3A, B). Next, we treated the cells with cycloheximide (CHX, HY-12320, MCE, China), a universal inhibitor of protein synthesis, with or without PR-619. The half-life results showed that protein level of G9a was declined faster in DUBs-deficient cells (Fig.3C, D). To further validate whether the ubiquitin-proteasome pathway is involved in the process of DUBs regulating stability of G9a, we treated DUBs-deficient cells with the proteasome inhibitor, MG132 (HY-13259; MCE). Results showed that we successfully restored the G9a protein abundance which was previously reduced by DUBs defects (Fig.3E, F). In short, these results indicated that DUBs stabilized G9a through deubiquitination enzyme activity.

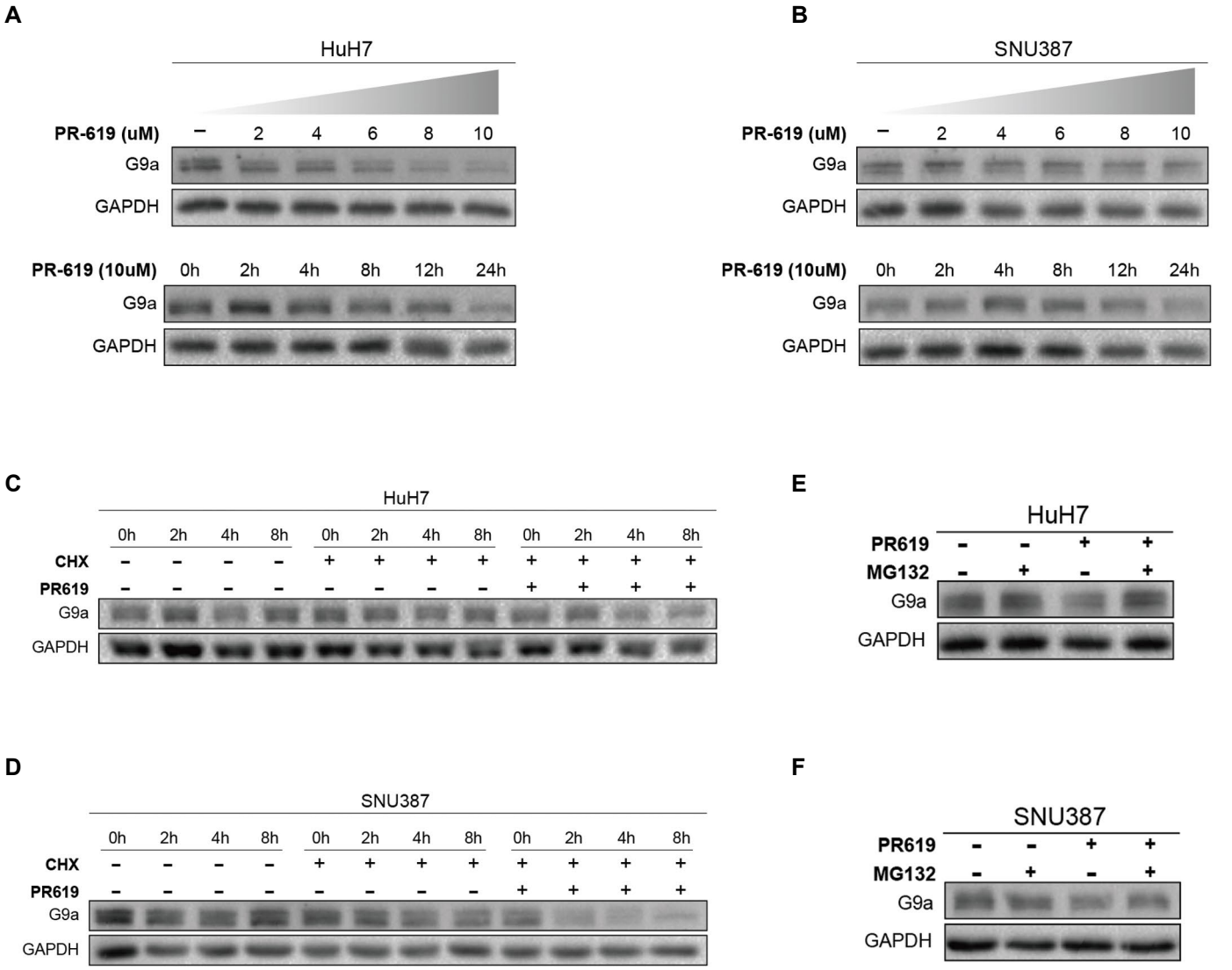

Fig.3: DUBs stabilizes G9a through deubiquitinase activity. **A.** HuH7 cells were treated with PR-619 and the cells were harvested for western blot analysis at the indicated concentration and time. **B.** SNU387 cells were treated with PR-619 and the cells were harvested for western blot analysis at the indicated concentration and time. **C.** HuH7 cells pretreated with or without PR-619 were incubated with or without CHX (50 μg/ml) and harvested for western blotting within the indicated time. **D.** SNU387 cells pretreated with or without PR-619 were incubated with or without CHX (50 μg/ml) and harvested for western blotting within the indicated time. **E.** HuH7 cells pretreated with or without PR-619 were incubated with or without MG132 (25 μM) for 5 hours, followed by western blotting with the indicated antibodies. **F.** SNU387 cells pretreated with or without PR-619 were incubated with or without MG132 (25 μM) for 5 hours, followed by western blotting with the indicated antibodies. h; Hour.

DUBs deletion impairs the cisplatin resistance of HCC cells

Next, we attempted to further confirm influence of DUBs on HCC cisplatin resistance. Upon treating DUBs-deficient cells with cisplatin, cell viability was declined more significantly, compared to the normal cells under the same conditions (Fig.4A, B). We next experimented with the apoptotic response of HCC cells to cisplatin. The results showed that cisplatin could significantly promote HCC cells apoptosis when DUBs were inhibited by PR-619 (Fig.4C, D). Similar results were obtained in the WB analysis; by that means, cisplatin could significantly boost expression of cleaved PARP in DUBs-deficient cells, which serves as a marker of cells undergoing apoptosis. It is worth noting that after treating DUBs-deficient cells with cisplatin, we further reduced protein abundance of G9a which were previously declined by DUBs depletion (Fig.4E, F). Taken together, high expression of DUBs may promote cisplatin resistance in HCC by stabilizing G9a.

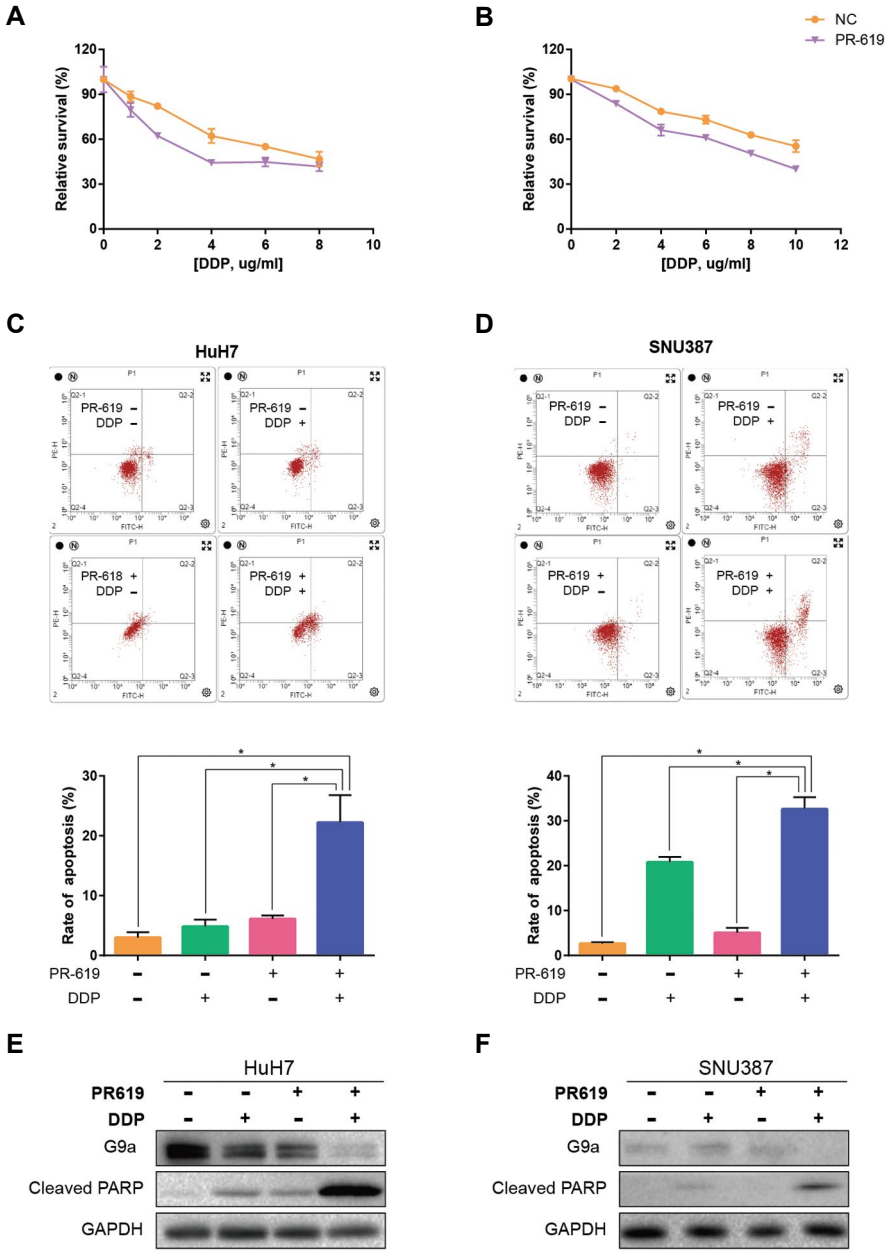

Fig.4: DUBs deletion impairs cisplatin resistance of HCC cells. **A.** HuH7 cells were treated with cisplatin (0, 1, 2, 4, 6, 8 μg/ml) with or without PR-619 and cell viability was measured at 24 hours post-treatment with the CCK-8 kit. **B.** SNU387 cells were treated with cisplatin (0, 2, 4, 6, 8, 10 μg/ml) with or without PR-619 and cell viability was measured at 24 hours post-treatment with the CCK-8 kit. **C.** Flow cytometry was performed to assess rate of apoptosis in HuH7 cells with DDP (4 μg/ml), PR-619 (6 μM) or the combination of DDP and BIX-01294 for 48 hours. Error bars represent SD (n = 3). *; P<0.05. Specifically, the P values from left to right are 2.03×10^{-003}, 3.09×10^{-003}, and 3.77×10^{-003}. **D.** Flow cytometry was performed to assess rate of apoptosis in SNU387 cells with DDP (10 μg/ml), PR-619 (12 μM) or combination of DDP and BIX-01294 for 48 hours. Error bars represent SD (n=3). *; P<0.05. Specifically, the P values from left to right are 3.99×10^{-005}, 2.05×10^{-003}, and 7.48×10^{-005}. **E.** Western blot analysis of Cleaved-PARP and G9a in HuH7 cells with treatment of DDP (4 μg/ml), PR-619 (6 μM) or combination of DDP and BIX-01294 for 48 hours. **F.** Western blot analysis of Cleaved-PARP and G9a in SNU387 cells with treatment of DDP (10 μg/ml), PR-619 (12 μM) or combination of DDP and BIX-01294 for 48 hours. HCC; Hepatocellular carcinoma and DDP; Cisplatin.

Discussion

Here, we showed that inhibition of G9a can promote sensitivity of HCC cells to cisplatin. Additionally, global mRNA expression profile analysis revealed that DNA repair and cell cycle progression were down-regulated in G9a-inhibited cells. Moreover, targeting DUBs with PR-619 could suppress expression of G9a and could significantly impair the resistance of HCC cells to cisplatin.

In the past few decades, despite many advances in diagnosis and treatment of HCC, prognosis of HCC patients is still poor, due to the lack of proper intervention targets which is also a pivotal challenge for treatment of HCC. Given the prevailing function of G9a overexpression in a variety of cancers, including HCC, identifying its upstream regulatory signals has emerged as an attractive strategy for treatment of HCC. Previous studies have found that G9a could enhance proliferation and migration of HCC cells, though paying little attention to the effects of G9a on HCC cisplatin resistance (20, 31, 32). In this study, we noticed that inhibition of G9a with BIX-01294 could attenuate cisplatin resistance of HCC cells. Simply put, cisplatin could significantly inhibit proliferation of G9a-deficient cells while further promoting their apoptosis, compared to the normal HCC cells. To investigate potential molecular mechanism of G9a inhibition to promote HCC cisplatin sensitivity, we conducted a series of evaluations through RNA-Seq. We identified 339 up-regulated and 87 down-regulated genes after inhibiting G9a with BIX-01294. In particular, we discerned that cell cycle and DNA repair processes of the BIX group was down-regulated, indicating that inhibition of G9a with BIX-01294 may enhance sensitivity of HCC cells to cisplatin by arresting cell cycle and DNA repair signaling pathways.

The ubiquitin-proteasome system (UPS) exerts a profound impact on initiation and progression of HCC. UPS regulates abundance and activity of proteins by adjusting the balance between ubiquitination and deubiquitination. As the reverse process of ubiquitination, deubiquitination process is mediated by DUBs; a hot research topic in recent years has been proven to provide a reliable alternative target for addressing the non-targetability properties of their substrates (33). DUBs positively regulate expression level of target proteins by erasing ubiquitin chain on them, which may be one of the explanations for overexpression of G9a in HCC. Our experimental results indeed confirmed this. Specifically, upon treating HuH7 with PR-619, as a broad-spectrum deubiquitinase inhibitor, we reduced protein level of G9a in concentration-dependent and time-dependent manners. Subsequent half-life tests showed that G9a protein levels was declined faster in DUBs-deficient cells. After treating DUB-deficient cells with proteasome inhibitor MG132, we restored G9a protein abundance that was previously consumed by DUBs inhibition. Similar results were also observed in another liver cancer cell line SNU387. The above results suggested that DUBs participated in overexpression of G9a in HCC via deubiquitination.

Previous studies have well proved that the imbalance between ubiquitination and deubiquitination, caused by the dysfunction of DUBs, played an essential role in a variety of cancers, including HCC. For instance, USP10 depletion was reported to inhibit proliferation of HCC by impairing the YAP/TAZ pathway (34). USP1 can drive growth and metastasis of HCC cells by stabilizing RPS16 (35). Moreover, inhibition of USP9X with WP1130 increased sensitivity of HCC cells to doxorubicin through destabilization of p53 (36). A recent study has also characterized that USP29-mediated HIF1α stabilization promoted sorafenib resistance in HCC cells, partly through up-regulation of glycolysis (37). Although so many struggles have been made to explore the effect of DUBs on HCC, their role in cisplatin resistance of HCC remains indistinct. Here in this study, we appraised the impact of global-level DUBs deficiency on cisplatin resistance in HCC cells. Similarly, colony formation assay and apoptosis analysis both convinced that PR-619 combined treatment sensitized HCC cells to cisplatin. We also discerned that after treating DUBs-deficient cells with cisplatin, protein abundance of G9a was reduced most significantly, hinting that DUBs depletion may increase cisplatin sensitivity in HCC cells, through degradation of G9a.

Conclusion

In summary, our study discovered that high expression of G9a is associated with the cisplatin resistance of HCC. Furthermore, the inhibition of DUBs with the broad-spectrum deubiquitination enzyme inhibitor PR-619 promoted sensitivity of HCC cells to cisplatin via destabilization of G9a. Therefore, targeting DUBs, instead of G9a, is a potential alternative strategy for treatment of HCC.

Acknowledgments

This work was supported by the Major Projects of Jinhua Science and Technology Plan Project, Jinhua, China (No. 2018-3-001a), Special Research Fund for Basic Research of Jinhua Central Hospital, Jinhua, China (JY2020-6-11), Jinhua Science and Technology Research Program, Jinhua, China (2021-3-098), Zhejiang Medical and Health Science and Technology Project, Hangzhou, China (2023KY381), and Key discipline of clinical laboratory medicine of Jinhua City, Jinhua, China (JYZDXK-2019-13). We declare that we have no conflict of interest in this work.

Authors' Contributions

J.H.F., M.Y.; Were involved in the experiments, data analysis, and participated in the writing the original draft. W.X.X., S.A.Y.; Were responsible for the research design and critical revision of the manuscript. S.A.Y.; Approved the final version of manuscript to submit. All authors read

and approved the final manuscript.

References

1. Vivarelli S, Salemi R, Candido S, Falzone L, Santagati M, Stefani S, et al. Gut microbiota and cancer: from pathogenesis to therapy. Cancers (Basel). 2019; 11(1): 38.
2. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021; 71(3): 209-249.
3. Cancer Genome Atlas Research Network. Electronic address: wheeler@bcm.edu; Cancer Genome Atlas Research Network. Comprehensive and integrative genomic characterization of hepatocellular carcinoma. Cell. 2017; 169(7): 1327-1341. e23.
4. Shokoohian B, Negahdari B, Aboulkheyr EsH, Abedi-Valugerdi M, Baghaei K, Agarwal T, et al. Advanced therapeutic modalities in hepatocellular carcinoma: novel insights. J Cell Mol Med. 2021; 25(18): 8602-8614.
5. Chen Z, Xie H, Hu M, Huang T, Hu Y, Sang N, et al. Recent progress in treatment of hepatocellular carcinoma. Am J Cancer Res. 2020; 10(9): 2993-3036.
6. Petrowsky H, Fritsch R, Guckenberger M, De Oliveira ML, Dutkowski P, Clavien PA. Modern therapeutic approaches for the treatment of malignant liver tumours. Nat Rev Gastroenterol Hepatol. 2020; 17(12): 755-772.
7. Heimbach JK, Kulik LM, Finn RS, Sirlin CB, Abecassis MM, Roberts LR, et al. AASLD guidelines for the treatment of hepatocellular carcinoma. Hepatology. 2018; 67(1): 358-380.
8. Chino F, Stephens SJ, Choi SS, Marin D, Kim CY, Morse MA, et al. The role of external beam radiotherapy in the treatment of hepatocellular cancer. Cancer. 2018; 124(17): 3476-3489.
9. Fang D, Guo Y, Zhu Z,Chen W. Silence of p15 expression by RNAi enhances cisplatin resistance in hepatocellular carcinoma cells. Bosn J Basic Med Sci. 2012; 12(1): 4-9.
10. Ghosh S. Cisplatin: the first metal based anticancer drug. Bioorg Chem. 2019; 88: 102925.
11. Zhang B, Cao K, Liu Z, Shan W, Wen Q, Wang R. Receptor interacting protein kinase 3 promotes cisplatin-induced necroptosis in apoptosis-resistant HepG2/DDP cells. Neoplasma. 2019; 66(5): 694-703.
12. Margueron R, Reinberg D. Chromatin structure and the inheritance of epigenetic information. Nat Rev Genet. 2010; 11(4): 285-296.
13. Momparler RL. Cancer epigenetics. Oncogene. 2003; 22(42): 6479-6483.
14. Nagaraju GP, Dariya B, Kasa P, Peela S, El-Rayes BF. Epigenetics in hepatocellular carcinoma. Semin Cancer Biol. 2022; 86(Pt 3): 622-632.
15. Shafabakhsh R, Arianfar F, Vosough M, Mirzaei HR, Mahjoubin-Tehran M, Khanbabaei H, et al. Autophagy and gastrointestinal cancers: the behind the scenes role of long non-coding RNAs in initiation, progression, and treatment resistance. Cancer Gene Ther. 2021; 28(12): 1229-1255.
16. Yokoyama M, Chiba T, Zen Y, Oshima M, Kusakabe Y, Noguchi Y, et al. Histone lysine methyltransferase G9a is a novel epigenetic target for the treatment of hepatocellular carcinoma. Oncotarget. 2017; 8(13): 21315-21326.
17. Albert M, Helin K. Histone methyltransferases in cancer. Semin Cell Dev Biol. 2010; 21(2): 209-220.
18. Shinkai Y, Tachibana M. H3K9 methyltransferase G9a and the related molecule GLP. Genes Dev. 2011; 25(8): 781-788.
19. Casciello F, Windloch K, Gannon F, Lee JS. Functional Role of G9a histone methyltransferase in cancer. Front Immunol. 2015; 6: 487.
20. Wei L, Chiu DK, Tsang FH, Law CT, Cheng CL, Au SL, et al. Histone methyltransferase G9a promotes liver cancer development by epigenetic silencing of tumor suppressor gene RARRES3. J Hepatol. 2017; 67(4): 758-769.
21. Bai K, Cao Y, Huang C, Chen J, Zhang X, Jiang Y. Association of histone methyltransferase G9a and overall survival after liver resection of patients with hepatocellular carcinoma with a median observation of 40 months. Medicine (Baltimore). 2016; 95(2): e2493.
22. Swatek KN, Komander D. Ubiquitin modifications. Cell Res. 2016; 26(4): 399-422.
23. Wilkinson KD. Regulation of ubiquitin-dependent processes by deubiquitinating enzymes. FASEB J. 1997; 11(14): 1245-1256.
24. Lv XY, Duan T, Li J. The multiple roles of deubiquitinases in liver cancer. Am J Cancer Res. 2020; 10(6): 1647-1657.
25. Ciechomska IA, Marciniak MP, Jackl J,Kaminska B. Pre-treatment or post-treatment of human glioma cells with BIX01294, the inhibitor of histone methyltransferase G9a, sensitizes cells to temozolomide. Front Pharmacol. 2018; 9: 1271.
26. Liu CW, Hua KT, Li KC, Kao HF, Hong RL, Ko JY, et al. Histone methyltransferase G9a drives chemotherapy resistance by regulating the glutamate-cysteine ligase catalytic subunit in head and neck squamous cell carcinoma. Mol Cancer Ther. 2017; 16(7): 1421-1434.
27. Trapnell C, Pachter L, Salzberg SL. TopHat: discovering splice junctions with RNA-Seq. Bioinformatics. 2009; 25(9): 1105-1111.
28. Trapnell C, Williams BA, Pertea G, Mortazavi A, Kwan G, van Baren MJ, et al. Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. Nat Biotechnol. 2010; 28(5): 511-515.
29. Wang L, Feng Z, Wang X, Wang X, Zhang X. DEGseq: an R package for identifying differentially expressed genes from RNA-seq data. Bioinformatics. 2010; 26(1): 136-138.
30. Nakatsuka T, Tateishi K, Kato H, Fujiwara H, Yamamoto K, Kudo Y, et al. Inhibition of histone methyltransferase G9a attenuates liver cancer initiation by sensitizing DNA-damaged hepatocytes to p53-induced apoptosis. Cell Death Dis. 2021; 12(1): 99.
31. Guo Y, Zhao YR, Liu H, Xin Y, Yu JZ, Zang YJ, et al. EHMT2 promotes the pathogenesis of hepatocellular carcinoma by epigenetically silencing APC expression. Cell Biosci. 2021; 11(1): 152.
32. Hu Y, Zheng Y, Dai M, Wang X, Wu J, Yu B, et al. G9a and histone deacetylases are crucial for Snail2-mediated E-cadherin repression and metastasis in hepatocellular carcinoma. Cancer Sci. 2019; 110(11): 3442-3452.
33. Harrigan JA, Jacq X, Martin NM, Jackson SP. Deubiquitylating enzymes and drug discovery: emerging opportunities. Nat Rev Drug Discov. 2018; 17(1): 57-78.
34. Zhu H, Yan F, Yuan T, Qian M, Zhou T, Dai X, et al. USP10 Promotes proliferation of hepatocellular carcinoma by deubiquitinating and Stabilizing YAP/TAZ. Cancer Res. 2020; 80(11): 2204-2216.
35. Liao Y, Shao Z, Liu Y, Xia X, Deng Y, Yu C, et al. USP1-dependent RPS16 protein stability drives growth and metastasis of human hepatocellular carcinoma cells. J Exp Clin Cancer Res. 2021; 40(1): 201.
36. Liu H, Chen W, Liang C, Chen BW, Zhi X, Zhang S, et al. WP1130 increases doxorubicin sensitivity in hepatocellular carcinoma cells through usp9x-dependent p53 degradation. Cancer Lett. 2015; 361(2): 218-225.
37. Gao R, Buechel D, Kalathur RKR, Morini MF, Coto-Llerena M, Ercan C, et al. USP29-mediated HIF1alpha stabilization is associated with Sorafenib resistance of hepatocellular carcinoma cells by upregulating glycolysis. Oncogenesis. 2021; 10(7): 52.