Gambogenic Acid Inhibits Basal Autophagy of Drug-Resistant Hepatoma Cells and Improves Its Sensitivity to Adriamycin

Meng Wang, a,b Fan Zhan, b,a Hui Cheng, a and Qinglin Li*, a

a Key Laboratory of Xin’an Medicine, Ministry of Education, Anhui Province Key Laboratory of R&D of Traditional Chinese Medicine, Anhui University of Chinese Medicine; Hefei 230038, China; and b Huaihe Maternity & Child Healthcare Hospital; Huaihe 235000, China.

Received June 16, 2021; accepted October 5, 2021

Gambogenic acid (GNA) is extracted from plant Gamboge, has a wide range of anti-tumor effects. In this paper, we study the inhibitory effect of GNA on the BEL-7402/ADM of hepatoma resistant cell lines and further study the mechanism of action. Cell viability test represented that GNA could improve the sensitivity of hepatoma drug-resistant cell line BEL-7402/ADM to Adriamycin (ADM), and further study by 4’,6-diamidino-2-phenylindole (DAPI) staining and flow cytometry found that GNA could improve the effect of ADM on promoting apoptosis in BEL-7402/ADM cells, and the activation of apoptosis-related protein was significantly increased, and the ratio of Bax to Bcl-2 was significantly increased. Monodansylcadaverine staining and transmission electron microscopy showed that the basal autophagy level of BEL-7402/ADM cells was higher than that of BEL-7402 cells. Further detection of protein expression found that the intracellular LC3-II to LC3-I ratio and Beclin 1 protein expression increased in the combination of GNA and ADM, but the protein level of p62 increased significantly. GNA inhibit protective autophagy in BEL-7402/ADM cells and promote the role of ADM in inducing apoptosis, thereby increasing ADM sensitivity to BEL-7402/ADM cells, and the effect of GNA inhibition of autophagy may be achieved by inhibiting the degradation of autophagosomes.

Key words gambogenic acid; autophagy; drug-resistant; Adriamycin (ADM); apoptosis

INTRODUCTION

Hepatic carcinoma (HCC) is the most common digestive tract tumor with high morbidity and mortality.1 At present, chemotherapy is still the main means of liver cancer treatment. In recent years, chemotherapeutic therapy has confronted great challenges. Chemotherapeutic drugs produce multidrug resistance (MDR) in the treatment of liver cancer, which ultimately makes the drug not effective, the prognosis is poor, and the final chemotherapy fails.2 Adriamycin is a first-line drug for the treatment of liver cancer, but its application has been limited by some side effects, especially development of MDR.

P-glycoprotein (P-gp) mediated multidrug resistance is a classical mechanism for tumor cells to produce multidrug resistance, is also the most in-depth and earliest drug resistance mechanism studied at present.3 Clinical studies have also illustrated that high expression of P-gps is closely related to cancer chemotherapy resistance, recurrence and prognosis.4 Thus, reversing multidrug resistance mediated by P-gps will perform a crucial function in the treatment of tumor.5

Recent studies have found that autophagy has become one of the essential mechanisms of tumor resistance and is a potential target for tumor therapy.6 Autophagy is under certain exogenous stimuli. Cells induce the formation of autophagosomes and autolysosomes through a variety of autophagy-related genes that self-digest damaged organelles as well as macromolecular substances to maintain homeostasis.7,8

Many chemotherapeutic drugs can induce autophagy in tumor cells, and there is an inevitable correlation between autophagy and the production of chemotherapeutic resistance in tumor cells.9 The study found that in the middle and late stages of tumor development, tumor cells can adapt to nutritional deficiency by autophagy metabolic stress such as insufficient blood supply and hypoxia to maintain its stable growth and reproduction.10 At this time, autophagy exerts a part in promoting tumor survival and development, that is, protective autophagy.11 In tumor therapy, apoptosis tolerance is one of the main reasons why tumor cells resist chemotherapeutic drugs.12 Autophagy can antagonize chemotherapeutic drug-induced apoptosis and maintain tumor cell survival, thus promoting the formation of tumor resistance.

Gambogenic acid (GNA) is a xanthone found in traditional Chinese medicine Gamboge, which has good anti-tumor effect.13 In previous studies, we found that GNA can resist cancer by regulating multiple programmed cell death patterns such as autophagy and apoptosis, and there was already a variety of evidence that GNA could reverse tumor resistance.14-16 However, the molecular mechanism of GNA regulates autophagy and apoptosis in Adriamycin (ADM) resistant hcc cells has not been elucidated. Therefore, in this study, we investigated the role of GNA in reversing ADM resistance in hcc cells through in vitro experiments and further explored the interaction between autophagy and apoptosis during the reversal.

MATERIALS AND METHODS

Reagents GNA (Fig. 1A) (purity ≥98%) was generously provided by Prof. Wang Xiaoshan’s laboratory as previously studied. Fetal bovine serum (FBS) and RPMI medium 1640 basic was obtained from Gibco (NYC, U.S.A.). 3-[4,5-Dimethyl-
thiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), Adriamycin and 4',6-diamidino-2-phenylindole (DAPI) was bought from Sigma-Aldrich (Shanghai, China). Antibodies P-gp, beclin1, LC3B, p62, Bax, Bcl-2, Caspase-3, Cleaved-caspase-3, β-actin were acquired from Cell Signaling Technology (MA, U.S.A.).

Cell Culture  The cells (BEL-7402/ADM and BEL-7402) which bought from the Cellbio (Shanghai, China), were maintained in RPMI medium 1640 basic containing 10% FBS. all the cells were grown at 37°C with 5% CO2.

**Cell Viability Test**  BEL-7402/ADM and BEL-7402 cells were inoculated on 96-well plates with blank control group and administration group with 6 holes in each group. When the cells adhered well, discard the medium, add the drug according to the group setting, and place 37°C, 5% CO2 incubator. The culture was terminated after each hole was added for 20μL MTT and incubated for 4h to terminate the discarded medium.
The optical density value (OD value) of each pore was determined at nm 490 wavelength by an enzyme labeling instrument after adding dimethyl sulfoxide 150 µL, to oscillate on shaker for 10 min. According to the above process, the cell proliferation inhibition rate was calculated three times repeatedly.

**DAPI Staining** BEL-7402/ADM cells were treated in groups, washed phosphate buffered saline (PBS) cells, centrifuged to remove supernatant, then incubated with DAPI dye solution 2–3 mL, room temperature for 15 min, and then washed again with PBS. After heavy suspension, a drop of cell suspension was added to the slide, covered with cover glass, and observed under fluorescence microscope.

**Annexin V-Fluorescein Isothiocyanate (FITC)/Propidium Iodide (PI) Staining** The BEL-7402/ADM cells were incubated in groups and treated with AnnexinV-FITC/PI kit (BestBio, Shanghai, China). The cells were collected according to the instructions and then detected by FACS Calibur flow cytometry (Becton-Dickinson, NJ, U.S.A.).

**Monodansylcadaverine (MDC) Staining** The BEL-7402/ADM cells were treated in groups according to the experi-

---

**Fig. 2.** GNA Enhanced Effects of ADM on Apoptosis of BEL-7402/ADM Cells

(A) The effects of GNA (0.5, 1.0, 2.0 µM) on the morphological changes in BEL-7402/ADM cells treated with 20 µM ADM for 24 h. (**×200).** The upper panels are morphological features, the bottoms are DAPI staining. (B) The effect of GNA (0.5, 1.0, 2.0 µM) on apoptosis rate of BEL-7402/ADM cells treated with 20 µM ADM for 24 h. (C) The effect of GNA (0.5, 1.0, 2.0 µM) on apoptotic proteins in BEL7402/ADM cells treated with 20 µM ADM for 24 h. (D, E) Relative expression of the cleaved-caspase-3 and Bax/Bcl-2 protein. **p < 0.01 vs. control (X ± S.E., n = 3); ##p < 0.01 vs. the ADM group.
mental requirements. The collected cells were washed PBS twice, cultured in a cell incubator, suspended with 1 mL of PBS, 1 storage solution (concentration 50 mmol/L) was added to the final concentration of 0.05, incubated for half an hour under mat 37, PBS washed three times, and then autophagy morphology was observed under fluorescence microscope.

**Transmission Electron Microscopy (TEM) Observation**

According to the experimental requirements, the BEL-7402/ADM cells were grouped and collected. Glutaraldehyde was slowly added along the tube wall, fixed cells and placed in 4 °C refrigerators. The next day, washed 3 times with PBS and fixed with ursolic acid at 4 °C. Dehydrated overnight and then cured in an oven at 45 °C for 12 h, stained with uranium acetate and lead citrate. Ultrastructure of cells was observed under transmission electron microscopy.

**Western Blot Analysis**

The total protein of each group was extracted, the protein concentration was detected BCAS the kit, and the sample amount of each group was adjusted to 20 µg. After sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) electrophoresis, the protein was transferred to the NC membrane, and the waste seal solution was sealed in 5% skim milk powder seal solution to wash the membrane. Add the corresponding first antibody 4°C incubate overnight, add the second antibody after washing the film, room temperature shaker slowly shakes 2 h, exposure treatment after washing the film. And the gray ratio of the target protein to the inner reference protein (β-actin) band was calculated to determine the relative expression of each histone. The above experiments were repeated three times.

**Statistical Analysis**

The results are represented by mean ± standard deviation (S.D.) calculated from three independent experiments. Student’s t-test was used for the mean comparison between two sets of samples. One-way ANOVA with a tukey post-test was used for multiple comparison.

**RESULTS**

**GNA Increased ADM Sensitivity of BEL-7402/ADM Cells**

Firstly, we found that after 12, 24, 48 h of cells treated with different concentrations of ADM, the IC_{50} value of BEL-7402/ADM cells was higher than that of BEL-7402 cells (Fig. 1B). Then WB results showed that the expression of P-gp protein in BEL-7402/ADM cells was larger than that of BEL-7402 cells (Fig. 1C). Then we used a safe concentration of GNA to BEL-7402/ADM cells for 12, 24, 48 h, the cell viability decreased significantly (Figs. 1D, E). The results showed that GNA could improve the sensitivity of drug-resistant cell BEL-7402/ADM to ADM.

**GNA Enhanced Effects of ADM on Apoptosis of BEL-7402/ADM Cells**

We used 20 µM ADM to treat BEL-7402/ADM cells, after adding different concentrations of GNA, we observed by inverted microscope. As shown in Fig. 2A,

![Fig. 3. BEL-7402/ADM Resistance to ADM by Increasing Basic Autophagy](image)
the upper panels are morphological features, the bottoms are DAPI staining. With the increase of GNA concentration, the cell viability decreased, and the cell morphology crumpled. When the GNA concentration was $2\mu M$, the cells appeared obvious fragmentation. DAPI staining showed that the number of cells showing highlights in the nucleus gradually increased with the increase of GNA concentration. Annexin V/PI staining results showed that the number of early and late apoptotic cells increased with the increase of GNA concentration (Fig. 2B). The expression level of cleaved-caspase-3 (apoptosis-related proteins) was significantly increased with the increase of GNA concentration, and the ratio of Bax to Bcl-2 was also significantly higher (Fig. 2C). The above results showed that GNA enhanced the ADM to promote the apoptosis of BEL-7402/ADM cells.

**BEL-7402/ADM Resistance to ADM by Increasing Basic Autophagy** To further investigate GNA mechanisms to increase ADM sensitivity, we first investigated the possible causes of MDR in BEL-7402/ADM cells. As shown in Fig. 3A, the upper panels are MDC staining, the bottoms are cell microfeatures observed by transmission electron microscopy. MDC staining showed that the basal autophagy level of BEL-7402/ADM cells was higher than that of BEL-7402 cells, and a large number of autophagosomes and autophagic vesicles could be observed in BEL-7402/ADM cells under transmission electron microscopy. The detection of autophagy-related protein expression found that the ratio of autophagy-related protein Beclin1 and LC3-II/LC3-I in BEL-7402/ADM cells was significantly higher than that in BEL-7402 cells, and the level of P62 protein was lower than that in middle cells (Fig. 3B). The above results suggest that BEL-7402/ADM cells may develop resistance to ADM by upregulating basal autophagy levels.

**GNA Promotes ADM Induced Apoptosis by Inhibiting Protective Autophagy in BEL-7402/ADM Cells** To investigate whether GNA reverse resistance by interfering with pro-

---

**Fig. 4. GNA Promotes ADM Induced Apoptosis by Inhibiting Protective Autophagy in BEL-7402/ADM Cells**

(A) The morphological effects of $2\mu M$ GNA on autophagy of BEL-7402/ADM cells treated with $20\mu M$ ADM for 24h. The upper panels are MDC staining, the bottoms are cell microfeatures observed by transmission electron microscopy. (B) The effects of $2\mu M$ GNA on autophagy and apoptotic related proteins in BEL-7402/ADM and BEL-7402 cells treated with $20\mu M$ ADM for 12 and 24h. (C–H) Relative expression of the LC3-II/LC3-I, P62 and Beclin1 cleaved-caspase-3 Bax/Bcl-2 and P-gp protein. **$p<0.01$, *$p<0.05$ vs. control (X ± S.E., $n = 3$); ##$p<0.01$ vs. the ADM group.**

tective autophagy in BEL-7402/ADM cells, we first observed by MDC staining and transmission electron microscopy. As show in Fig. 4A, the upper panels are MDC staining, the bottoms are cell microfeatures observed by transmission electron microscopy. Using the ADM group alone, the cell fluorescence intensity and the number of autophagosomes increased, indicating increased autophagic activity. While 2 µM GNA pre-treated 3 h, then 20 µM ADM after treatment 24 h, it was found that the autophagic fluorescence intensity and the number of autophagosomes in the combined drug group were higher than those in the ADM group, indicating that the degradation of ADM induced autophagosomes was prevented after combined ADM administration, resulting in the accumulation of autophagic vesicles. Moreover, the effects of different time GNA on ADM induced autophagy protein levels and related apoptotic proteins were detected. The results showed that the expression level of Beclin-1 and LC3-II proteins in the time GNA on ADM induced autophagy protein levels and related apoptotic proteins were detected. The results showed that the expression level of Beclin-1 and LC3-II proteins in the combined drug group was significantly increased, but the P62 protein level was significantly increased, suggesting that GNA may inhibit BEL-7402/ADM protective autophagy by inhibiting the late process of autophagy. The expression of apoptosis-related protein expression levels showed that the cleaved-caspase-3 of the combined drug group increased significantly, and the ratio of Bax to Bcl-2 increased significantly, which further indicated that GNA inhibited ADM induced protective autophagy, and finally made BEL-7402/ADM cells more sensitive to ADM and promoted apoptosis (Fig. 4B).

DISCUSSION

GNA is one of the main active components of Gamboge. In recent years, the anticancer effect of GNA has gradually attracted wide attention. Studies have shown that GNA can reverse the MDR of HepG2/ADR cells by inhibiting the expression of P-gp. Based on the results of previous studies, we also found that GNA induced tumor cell death by inhibiting cytoprotective autophagy. This study investigated the effects of GNA on BEL-7402/ADM cells and their relationship with autophagy. MTT results showed that treatment of BEL-7402/ADM cells with 2 µmol/L GNA combined with 20 µmol/L ADM significantly increased the sensitivity of BEL-7402/ADM to ADM.

The mechanism of MDR occurrence is complex, including increased efflux of overexpressed ATP binding cassette membrane transporters; decreased drug intake; alterations and inhibition of apoptosis-related pathways; changes in drug targets; and enhanced DNA repair capacity. But the specific mechanism is not clear. P-gp are encoded by MDR1 genes and high levels of MDR1 expression can be detected in tumor cells such as kidney, colon and liver cancer. A number of studies have shown that expression of P-gp is associated with the production of cancer cell resistance. The expression of P-gp was also found to be higher in BEL-7402/ADM cells but lower in BEL-7402 cells. GNA significantly decreased P-gp protein levels in BEL-7402/ADM cells were also observed, suggesting that GNA may reverse MDR by lowering protein levels.

Autophagy, also known as the procedural death of type II cells, has increasingly shown studies that autophagy levels of tumor cells affect cell resistance, and the expression of autophagy and resistant genes is closely associated with. While the underlying level of autophagy seems to support cell survival, continuous activation of autophagy may in turn cause cell death through the gradual self-degradation of the tumor cell. ABCB1 (member of the ATP binding box B subfamily 1) belongs to the ABC protein family and is one of the most important mechanisms for MDR. In a study of tumor specimens in patients with colorectal cancer, ABCB1 and LC3 (light chain 3) were positively correlated with the expression levels of Beclin 1 and Rictor. Instead, there is a negative correlation between the expression levels of ABCB1 and Raptor (regulatory-binding protein of mammalian target of rapamycin (mTOR)). It indicates that autophagy can be associated with cancer progression in MDR. In addition, Atg5/Atg7 (autophagy-related proteins 5/7) knockdown and chloroquine (CQ) or hydroxychloroquine (HCQ) reduce the vitality of drug inhibition of drug-resistant breast cancer cells. Therefore, it can be said that autophagy plays a dual role in cancer. When autophagy is activated as a cell protection mechanism, MDR is more difficult to treat. Therefore, inhibiting autophagy can resensitize drug-resistant cancer cells and making chemotherapy more effective.

We examined the basal autophagy levels of BEL-7402/ADM and BEL-7402 cells by MDC staining and transmission electron microscopy and expressed the associated autophagy proteins. As a result, BEL-7402/ADM has high autophagy activity, indicating that autophagy is involved in the formation of drug resistance. Autophagy includes activation of autophagy activity, autophagy formation, fusion of autophages and lysoomes, and the degradation process. During autophagy, p62 binds to the ubiquitinated protein and forms a complex with the LC3-II protein localized on the autophagy in vivo membrane and degrade together within the autophagy lysosome. When the autophagy activity is weakened or the autophagy system is damaged, the p62 protein constantly accumulates in the cytoplasm. The protein content of the p62 indirectly reflects the autophagosome clearance level and is negatively associated with the autophagy flow. More and more studies have found that when autophagy is activated, and when the late stage of autophagy process is inhibited, the accumulation of autophagosomes and emergent autophagy activation are shown in both cases, but the blocking of late autophagy process leads to the final inhibition of autophagy. For example, when cells undergo chemotherapy, hunger, etc., a complete autophagy process occurs, and both the protein expression of P62 and LC3-II decreases. However, when the autolysosome inhibitor chloroquine is added, the degradation process of the autolysosome is inhibited and the LC3-II protein expression continues to increase. The protein expression of the autophagy lysosome substrate P62 has increased significantly.

Interestingly, after combined combination of GNA and Adriamycin on BEL-7402/ADM cells for 24 h, the number of autophagosomes was significantly increased, with the ratio of LC3-II to LC3-I and Beclin-1 expression, indicating enhanced autophagy activity. While P62 protein levels, showed that GNA pretreatment can activate the activation of autophagy, autophagosome formation, autophagosome and lyosome fusion. But blocking late process of autophagy can hinder the degradation of autolysosome. In sum, Adriamycin activates protective autophagy of resistant cell lines, while GNA inhibits protective autophagy of BEL-7402/ADM cells.

Moreover, after adding GNA and ADM into BEL-7402/ADM...
cells, the nucleus became smaller and the chromatin was crumpled. DAPI staining could observe obvious spots under fluorescence microscope. It was preliminarily judged that the apoptosis levels of each group increased with the increase of GNA concentration, and the apoptosis rate and the ratio cleaved-caspase-3, Bax/Bcl-2 apoptosis-related proteins increased by Western blotting, which further indicated that GNA could improve the apoptosis-promoting effect of ADM on BEL-7402/ADM cells. Autophagy and apoptosis, as two major pathways in the mechanism of programmed cell death (PCD), jointly maintain processes such as the synthesis and decomposition of intracellular related proteins. In general, in order to maintain their own homeostasis and maintain the normal synthesis and decomposition of various substances in the cell, the intracellular autophagy level is in a stable state.

When additional overload of chemotherapeutic drugs, radiation and other anti-tumor means are applied to cancer cells, the intracellular autophagy level is elevated and the damaged organelles and proteins in the cells are decomposed, while recycling the decomposition products to provide more abundant nutrition and energy for cancer cells. So autophagy increases the killing effect of chemotherapeutic drugs on cancer cells but eventually leads to the formation of tumor resistance. Protecting tumor cells from anti-tumor drug-induced apoptosis by degrading apoptosis-related proteins is a survival mechanism of drug-resistant tumor cells.

Based on the above results, GNA may enhance ADM sensitivity and promote ADM induced apoptosis by inhibiting basal autophagy levels in BEL-7402/ADM cells. In addition, the effect of GNA inhibition of autophagy may be achieved by hindering the degradation of autolysosome and thus blocking the late process of autophagy.

Acknowledgments This work was supported by the National Natural Science Foundation of China [Grant numbers 81673650 and 81173600 to Q.L.]

Conflict of Interest The authors declare no conflict of interest.

Supplementary Materials The online version of this article contains supplementary materials.

REFERENCES

1. Forner A, Reig M, Bruix J. Hepatocellular carcinoma. *Lancet*, 391, 1301–1314 (2018).
2. Tanwar J, Das S, Fatima Z, Hameed S. Multidrug resistance: an emerging crisis. *Interdiscip. Perspect. Infect. Dis.*, 2014, 541340– (2014).
3. Fito T, Coley HM. The role of efflux pumps in drug-resistant metastatic breast cancer: new insights and treatment strategies. *Clin. Breast Cancer*, 7, 749–756 (2007).
4. Silva R, Vilas-Boas V, Carmo H, Dinis-Oliveira RJ, Carvalho F, de Lourdes Bastos M, Remiao F. Modulation of P-glycoprotein efflux pump: induction and activation as a therapeutic strategy. *Pharmacol. Ther.*, 149, 1–123 (2015).
5. Shaaban S, Negm A, Ibrahim EE, Elrazak AA. Chemotherapeutic agents for the treatment of hepatocellular carcinoma: efficacy and mode of action. *Oncotherapy*, 8, 246 (2014).
6. Chen C, Lu L, Yan S, Yi H, Yao H, Wu D, He G, Tao X, Deng X. Autophagy and doxorubicin resistance in cancer. *Anticancer Drugs*, 29, 1–9 (2018).
7. Lee YG, Jeon T. Modulation of the autophagy-lysosomal pathway in hepatocellular carcinoma using small molecules. *Molecules*, 25, 1580 (2020).
8. Das CK, Mandal M, Kögel D. Pro-survival autophagy and cancer cell resistance to therapy. *Cancer Metastasis Rev.*, 37, 749–766 (2018).
9. Li Y-J, Lei Y-H, Yao N, Wang C-R, Hu N, Ye W-C, Zhang D-M, Chen Z-S. Autophagy and multidrug resistance in cancer. *Chin. J. Cancer*, 36, 52 (2017).
10. Garg M. Epithelial plasticity, autophagy and metastasis: potential modifiers of the crosstalk to overcome therapeutic resistance. *Stem Cell Rev. Rep.*, 16, 503–510 (2020).
11. Li J, Liu G, Li L, Yao Z, Huang J. Research progress on the effect of autophagy-lysosomal pathway on tumor drug resistance. *Exp. Cell Res.*, 389, 11925–11926 (2020).
12. Pallis M, Turzanski J, Hijashi Y, Russell N. P-glycoprotein in acute myeloid leukaemia: therapeutic implications of its association with both a multidrug-resistant and an apoptosis-resistant phenotype. *Leuk. Lymphoma*, 43, 1221–1228 (2002).
13. Yan F, Wang M, Li J, Cheng H, Su J, Wang X, Wu H, Xia L, Li X, Chang HC, Li Q. Gambogenic acid induced mitochondrial-dependent apoptosis and referred to Phospho-eRk1/2 and Phospho-p38 MAPK in human hepatoma HepG2 cells. *Environ. Toxicol. Pharmacol.*, 33, 181–190 (2012).
14. Su J, Xu T, Jiang G, Hou M, Liang M, Cheng H, Li Q. Gambogenic acid triggers apoptosis in human nasopharyngeal carcinoma CNE-2Z cells by activating volume-sensitive outwardly rectifying chloride channel. *Fitoterapia*, 133, 150–158 (2019).
15. Mei W, Dong C, Hui C, Bao L, Fenggen Y, Jingjing S, Cheng P, Meiling S, Yawen H, Xiaoshan W, Guanghui W, Zhivu C, Jinglin L. Gambogenic acid kills lung cancer cells through aberrant autophagy. *PLOS ONE*, 9, e83604 (2014).
16. He Y, Ding J, Lin Y, Li J, Shi Y, Wang J, Zhu Y, Wang K, Hu X. Gambogenic acid alters chemosensitivity of breast cancer cells to Adiriamycin. *BMC Complement. Altern. Med.*, 15, 181 (2015).
17. Li Q, Cheng H, Zhu G, Yang L, Zhou A, Wang X, Fang N, Xia L, Su J, Wang M, Peng D, Xu Q. Gambogenic acid inhibits proliferation of A549 cells through apoptosis-inducing and cell cycle arrest. *Biol. Pharm. Bull.*, 33, 415–420 (2010).
18. Xu Q, Guo J, Chen W. Gambogenic acid reverses P-glycoprotein mediated multidrug resistance in HepG2/Adr cells and its underlying mechanism. *Biochem. Biophys. Res. Commun.*, 508, 882–888 (2019).
19. Soo S-B, Hur J-G, Kim M-J, Lee J-W, Kim H-B, Bae J-H, Kim D-W, Kang C-D, Kim S-H. TRAIL sensitize MDR cells to MDR-related drugs by down-regulation of P-glycoprotein through inhibition of DNA-Pkcs/Akt/GSK-3β pathway and activation of caspases. *Mol. Cancer*, 9, 199 (2010).
20. De Vera AA, Gupta P, Lei Z, Liao D, Narayanan S, Teng Q, Reznik SE, Chen Z-S. Immuno-oncology agent IPI-549 is a modulator of P-glycoprotein (P-gp, MDR1, ABCB1)-mediated multidrug resistance (MDR) in cancer. *In vitro and in vivo. Cancer Lett.*, 442, 91–103 (2019).
21. Gao B, Yang F-M, Yu Z-T, Li R, Xie F, Chen J, Luo H-J, Zhang J-C. Relationship between the expression of MDR1 in hepatocellular cancer and its biological behaviors. *Int. J. Clin. Exp. Pathol.*, 8, 6995–7001 (2015).
22. Wu M, Lin X, Tan X, Li J, Wei Z, Zhang D, Zheng Y, Zheng A, Zhao B, Zeng Y, Liu X, Liu J. Photoresponsive nanovehicle for two independent wavelength light-triggered sequential release of P-gp shRNA and doxorubicin to optimize and enhance therapeutic effect of multidrug-resistant cancer. *ACS Appl. Mater. Interfaces*, 10, 19416–19427 (2018).
23. Kanzawa T, Gernano IM, Komata T, Ito H, Kondo Y, Kondo S, Role of autophagy in temozolomide-induced cytotoxicity for malig-
nant glioma cells. *Cell Death Differ.*, **11**, 448–457 (2004).

24) Zhou J, Kang Y, Chen L, Wang H, Liu J, Zeng S, Yu L. The drug-resistance mechanisms of five platinum-based antitumor agents. *Front. Pharmacol.*, **11**, 343 (2020).

25) Pan ST, Li ZL, He ZX, Qiu JX, Zhou SF. Molecular mechanisms for tumour resistance to chemotherapy. *Clin. Exp. Pharmacol. Physiol.*, **43**, 723–737 (2016).

26) Tabata M, Tsubaki M, Takeda T, Tateishi K, Tsurushima K, Imano M, Satou T, Ishizaka T, Nishida S. Dasatinib reverses drug resistance by downregulating MDR1 and Survivin in Burkitt lymphoma cells. *BMC Complement. Med. Ther.*, **20**, 84 (2020).

27) Shuhua W, Chenco S, Yangyang L, Xiangqian G, Shuang H, Tanguye L, Dong T. Autophagy-related genes Raptor, Rictor, and Beclin1 expression and relationship with multidrug resistance in colorectal carcinoma. *Hum. Pathol.*, **46**, 1752–1759 (2015).

28) Chittaranjan S, Bortnik S, Dragowska WH, Xu J, Abeyssundara N, Leung A, Goe NE, DeVorkin L, Weaver SA, Gelmon K, Yapp DJ, Bally MB, Gorski SM. Autophagy inhibition augments the anticancer effects of epirubicin treatment in anthracycline-sensitive and -resistant triple-negative breast cancer. *Clin. Cancer Res.*, **20**, 3159–3173 (2014).

29) Levy JMM, Towers CG, Thorburn A. Targeting autophagy in cancer. *Nat. Rev. Cancer.*, **15**, 528–542 (2015).

30) Bartolini D, Dallaglio K, Torquato P, Pirrodi M, Galli F. Nrf2-p62 autophagy pathway and its role to oxidative stress in hepatocellular carcinoma. *Transl. Res.*, **193**, 54–71 (2018).

31) Zaffagnini G, Savoya A, Danieli A, Romanov J, Tremel S, Ebner M, Peterbauer T, Sztabo M, Trapanone R, Tafadar AK, Sachse C, Martens S. p62 filaments capture and present ubiquitinated cargos for autophagy. *EMBO J.*, **37**, e98308 (2018).

32) Pasquier B. Autophagy inhibitors. *Cell. Mol. Life Sci.*, **73**, 985–1001 (2016).

33) Qu X, Shen J, Shen L, Su J, Xu Y, Xie Q, Wu Y, Zhang X, Sun L. Autophagy inhibitor chloroquine increases sensitivity to cisplatin in QBC939 cholangiocarcinoma cells by mitochondrial ROS. *FRANCO R. PLOS ONE*, **12**, e0173712 (2017).

34) Xu R, Ji Z, Xu C, Zhu J. The clinical value of using chloroquine or hydroxychloroquine as autophagy inhibitors in the treatment of cancers: a systematic review and meta-analysis. *Medicine*, **97**, e12912 (2018).

35) Ryter SW, Mizumura K, Choi AMK. The impact of autophagy on cell death modalities. *Int. J. Cell Biol.*, **2014**, 502676 (2014).

36) Chaabane W, User SD, El-Gazzah M, Jaksik R, Sajjadi E, Kreszowska-Wolny J, Los MJ. Autophagy, apoptosis, mitoptosis and necrosis: interdependence between those pathways and effects on cancer. *Arch. Immunol. Ther. Exp. (Warsz)*, **61**, 43–58 (2013).

37) Jo GH, Bögler O, Chwae Y-J, Yoo H, Lee SH, Park JB, Kim Y-J, Kim JH, Gowak H-S. Radiation-induced autophagy contributes to cell death and induces apoptosis partly in malignant glioma cells. *Cancer Res. Treat.*, **47**, 221–241 (2015).