Original Article

DOI: 10.5582/irdr.2017.01069

Differences of basic and induced autophagic activity between K562 and K562/ADM cells

Feifei Wang[1,2,3,4], Jing Chen[5], Zhewen Zhang[5], Juan Yi[5], Minmin Yuan[1,2,3,4], Mingyan Wang[1,2,3,4], Na Zhang[1,2,3,4], Xuemin Qiu[1,2,3,4], Hulai Wei[5,*], Ling Wang[1,2,3,4,*]

[1] *Obstetrics and Gynecology Hospital, Fudan University, Shanghai, China;*
[2] *The Academy of Integrative Medicine of Fudan University, Shanghai, China;*
[3] *Laboratory for Reproductive Immunology, Hospital & Institute of Obstetrics and Gynecology, Fudan University Shanghai Medical College, Shanghai, China;*
[4] *Shanghai Key Laboratory of Female Reproductive Endocrine Related Diseases, Shanghai, China;*
[5] *Key Laboratory of Preclinical Study for New Drugs of Gansu Province, School of Basic Medical Sciences, Lanzhou University, Lanzhou, Gansu Province, China.*

Summary **Patients with acute myeloid leukemia (AML) often have a poor prognosis due to drug resistance, which is regarded as a tough problem during the period of clinical therapeutics. It has been reported that autophagy, an important event in various cellular processes, plays a crucial role in mediating drug-resistance to cancer cells. Our study attempts to comparatively investigate the differences of basic and induced autophagic activity between drug-sensitive and multidrug-resistant AML cells. The level of basic autophagy in K562/ADM cells was higher than that in K562 cells, which could be characterized by more cytosolic contents-packaged autophagic vacuoles in K562/ADM cells when compared to that in K562 cells. The observation of MDC staining showed that the fluorescent intensity of autophagosomes in K562/ADM cells was stronger than that in K562 cells. The expression of Beclin1 and the ratio of LC3-II to LC3-I were distinctly higher in K562/ADM cells, however, P62 protein was relatively lower in K562/ADM cells. Furthermore, we found that nutrient depletion could induce autophagic activity of both cell lines. However, autophagic activity of K562/ADM cells was always maintained at a higher level in contrast with K562 cells. ADM (Adriamycin) was also capable of inducing autophagic activity of K562 and K562/ADM cells, but the autophagic alteration in K562 cells appeared earlier. Taken together, our findings suggest that autophagy exerts an important effect on formation and maintenance of drug-resistance in AML cells.**

Keywords: Autophagic activity, drug-resistance, acute myeloid leukemia

1. Introduction

Acute myeloid leukemia (AML) is a highly malignant hematological neoplasm, which is characterized by high morbidity, poor prognosis and high cost of treatment, leading it to be a malignant neoplasm with one of the highest mortality rates (*1*). Plenty of therapeutic drugs have been used to treat patients with AML and good efficacy has been achieved (*2*). However, among those refractory patients, both drug resistance and poor prognosis appeared (*3*). Therefore, it is urgent to investigate the differences between drug-resistant and -sensitive AML cells, which may contribute to dissection of mechanisms in drug resistance.

Autophagy is an important cellular process associated with lysosome-mediated self-degradation in eukaryotic cells (*4*). According to different manners of substrate

Released online in J-STAGE as advance publication November 14, 2017.

**Address correspondence to:*
Dr. Ling Wang, Obstetrics and Gynecology Hospital, Fudan University, 413 Zhaozhou Road, Shanghai 200011, China.
E-mail: Dr.wangling@fudan.edu.cn

Dr. Hulai Wei, Key Laboratory of Preclinical Study for New Drugs of Gansu Province, School of Basic Medical Sciences, Lanzhou University, 199 Donggang West Road, Lanzhou, 730000 Gansu Province, China.
E-mail: weihulai@lzu.edu.cn

degradation, autophagy is mainly classified into three categories including microautophagy, chaperone-mediated autophagy (CMA) and macroautophagy (*5*). Macroautophagy, referred to autophagy hereinafter, exerts its function through formation of autophagosomes and subsequently being fused with lysosomes, triggering substances being degraded by acid hydrolases. Consequently, the catabolites including amino acids, fatty acids, nucleotides and so on are recycled in cytoplasm to reenter biological energy pathways, ultimately maintaining structure and metabolism of cells (*6*).

It has been reported that multiples of ATG (autophagy-related) proteins are recruited to tightly regulate the occurrence of autophagy, during which LC3 (microtubule- associated protein 1 light-chain 3) plays critical roles in elongation and completion of the autophagosome. In this process, the protein LC3 is cleaved at its C-terminus by Atg4B, resulting in formation of LC3-I. Subsequently, LC3-I conjugates with PE (phosphatidyl ethanolamine) and leads generation of the form of membrane-bound LC3 that called LC3-II. LC3-II is employed to the membrane of the autophagosome and specifically mediates elongation of the autophagosome. Therefore, the ratio of LC3-II to LC3-I will become larger when the quantity of autophagosomes increases (*7*). In addition to the shift of LC3-I to LC3-II, another crucial ATG protein P62 that interacts with ubiquitinated proteins through its UBA domain (ubiquitin-associated domain) and then delivers them to LC3-II *via* another domain LIR (LC3-interacting region) will be finally degraded in the autolysosome, so the level of P62 has a contrary relationship with autophagic activity (*8*).

Over the past few decades, basal autophagy was reported to remove misfolded proteins and damaged organelles to maintain cellular quality and health, involved in many physical processes of cells such as cellular turnover, differentiation and development (*9-11*). Abnormal basal autophagy can lead to various human diseases including cancer, neurodegenerative disorders, autoimmunity disease and inflammation (*12-15*). Autophagy can be induced in response to several stressors including: nutrient deprivation, chemotherapeutics, hypoxia, pathogen infection and endoplasmic reticulum stress (*16-18*). A larger body of research has reported that autophagy which is promoted by chemotherapeutic drugs in cancer cells, is related to drug-resistance of tumor cells (*19-21*). Autophagy is activated by doxorubicin in human colon cancer LoVo cells, and this autophagic activation reduces the sensitivity of cancer cells to doxorubicin (*22*). Enhanced autophagy is a survival mechanism of drug-resistant esophageal cancer cells treated with cisplatin (*23*). In contrast, elemene can reverse the drug resistance of the cisplatin-resistant lung adenocarcinoma cells by promoting autophagy (*24*). To clarify the relationship between autophagy and drug-resistance of AML cells, we tried to compare autophagic activity between K562/ADM cells and ADM-sensitive K562 cells in different environments such as the basic state, starvation and ADM treatment.

2. Materials and Methods

2.1. *Chemicals and antibodies*

ADM was purchased from Kangbao biochemical industry company (Wuhan); HCQ (hydroxychloroquine) was bought from Tokyo Chemical Industry; MTT was obtained from Sigma; newborn bovine serum was from Rongye biotech company (Lanzhou); RPMI 1640 medium from Gibco; anti-P62, anti-LC3, anti-Beclin1 was from Cell Signaling Technology; anti-β-actin was from Biovision; HRP-linked anti-rabbit or anti-mouse IgG antibodies were from Boster biological technology company (Wuhan).

2.2. *Cell lines and culture*

Human ADM-resistant AML cell line (K562/ADM cells) and its parental cell line (K562 cells) were both from Medical experimental center of Lanzhou University. K562/ADM and K562 cells were grown in RPMI 1640 Medium supplied with 10% inactivated newborn bovine serum and 2 mmol/L L-glutamine at 37℃ in a humid atmosphere containing 5% CO_2. The viability of all cultured cells was determined for up to 95% before experiments were performed.

2.3. *Cell proliferation assay (MTT)*

Cells were seeded in 96-well plates at a density of 1×10^5 cells/mL in triplicate and were cultured with ADM at indicated concentrations and times at 37℃. MTT (5 mg/mL, 10 μL) was added to each well and cultured for another 4 h, and then 100 μL of acidifying sodium dodecyl sulfate (10%) was added to dissolve the formazan. 24 hours later, optical density (OD) was measured at 570 nm with a Powerwave X plate reader (Bio-Tek, USA). Cell proliferation inhibition rates were calculated using the following formula: cell proliferation inhibition rate = $[(OD_{control} - OD_{experiment})/OD_{control}] \times 100\%$ (*25*).

2.4. *MDC staining*

MDC has been reported to distinctively mark autophagic vesicles (*26*). Cells were harvested and washed twice with PBS. After resuspending in 1 mL of PBS, cells were stained with 1 μL of MDC stock-solution (50 mmol/L) of which terminal concentration reached 0.05 mmol/L for 30 min at 37℃ in the dark (*27*). Subsequently, cells were gently washed with PBS three times and observed under an IX81 inverted

microscope (Olympus, Japan).

2.5. *Transmission electron microscope detection*

Cells were fixed with glutaraldehyde overnight at 4℃. On the next day, after rinsing with PBS three times each for 5 min, cells were fixed for 1.5 h with osmic acid. Next, cells were rinsed with PBS again and dehydrated in the following steps: 50% alcohol for 10 min, 70% alcohol for 10 min, 90% alcohol for 10 min, 100% alcohol for 10 min, 100% acetone for 10 min twice. Subsequently, cells were embedded in an epoxy resin overnight and then solidified for 12 h at 45℃. Following slicing into sections, cells were stained with uranyl acetate and lead citrate. Finally, the ultrastructure of cells was observed under a JEM1230 transmission electron microscope (JEOL, Japan) (*28*).

2.6. *Real-Time quantitative RT-PCR*

Total RNA was isolated from cells with a Trizol kit following the manufacturer's specifications. Both the concentrations and purity of extracted RNA samples were determined by spectrophotometer. 500 ng of each RNA sample was used to synthesize cDNA with Prime Script RT Master Mix (Takara). Real-time PCR was performed with SYBR Premix Taq II (Takara) and primers. The primers used are as follows: *beclin1* forward: 5'-ACCTCAGCCGAAGACTGAAG-3', *beclin1* reverse: 5'-AACAGCGTTTGTAGTTCTGAC-3'; *β-actin* forward: 5'-TGCTCCTCCTGAGCGCAAGTA-3', *β-actin* reverse: 5'–CCACATCTGCTGGAAGGTGGA-3'. After the procedure was completed, the relative mRNA levels of genes indicated were analyzed by software Roto-Gene6.0 (*29*).

2.7. *Western blotting*

Cells were lysed in RIPA buffer with PMSF (PMSF:RIPA=1:100) for 30 min on ice and then centrifuged for 15 min. Protein concentration of the supernatant was measured using a BCA protein assay kit. Equal amounts of protein from cell extracts were separated by SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) and transferred onto a PVDF (polyvinylidene fluoride) membrane (*30*). The membrane was incubated with primary antibodies overnight at 4℃ and then with secondary antibodies conjugated with horseradish peroxidase. Finally, the protein level was determined using a chemiluminescent approach in a dark room.

2.8. *Statistical analysis*

All data were statistically analyzed with the student's *t*-test by utilizing SPSS 17.0 software. *P*-value < 0.05 was considered statistically significant.

3. Results

3.1. *ADM inhibits the proliferation of K562 cells greater than K562/ADM cells*

To investigate the sensitivities of K562/ADM and K562 cells to chemotherapeutic drug, we treated these two cell lines with a gradually increased concentration of ADM for different times as indicated. The MTT assay showed that ADM inhibited the growth of K562/ADM and K562 cells to various extents, and ADM-induced cytotoxicity in both cell lines was dose- and time-dependent (Figure1A and 1B). We calculated IC_{50} values of ADM for K562 and K562/ADM cells, which showed that IC_{50} values of ADM for K562 cells for 12 h, 24 h, 48 h were 2.63 ± 0.12, 1.04 ± 0.04, 0.25 ± 0.05 μmol/L respectively and the corresponding values for K562/ADM cells were 54.19 ± 0.87, 46.46 ± 4.36, 15.44 ± 0.81 μmol/L (Figure1C). The degree of ADM-resistance for K562/ADM cells was 20.6~62.9 fold greater when compared with that of K562 cells, suggesting K562/ADM cells have more potential for resistance to ADM than K562 cells.

3.2. *K562/ADM cells show higher basic autophagic activity than K562 cells*

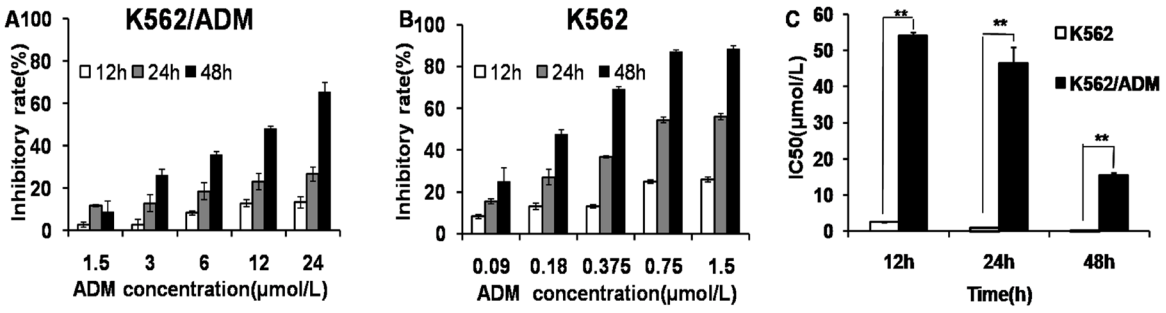

Figure 1. ADM inhibits the proliferation of K562 cells greater than K562/ADM cells. (A) K562/ADM cells were exposed to 1.5, 3, 6, 12 and 24 μmol/L ADM for 12, 24, 48 h, and then MTT assay was performed to detect cell viability; **(B)** K562 cells were treated with 0.09, 0.18, 0.375, 0.75, 1.5 μmol/L ADM for 12, 24, 48h separately, and then MTT assay was performed to determinate cell proliferation; **(C)** IC_{50} values of ADM for K562 and K562/ADM cells. Results are all presented as the mean ± Standard Deviation (SD) of four separate experiments. ***p* < 0.01 versus K562 cells.

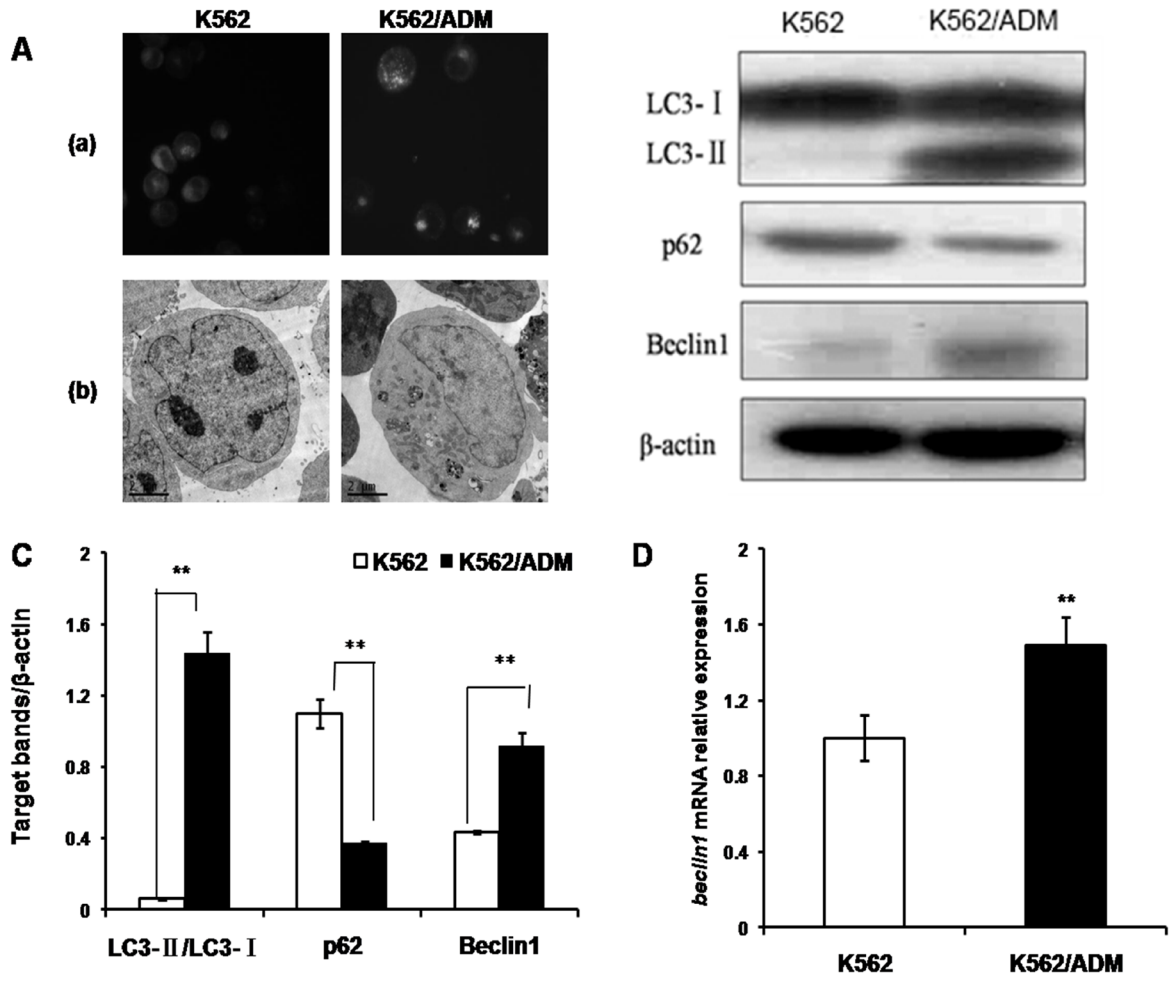

Figure 2. K562/ADM cells show higher basic autophagic activity than K562 cells. (A) Autophagic morphology was observed by fluorescent microscopy followed by MDC staining (×100) (a); Autophagosomes were observed by transmission electron microscope (×8,000) (b); **(B, C)** Expression levels of LC3, P62 and Beclin1 were detected by Western blot; **(D)** Relative expressions of *beclin1* mRNA in two cell lines were analyzed by real-time quantitative RT-PCR. Values are mean ± SD of three separate experiments. $^{**}p < 0.01$ versus K562 cells.

To explore the relationship between autophagy and ADM resistance, we first measured the basic autophagy activities of K562/ADM and K562 cells. After observation of autophagosomes in cells under the fluorescent microscope followed by MDC staining, we found that K562 cells exhibited weak fluorescent intensity in the cytosol, whereas some bright foci could be seen in K562/ADM cells (Figure 2A). A similar result was also found using the transmission electron microscope, a gold standard for determining autophagic activity, which showed more cytosolic contents-packaged autophagic vacuoles in K562/ADM cells when compared with K562 cells (Figure 2A). To further verify the results above, we examined other autophagic indicators such as LC3 and P62/SQSTM1. As shown in Figure 2B and 2C, K562/ADM cells showed a much higher ratio of LC3-II to LC3-I and relatively lower P62 levels when compared with K562 cells, indicating that K562/ADM cells had a higher level of autophagy flux. In addition, *beclin1* is one of the specific genes involved in autophagy regulation. Our results displayed the level of Beclin1, mRNA expression or protein expression, was much higher in K562/ADM cells compared to K562 cells, indicating that Beclin1 might be involved in regulating basic autophagy of these two cell lines (Figure 2C and 2D).

3.3. *Starvation-induced autophagic activity of K562/ADM and K562 cells*

To compare the different autophagic variations between K562/ADM and K562 cells in nutrition depletion, we cultured the cells in serum- and amino acid-free EBSS (Earle's Balanced Salt Solution) for different times. After observing with the fluorescent microscope, we found abundant fluorescent puncta distributed in the cytosol of K562/ADM and K562 cells starved for 1h, which showed stronger fluorescent intensity relative to untreated cells (Figure 3A). A huge number of typical autophagic vesicles were also observed using the transmission electron microscope in the cytosol of starved cells (Figure 3B). Western blot results

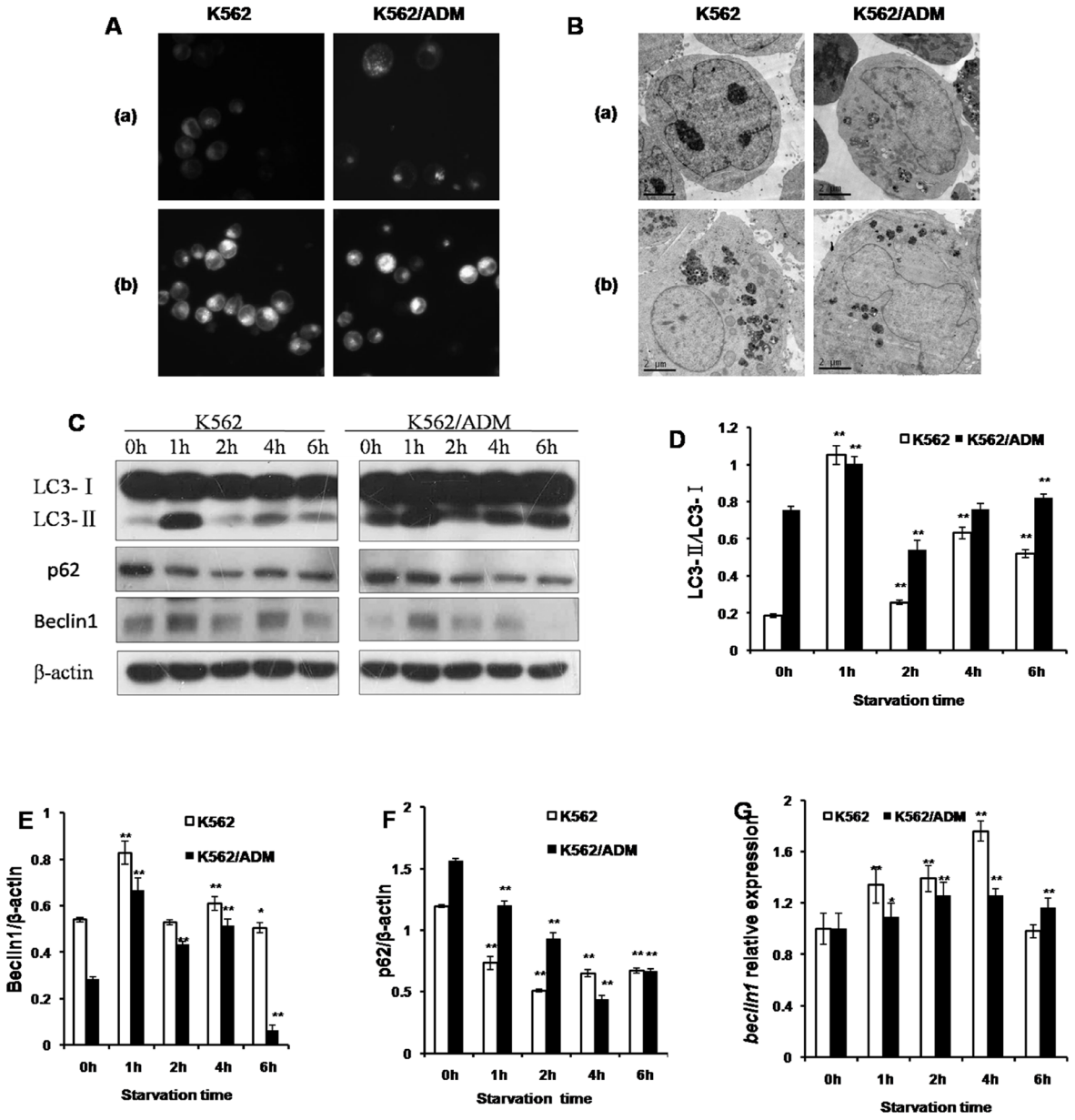

Figure 3. Starvation-induced autophagic activity of K562/ADM and K562 cells. (A) K562/ADM and K562 cells were cultured in EBSS for 0 h (a), 1 h (b), autophagic morphology was observed under a fluorescent microscope (×100); **(B)** Two cell lines were cultivated in EBSS for 0 h (a), 1 h (b), autophagosomes were observed by transmission electron microscopy (×8,000); **(C)** K562/ADM and K562 cells were cultured in EBSS for 0 h,1 h, 2 h, 4 h, 6 h. The levels of LC3, P62 and Beclin1 were determined by Western blot. **(D, E, F)** Densitometric analysis of LC3-II/LC3-I, Beclin1 and P62 normalized to β-actin in K562/ADM and K562 cells starved for 0 h,1 h, 2 h, 4 h, 6 h**; (G)** Relative expressions of *beclin1* mRNA in two cell lines starved for 0 h,1 h, 2 h, 4 h, 6 h were analyzed by real-time quantitative RT-PCR. Values are presented as mean ± SD of three separate experiments. $^{*}p < 0.05$, $^{**}p < 0.01$ *versus* control cells (starvation for 0 h).

described that both the expression of Beclin1 and the ratio of LC3-II to LC3-I reached peak value in 1hour of starvation and then gradually decreased while the degradation of P62 reached peak value after 2~4 hours of starvation. However, according to LC3-II level variation, it was found that autophagic activity of K562/ADM cells always maintained a higher level in contrast with that of K562 cells (Figure 3C-3F). Additionally, the results of RT-PCR indicated *beclin1* mRNA expression was enhanced in two cell lines after starvation (Figure 3G). Taken together, autophagy is induced in K562/ADM and K562 cells in a state of nutrition depletion, however, starvation-induced autophagic activity of K562/ADM cells is significantly stronger than K562 cells.

3.4. ADM potentiates autophagic activity of K562/ADM and K562 cells

To explore the different autophagic appearance in two cell lines with ADM exposure, K562/ADM and K562 cells were treated with 35 μmol/L and 0.75 μmol/L of ADM separately for 12 h or 24 h. Observing through the fluorescent microscope and transmission electron

microscope, ADM enhanced the intensity of fluorescent dots and the amount of autophagic vacuoles in cytosol of both cell lines (Figure 4). The same results were also obtained in cells with exposure to HCQ, a recognized autophagy inhibitor (Figure 4). To investigate whether HCQ can inhibit ADM-induced autophagy, K562/ADM and K562 cells were pre-treated with 16 μmol/L and 4 μmol/L HCQ respectively for 3 h before exposure to ADM. We found HCQ resulted in upregulated intensity of fluorescent dots and increased autophagic vacuoles in K562/ADM and K562 cells with ADM treatment (Figure 4).

It is acknowledged that the accumulation of autophagic vacuoles is attributed either to up-regulated autophagosome generation or to down-regulated autophagosome degradation (*31*). As Figure 5 showed, ADM induced the rise of the LC3-II/LC3-I ratio and Beclin1 expression along with a fall of P62 level, which suggested that ADM promoted autophagy flux and the accumulation of autophagic vacuoles induced by ADM should be attributable to up-regulated autophagosome generation. However, ADM-induced autophagic alteration happened earlier in K562 cells with ADM treatment for 12 h than in K562/ADM cells exposed to ADM for 24 h. In addition, HCQ led to an increased ratio of LC3-II/LC3-I and up-regulated P62 level in

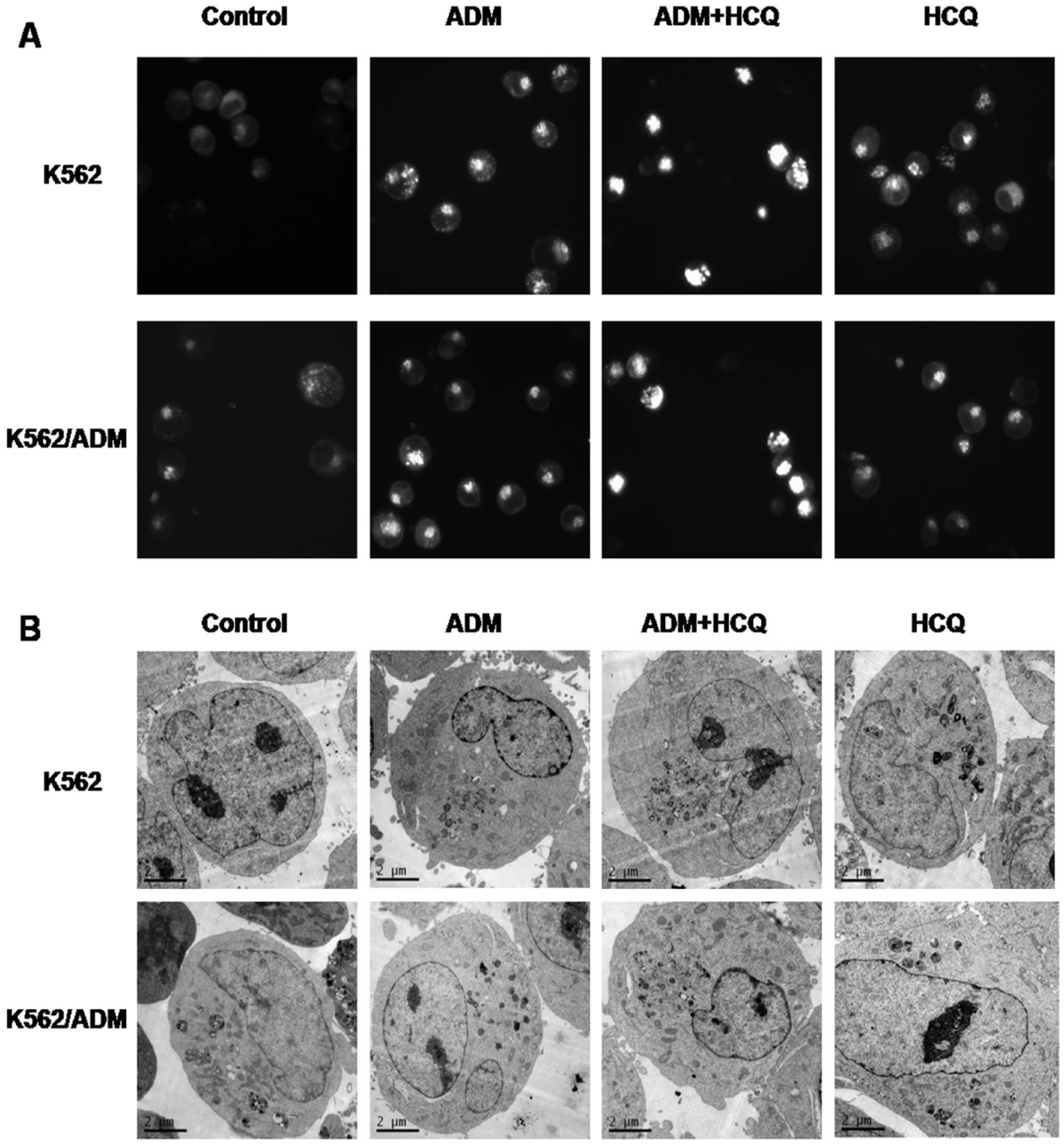

Figure 4. The morphological changes of autophagy in ADM-induced K562 and K562/ADM cells. K562/ADM and K562 cells were respectively treated with 35 and 0.75 μmol/L of ADM alone for 24 h, or 16 and 4 μmol/L of HCQ alone for 12 h, or were pre-treated with HCQ for 3 h before exposure to ADM for 24 h. **(A)** Autophagy morphology was observed under a fluorescent microscope (×100); **(B)** Cellular ultrastructure was observed by transmission electron microscopy (×8,000).

the two cell lines, indicating HCQ hindered autophagy progression and the accumulation of autophagic vesicles induced by HCQ was derived from restrained degradation of autophagy. Furthermore, HCQ increased the level of LC3-II and P62, and had a diminishing effect on Beclin1 level in the case of cells exposed to ADM, implying that HCQ was capable of inhibiting ADM-induced autophagic activity by blocking the degradation of the autophagosome. The relative expression alteration of *beclin1* mRNA detected by RT-PCR was consistent with that analyzed by Western blot.

4. Discussion

Most AML patients are prone to acquire chemotherapy resistance, which remains a troublesome obstacle for

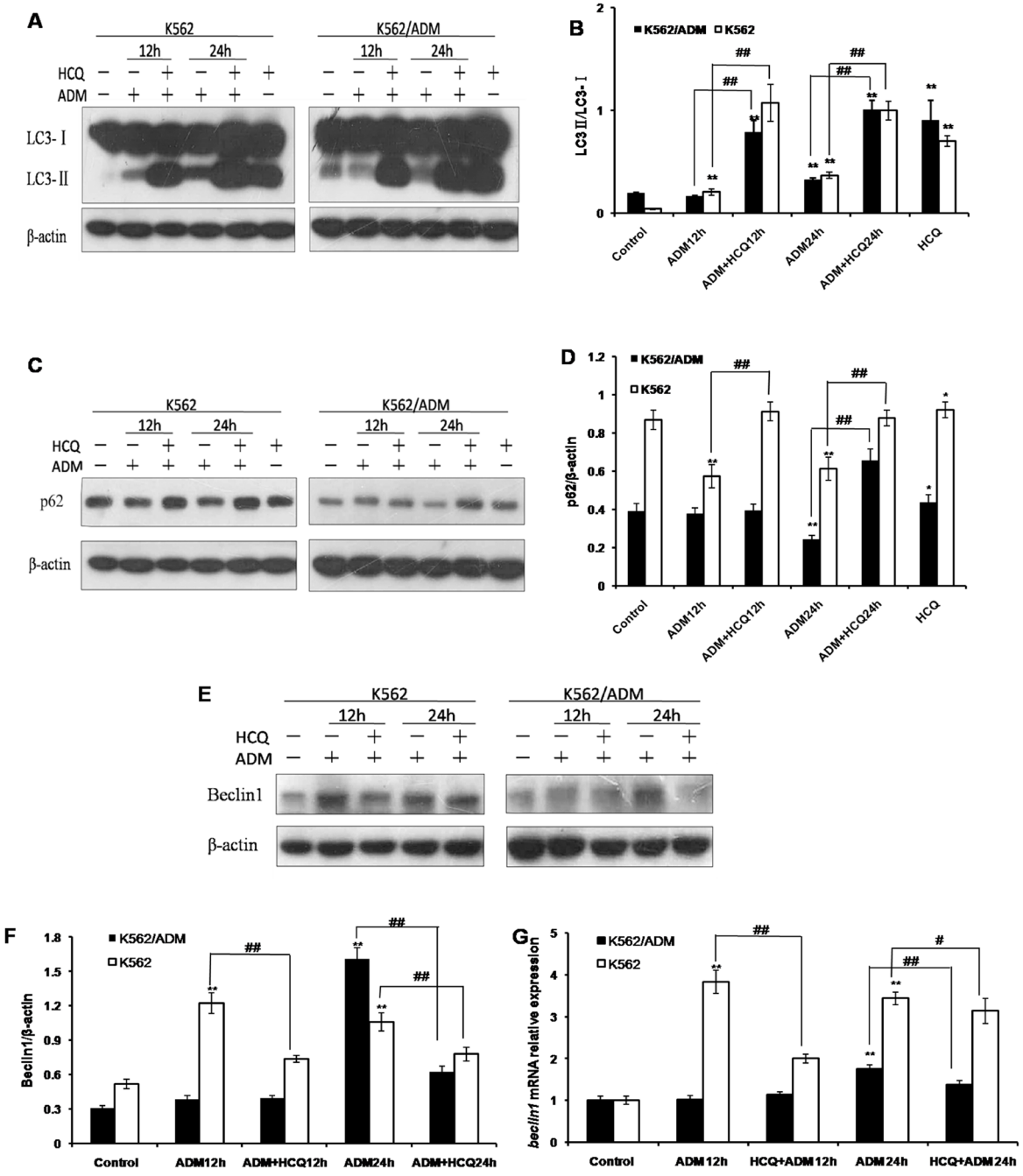

Figure 5. ADM potentiates autophagic activity of K562/ADM and K562 cells. K562/ADM cells were exposed to ADM (35 μmol/L) alone for 12 or 24 h, or treated with HCQ (16 μmol/L) alone for 12 h, or pre-treated with HCQ for 3 h before treatment with ADM for 12 or 24 h. The same procedures were applied to K562 cells with different drugs concentration. In detail, K562 cells were treated with ADM (0.75μmol/L) alone for 12 or 24 h, or HCQ (4 μmol/L) alone for 12 h, or treated with ADM for 12 or 24 h followed by administration of HCQ for 3 h. **(A)** Western blot analysis for translation of LC3-I to LC3-II was performed on lysates from cells treated as above; **(B)** Densitometric analysis of LC3-II/LC3-I normalized to β-actin; **(C)** Expression level of P62 was quantified by Western blot; **(D)** Densitometric analysis of P62 was normalized to β-actin; **(E)** Beclin1 level was monitored by Western blot; **(F)** Densitometric analysis of Beclin1 normalized to β-actin; **(G)** *Beclin1* mRNA relative expression was detected by RT-PCR. Values are mean ± SD of three separate experiments. $^{**}p < 0.01$*versus* untreated cells. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ *versus* ADM-treated cells.

clinical therapy. Emerging evidence suggests that anti-cancer therapeutic agents can induce autophagy of cancer cells (*32*), which may have a vital effect on drug-resistance. Autophagy inhibition by CQ (chloroquine) significantly enhances the sensitivity of DOX-resistant human acute myelocytic leukemia HL60 cells to DOX (*33*). Heterogeneous nuclear ribonucleoprotein K (hnRNP K) may be associated with the development of ADM resistance in AML by augmenting autophagy (*34*). Inhibition of WAVE1 expression enhances the sensitivity of leukemia cells to chemotherapy by downregulating autophagy (*35*). All this research indicates that autophagy is a mechanism involved in chemotherapy resistance of various leukemia cells. To further elucidate this problem, it is required to analyze the differences of autophagic variation between AML chemoresistant K562/ADM cells and sensitive K562 cells.

K562/ADM cells are a multidrug-resistant cell line acquired by exposing K562 cells to step-wise increasing concentrations of ADM. It is characterized by being resistant not only to ADM, but also to mitoxantrone, homoharringtonine, rubidomycin and etoposide (*36*). K562/ADM cells appear to have sophisticated cross drug-resistance to all these anti-cancer drugs with quite different structures and functions. Our experiments also showed that the IC_{50} of K562/ADM cells was 20.6~62.9 fold greater than that of K562 cells, verifying that K562/ADM cells were highly resistant to ADM-induced proliferation inhibition and apoptosis.

Here, we found that basic autophagy in K562/ADM cells was distinctly higher than that in K562 cells, which could be indicated by more cytosolic contents-packaged autophagic vacuoles, higher Beclin1 expression level and LC3-II/LC3-I ratio, and lower P62 level in K562/ADM cells. Similar results are also obtained in a multiple myeloma cell line, Pan *et al*. (*37*) reported that expression level of LC3-II was higher in DOX-resistant RPMI8226/DOX cells than in DOX-sensitive RPMI8226/S cells. Similarly, compared with K562 cells, there is an increased PI3K/AKT/mTOR signaling and enhanced autophagic activity in imatinib-resistant human chronic myelogenous leukemia K562R cells (*38*).

We further investigated the different autophagic appearance between K562 and K562/ADM cells under serum depletion. A report has described that activation of autophagy is critical for cancer cellular survival under nutrient starvation (*39*). Another report also demonstrates that CD133-positive glioma cells exhibit higher survival under starvation conditions, which depends on induction of autophagy (*40*). Furthermore, autophagy induced by amino acid starvation is accelerated in the multidrug-resistant cells when compared to parental cells, resulting in enhanced cell survival capacity (*41*). Here, we found that starvation-induced autophagic activity of K562/ADM cells was significantly stronger relative to that of K562 cells, which may decipher why K562/ADM cells possess potent resistance to extra stressors. In conclusion, our results showed K562/ADM cells exhibited a higher basic and starvation-induced autophagy, indicating autophagy exerts a vital effect on formation and maintenance of drug-resistance in AML cells.

It is generally accepted that ADM, a canonical anthracycline chemotherapeutic drug, can induce cell death with apoptosis being involved (*42*). More evidence shows that ADM is also capable of inducing autophagy (*43*). Here we found that ADM induced autophagy in both K562/ADM and K562 cells, but the autophagic alteration in K562 cells appeared earlier, which might be attributed to its lower basic autophagic activity. In addition, HCQ suppressed autophagy flux through blocking degradation of autophagosomes. In line with this result, a recent study showed HCQ could inhibit late stage of the autophagy process by hindering lysosomal acidification (*44*). We also found that HCQ inhibited ADM-induced autophagic activity by restraining the degradation of the autophagosome.

Taken together, our findings suggest that autophagy exerts an important effect on formation and maintenance of drug-resistance in AML cells, however, the mechanism is still not clearly defined and is required to be investigated further and more deeply. However, our findings provide some insight into understanding the relationship between autophagy and chemoresistance in AML patients.

Acknowledgements

The present study was supported by the National Natural Science Foundation of China No. 81541025 (to HL Wei), the Fundamental Research Funds for the Central Universities No. lzujbky-2016-174 (to HL Wei) and the Natural Science Fund of Gansu No. 1208RJZA183 (to HL Wei). This work was also supported by the National Natural Science Foundation of China No. 31571196 (to L Wang), the Science and Technology Commission of Shanghai Municipality 2015 YIXUEYINGDAO project No. 15401932200 (to L Wang), the FY2008 JSPS Postdoctoral Fellowship for Foreign Researchers P08471 (to L Wang), the National Natural Science Foundation of China No. 30801502 (to L Wang), the Shanghai Pujiang Program No. 11PJ1401900 (to L Wang), Development Project of Shanghai Peak Disciplines-Integrative Medicine No. 20150407.

References

1. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, Bloomfield CD, Cazzola M, Vardiman JW. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016; 127:2391-2405.

2. Kavanagh S, Murphy T, Law A, Yehudai D, Ho JM, Chan S, Schimmer AD. Emerging therapies for acute myeloid leukemia: Translating biology into the clinic. JCI insight. 2017; 2:e95679. doi: 10.1172/jci.insight.95679.
3. Gao W, Estey E. Moving toward targeted therapies in acute myeloid leukemia. Clin Adv Hematol Oncol. 2015; 13:748-754.
4. Zhi X, Feng W, Rong Y, Liu R. Anatomy of autophagy: From the beginning to the end. Cell Mol Life Sci. 2017. doi: 10.1007/s00018-017-2657-z.
5. Galluzzi L, Baehrecke EH, Ballabio A, et al. Molecular definitions of autophagy and related processes. EMBO J. 2017; 36:1811-1836.
6. Lee YK, Lee JA. Role of the mammalian ATG8/LC3 family in autophagy: Differential and compensatory roles in the spatiotemporal regulation of autophagy. BMB Rep. 2016; 49:424-430.
7. Wild P, McEwan DG, Dikic I. The LC3 interactome at a glance. J Cell Sci. 2014; 127:3-9.
8. Liu WJ, Ye L, Huang WF, Guo LJ, Xu ZG, Wu HL, Yang C, Liu HF. p62 links the autophagy pathway and the ubiqutin-proteasome system upon ubiquitinated protein degradation. Cell Mol Biol Lett. 2016; 21:29.
9. Vescarelli E, Pilloni A, Dominici F, Pontecorvi P, Angeloni A, Polimeni A, Ceccarelli S, Marchese C. Autophagy activation is required for myofibroblast differentiation during healing of oral mucosa. J Clin Periodontol. 2017; 44:1039-1050.
10. Rademacher BL, Matkowskyj KA, Meske LM, Romero A, Sleiman H, Carchman EH. The role of pharmacologic modulation of autophagy on anal cancer development in an HPV mouse model of carcinogenesis. Virology. 2017; 507:82-88.
11. Nguyen-McCarty M, Klein PS. Autophagy is a signature of a signaling network that maintains hematopoietic stem cells. PloS One. 2017; 12:e0177054.
12. Levy JMM, Towers CG, Thorburn A. Targeting autophagy in cancer. Nat Rev Cancer. 2017; 17:528-542.
13. Budini M, Buratti E, Morselli E, Criollo A. Autophagy and its impact on neurodegenerative diseases: New roles for TDP-43 and C9orf72. Front Mol Neurosci. 2017; 10:170.
14. Morandi E, Jagessar SA, t Hart BA, Gran B. EBV infection empowers human B cells for autoimmunity: Role of autophagy and relevance to multiple sclerosis. J Immunol. 2017; 199:435-448.
15. Cosin-Roger J, Simmen S, Melhem H, Atrott K, Frey-Wagner I, Hausmann M, de Valliere C, Spalinger MR, Spielmann P, Wenger RH, Zeitz J, Vavricka SR, Rogler G, Ruiz PA. Hypoxia ameliorates intestinal inflammation through NLRP3/mTOR downregulation and autophagy activation. Nat Commun. 2017; 8:98.
16. Maertin S, Elperin JM, Lotshaw E, Sendler M, Speakman SD, Takakura K, Reicher BM, Mareninova OA, Grippo PJ, Mayerle J, Lerch MM, Gukovskaya AS. Roles of autophagy and metabolism in pancreatic cancer cell adaptation to environmental challenges. Am J Physiol Gastrointest Liver Physiol. 2017; ajpgi.00138.02017. doi: 10.1152/ajpgi.00138.2017.
17. Hu F, Guo XL, Zhang SS, Zhao QD, Li R, Xu Q, Wei LX. Suppression of p53 potentiates chemosensitivity in nutrient-deprived cholangiocarcinoma cells *via* inhibition of autophagy. Oncol Lett. 2017; 14:1959-1966.
18. Zhang W, Wan X, Liu Z, Xiao L, Huang H, Liu X. The emerging role of oxidative stress in regulating autophagy: Applications of cancer therapy. Cell Mol Biol (Noisy-le-grand). 2017; 63:67-76.
19. Pagotto A, Pilotto G, Mazzoldi EL, Nicoletto MO, Frezzini S, Pasto A, Amadori A. Autophagy inhibition reduces chemoresistance and tumorigenic potential of human ovarian cancer stem cells. Cell Death Dis. 2017; 8:e2943.
20. Xu XD, Zhao Y, Zhang M, He RZ, Shi XH, Guo XJ, Shi CJ, Peng F, Wang M, Shen M, Wang X, Li X, Qin RY. Inhibition of autophagy by deguelin sensitizes pancreatic cancer cells to doxorubicin. Int J Mol Sci. 2017; 18:370.
21. Zhang P, Liu X, Li H, Chen Z, Yao X, Jin J, Ma X. TRPC5-induced autophagy promotes drug resistance in breast carcinoma *via* CaMKKβ/AMPKα/mTOR pathway. Sci Rep. 2017; 7:3158.
22. Fang LM, Li B, Guan JJ, Xu HD, Shen GH, Gao QG, Qin ZH. Transcription factor EB is involved in autophagy-mediated chemoresistance to doxorubicin in human cancer cells. Acta Pharmacol Sin. 2017; 38:1305-1316.
23. Cheng CY, Liu JC, Wang JJ, Li YH, Pan J, Zhang YR. Autophagy inhibition increased the anti-tumor effect of cisplatin on drug-resistant esophageal cancer cells. J Biol Regul Homeost Agents. 2017; 31:645-652.
24. Zhou K, Wang L, Cheng R, Liu X, Mao S, Yan Y. Elemene increases autophagic apoptosis and drug sensitivity in human cisplatin (DDP)-resistant lung cancer cell line SPC-A-1/DDP by inducing beclin-1 expression. Oncol Res. 2017. doi: 10.3727/096504017X14954936991990.
25. Li LJ, Li GD, Wei HL, Chen J, Liu YM, Li F, Xie B, Wang B, Li CL. Insulin resistance reduces sensitivity to Cis-platinum and promotes adhesion, migration and invasion in HepG2 cells. Asian Pac J Cancer Prev. 2014; 15:3123-3128.
26. Biederbick A, Kern HF, Elsasser HP. Monodansylcadaverine (MDC) is a specific *in vivo* marker for autophagic vacuoles. Eur J Cell Biol. 1995; 66:3-14.
27. Belounis A, Nyalendo C, Le Gall R, Imbriglio TV, Mahma M, Teira P, Beaunoyer M, Cournoyer S, Haddad E, Vassal G, Sartelet H. Autophagy is associated with chemoresistance in neuroblastoma. BMC Cancer. 2016; 16:891.
28. Li CL, Wei HL, Chen J, Wang B, Xie B, Fan LL, Li LJ. Ebb-and-flow of macroautophagy and chaperone-mediated autophagy in Raji cells induced by starvation and arsenic trioxide. Asian Pac J Cancer Prev. 2014; 15:5715-5719.
29. Cheng J, Chen J, Xie B, Wei HL. Acquired multidrug resistance in human K562/ADM cells is associated with enhanced autophagy. Toxicol Mech Methods. 2013; 23:678-683.
30. Li CL, Wei HL, Chen J, Wang B, Xie B, Fan LL, Li LJ. Arsenic trioxide induces autophagy and antitumor effects in Burkitt's lymphoma Raji cells. Oncol Rep. 2014; 32:1557-1563.
31. Yoshii SR, Mizushima N. Monitoring and measuring autophagy. Int J Mol Sci. 2017; 18: 1865.
32. Fulda S. Autophagy in cancer therapy. Front Oncol. 2017; 7:128.
33. Gao M, Xu Y, Qiu L. Sensitization of multidrug-resistant malignant cells by liposomes co-encapsulating doxorubicin and chloroquine through autophagic inhibition. J Liposome Res. 2017; 27:151-160.
34. Zhang J, Liu X, Lin Y, Li Y, Pan J, Zong S, Li Y, Zhou Y.

HnRNP K contributes to drug resistance in acute myeloid leukemia through the regulation of autophagy. Exp Hematol. 2016; 44:850-856.
35. Zhang Z, Wu B, Chai W, Cao L, Wang Y, Yu Y, Yang L. Knockdown of WAVE1 enhances apoptosis of leukemia cells by downregulating autophagy. Int J Oncol. 2016; 48:2647-2656.
36. Li GY, Liu JZ, Zhang B, Wang LX, Wang CB, Chen SG. Cyclosporine diminishes multidrug resistance in K562/ADM cells and improves complete remission in patients with acute myeloid leukemia. Biomed Pharmacother. 2009; 63:566-570.
37. Pan YZ, Wang X, Bai H, Wang CB, Zhang Q, Xi R. Autophagy in drug resistance of the multiple myeloma cell line RPMI8226 to doxorubicin. Genet Mol Res. 2015; 14:5621-5629.
38. Liu J, Zhang Y, Liu A, Wang J, Li L, Chen X, Gao X, Xue Y, Zhang X, Liu Y. Distinct dasatinib-induced mechanisms of apoptotic response and exosome release in imatinib-resistant human chronic myeloid leukemia cells. Int J Mol Sci. 2016; 17:531.
39. Palorini R, Votta G, Pirola Y, et al. Protein kinase a activation promotes cancer cell resistance to glucose starvation and anoikis. PLoS Genet. 2016; 12:e1005931.
40. Sun H, Zhang M, Cheng K, Li P, Han S, Li R, Su M, Zeng W, Liu J, Guo J, Liu Y, Zhang X, He Q, Shen L. Resistance of glioma cells to nutrient-deprived microenvironment can be enhanced by CD133-mediated autophagy. Oncotarget. 2016; 7:76238-76249.
41. Ding R, Jin S, Pabon K, Scotto KW. A role for ABCG2 beyond drug transport: Regulation of autophagy. Autophagy. 2016; 12:737-751.
42. Zhu C, Yan X, Yu A, Wang Y. Doxycycline synergizes with doxorubicin to inhibit the proliferation of castration-resistant prostate cancer cells. Acta Biochim Biophys Sin (Shanghai). 2017;1-9. doi: 10.1093/abbs/gmx097.
43. Friedhuber AM, Chandolu V, Manchun S, Donkor O, Sriamornsak P, Dass CR. Nucleotropic doxorubicin nanoparticles decrease cancer cell viability, destroy mitochondria, induce autophagy and enhance tumour necrosis. J Pharm Pharmacol. 2015; 67:68-77.
44. Folkerts H, Hilgendorf S, Wierenga ATJ, Jaques J, Mulder AB, Coffer PJ, Schuringa JJ, Vellenga E. Inhibition of autophagy as a treatment strategy for p53 wild-type acute myeloid leukemia. Cell Death Dis. 2017; 8:e2927.

(Received October 13, 2017; Revised October 25, 2017; Accepted November 1, 2017)