Arsenic trioxide (As₂O₃), exhibiting potent antitumor effects both in \textit{in vitro} and \textit{in vivo}, has been used successfully in the treatment of patients with newly diagnosed and relapsed or refractory acute promyelocytic leukemia (APL) (Chen et al., 1997; Shen et al., 1997; Soignet et al., 1998). \textit{In vitro} experiments have shown As₂O₃ can exert apoptosis and induces partial differentiation effects on APL cells (Chen et al., 1997). In addition, it has been reported that the effects of As₂O₃ are not confined to APL cells but can also be observed in a variety of malignant hematopoietic cells such as lymphoid (Akao et al., 1998; Hu et al., 2003), myeloid (Li and Broome et al., 1999), megakaryocytic (Lu et al., 1999), plasma cells (Rousselot et al., 1999; Gartenhaus et al., 2002) and solid tumors such as advanced hormone-refractory prostate cancer, renal cell cancer, cervical cancer and refractory transitional cell carcinoma of the bladder (Anthony, 2001). In cell based assays, As₂O₃ can induce apoptosis on K562 and K562/ADM cell lines. Numerous large cytoplasmic inclusions, abundant autophagic vacuoles, phagocytizing cytoplasm and organelles were observed in As₂O₃-treated cells using electron microscope. MDC-labeled autophagic vacuoles were observed by fluorescent inverted phase contrast microscopy and the enhanced MDC fluorescent staining was detected by flow cytometry in As₂O₃-treated cells. Furthermore, real-time quantitative RT-PCR revealed that the expression levels of Beclin-1 and LC3 genes, which play key roles in autophagy, increased in As₂O₃ treated samples than in controls, indicating that autophagy can potentially be involved in the antitumor properties of As₂O₃. The expression level of Bcl-2 gene, an anti-apoptotic molecule, decreased in As₂O₃ treated samples than in controls, suggesting that Bcl-2 may be involved in accumulating Beclin-1 and triggering autophagic cell death in As₂O₃-treated leukemia cells. Western blotting also showed that As₂O₃ up-regulated Beclin-1. Altogether, our data provide direct evidence that autophagic cell death is critical for the effects of As₂O₃ on acute myelogenous leukemia cells.

Keywords: Arsenic trioxide, autophagy, acute myelogenous leukemia

Introduction

Arsenic trioxide (As₂O₃), exhibiting potent antitumor effects both in \textit{in vitro} and \textit{in vivo}, has been used successfully in the treatment of patients with newly diagnosed and relapsed or refractory acute promyelocytic leukemia (APL) (Chen et al., 1997; Shen et al., 1997; Soignet et al., 1998). \textit{In vitro} experiments have shown As₂O₃ can exert apoptosis and induces partial differentiation effects on APL cells (Chen et al., 1997). In addition, it has been reported that the effects of As₂O₃ are not confined to APL cells but can also be observed in a variety of malignant hematopoietic cells such as lymphoid (Akao et al., 1998; Hu et al., 2003), myeloid (Li and Broome et al., 1999), megakaryocytic (Lu et al., 1999), plasma cells (Rousselot et al., 1999; Gartenhaus et al., 2002) and solid tumors such as advanced hormone-refractory prostate cancer, renal cell cancer, cervical cancer and refractory transitional cell carcinoma of the bladder (Anthony, 2001). In cell based assays, As₂O₃ can induce apoptosis on K562 and K562/ADM cell lines. The apoptosis mechanism may involve both the mitochondrial and endoplasmic reticulum cell death pathway. As₂O₃ is able to reverse the apoptosis resistance in drug-resistant K562/ADM cells by modulating expression or activity of key factors associated with apoptosis induction (Jing et al., 2009; Wang et al., 2005). The mechanisms of action of arsenic trioxide in malignant cells are not well understood though extensive research has been conducted over the years.

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Autophagy, defined as type II programmed cell death (PCD), is independent of phagocytes and differs from apoptosis by the presence of autophagosomes, autolysosomes, and an intact nucleus in the cell (Levine and Klionsky, 2004). Studies have shown that autophagy is not only a survival response to either nutrient deprivation or growth factor but an important mechanism for tumor cell apoptosis and suppression (Gozuacik and Kimchi, 2004). More importantly, some investigations have demonstrated that the co-regulation of both apoptosis and autophagy can participate in mammalian cell death (Cheng et al., 2008). In addition, more recent work shows that As₂O₃ can induce autophagic cell death in malignant glioma cells by up-regulation of mitochondrial cell death protein BNIP3 (Kanzawa et al., 2005); and induce autophagic cell death and apoptosis both in Molt-4 and Mutz-1 cell lines (Qian et al., 2007).

We report here the autophagy effect of As₂O₃ in human leukemia K562 cells and its drug-resistant line K562/ADM cells.

Materials and methods

Cell line and cell culture

Adriamycin-selected and P-gp positive multidrug-resistant human leukemia cell K562/ADM and its parental K562 cells were cultured and passaged in the Central Laboratory of Medical Science, School of Medicine, Lanzhou University. The cells were maintained in RPMI-1640 medium (Gibco, Rockville, MD) supplemented with 10% heat-inactivated fetal bovine serum (Sijiqing Biological Engineering Company Limited, Hangzhou, China), 100 U/mL of penicillin and 100 µg/mL of streptomycin at 37°C in a 5% CO₂ incubator. The cells in mid-log phase were used for experiments. As₂O₃ was dissolved in sterile double-distilled water at a stock concentration of 40 mg/mL, stored at 4°C. RPMI-1640 medium was used to obtain the desired concentration for experiments. Methyladenine (3-MA) (Sigma, USA) was dissolved in heated sterile double-distilled water to make a 100 mmol/L stock solution and then added to the medium after heated for a final concentration of 2 mmol/L.

MDC staining of autophagic vacuoles

Monodansylcadaverine (MDC) (Invitrogen, USA) staining of autophagic vacuoles was performed for autophagy analysis as previously described (Munafó and Colombo, 2001). K562 and K562/ADM cells were divided into control (0 µmol/L As₂O₃ treatment group), As₂O₃ (2 µmol/L and 4 µmol/L) treatment and As₂O₃ combined with 3-MA (Sigma, USA) treatment for experiments. The cells were incubated for 24 h and 48 h. Autophagic vacuoles were labeled with 0.05 mmol/L MDC in PBS at 37°C for 10 min. After incubation, the cells were washed three times with PBS and immediately analyzed under fluorescent inverted phase contrast microscope (Olympus, Tokyo, Japan). Fluorescence of MDC was measured at the excitation wavelength 488 nm with an emission filter at 530 nm.

Transmission electron microscope

K562 cells (2 µmol/L As₂O₃ treated for 48 h) and K562/ADM cells (4 µmol/L As₂O₃ treated for 24 h) were fixed with 2.5% glutaraldehyde in 0.1 mol/L PBS (pH 7.4) for 90 min at room temperature, and post-fixed in 1% osmium tetroxide for 30 min. After being washed with PBS, the cells were progressively dehydrated in a 10% graded series of 50–100% ethanol and propylene oxide, and embedded in Epon 812 resin. The blocks were cut into ultrathin sections with a microtome, which were then stained with saturated uranyl acetate and lead citrate. The ultrastructure of the cells was examined under a transmission electron microscope (JEM-1230, JEOL, Japan).

Flow cytometry

After being treated with As₂O₃, the K562 and K562/ADM cells were collected, labeled with MDC, and the fluorescence staining of cells was analyzed by flow cytometry (EPICS XL, Beckman Coulter, USA).

Real-time quantitative RT-polymerase chain reaction

K562 and K562/ADM cells were cultured and then collected after treatment with As₂O₃ for 24 and 48 h, respectively. Total RNA was isolated from cells using Trizol reagent (Invitrogen, USA) according to its manufacturer’s protocol. RNA concentration and purity were measured with a spectrophotometer at A260 and A260/280, respectively. RNA was reverse-transcribed into cDNA using a PrimeScript™ RT reagent kit (TaKaRa, Dalian, China) according to its manufacturer’s instructions. Real-time quantitative polymerase chain reaction (PCR) was carried out with the SYBR Green fluorescent dye method (SYBR® Premix Ex Taq™ II, TaKaRa, Dalian, China) and a Rotor Gene 3000 real-time PCR apparatus (Corbett Research Company, Australia). The sequences of primers used are as follows:

forward: 5'-AGAGCGATGTTAGTTCTGGAGGC-3' and reverse: 5'-TGTTTTGCTGGCGTGGTGTA-3' for Beclin-1;
forward: 5'-TGCCCTACGACCGGCCCTTCAA-3' and reverse: 5'-TTGTCCTTGGCTCAAGGGCGCA-3' for LC3;
forward: 5'-CGTCAACCGGGAGATGTCGCC-3' and reverse: 5'-CTGGGGCCGTACATTCACCACA-3' for bcl-2;
forward: 5'-GTGGGCGCCCGCCAGCCACCA-3' and reverse: 5'-CTCCTTAATGTGACAGATTTC-3' for β-actin.

β-actin was used as an internal control to evaluate the relative expressions of Beclin-1, LC3 and bcl-2. The PCR conditions were as follows: a pre-denaturing at 95°C for 2 min, followed by 40 cycles of denaturation at 95°C for 1 min, annealing/extension at 60°C for 30 s. The 2-ΔΔCT method was used to calculate the relative abundance of target gene expression generated by Rotor-Gene Real-Time Analysis Software 6.1.81. For each cDNA, the target gene mRNA level was normalized to β-actin mRNA level. Results were expressed as the ratio of normalized target gene mRNA level in cells treated with arsenic trioxide to...
that in cells untreated with arsenic trioxide. The experiments were performed in triplicate.

**Western blotting analysis**
The cells were lysed by adding 2 × SDS lysis buffer (100 mM Tris-HCl, pH 6.8, 4% SDS and 20% glycerol). Samples were then vortexed briefly and incubated in lysis buffer for 10 min in boiling water followed by centrifugation at 12,000 g for 10 min. Protein concentration was determined by the Pierce® BCA Protein Assay Kit. Samples were subjected to sodium dodecyl sulfatepolyacrylamide gel electrophoresis (SDS-PAGE), proteins were transferred onto a nitrocellulose membrane (Roche), and probed with primary antibodies, including Beclin-1 (#3738, Cell signaling, Technology, USA). After incubation with secondary antibodies (IRDye 800CW Donkey anti-Rabbit IgG (H+L), 926–32213, LI-COR; IRDye 800CW Donkey anti-Mouse IgG (H+L), 926–32212, LI-COR), Immunoblots were visualized by Infrared Imaging System (ODYSSEY, LI-COR).

**Statistical analysis**
All data were expressed as mean ± SD. Statistical analysis was performed using the SPSS 16.0 for Window. One-way analysis of variance (ANOVA) was used to analyze statistical differences between groups under different conditions. p < 0.05 was considered statistically significant.

**Results**

**Observation of autophagic vacuolization in cytoplasm by fluorescent inverted phase contrast microscopy**
MDC is a specific marker for autophagic vacuoles. When the cells were viewed under a fluorescent inverted phase contrast microscope, MDC-labeled autophagic vacuoles appeared as distinct dot like structures distributed in cytoplasm or in perinuclear space. The fluorescent density and MDC-labeled particles of K562 and K562/ADM cells were higher in As2O3 treatment group than in control group (Figures 1 and 2), indicating that As2O3 induces formation of MDC-labeled vacuoles. 3-MA, which is generally accepted as a specific inhibitor of autophagy, can decrease the number of autophagic vacuoles (Zhang et al., 2010). Lower fluorescent density was observed in combined 3-MA and As2O3 treatment group, showing that 3-MA exerts its inhibitory effects on As2O3-treated autophagy.

**Transmission electron microscopy revealed formation of autophagosomes in As2O3-treated K562 and K562/ADM cells**
Transmission electron microscopy (TEM), the standard method to detect autophagy (Mizushima, 2004), to further clarify whether the cell vacuolization induced by As2O3 is involved in autophagy, we performed TEM to detect the K562 cells treated with As2O3 at the concentration of 2 µmol/L for 48 h, the K562/ADM cells treated with As2O3 at the concentration of 4 µmol/L for 24 h. The cells not treated with As2O3 exhibited the normal ultrastructural morphology of cytoplasm, organelles and nuclei (Figure 3). The most apparent morphological change in As2O3-treated cells was the formation of double-membranes, abundant autophagic vacuoles phagocytizing cytoplasm and organelles, such as mitochondria and endoplasmic reticulum. Giant autophagosomes filled with degraded organelles, variant mitochondria and autolysosomes were frequently observed. These results

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**Figure 1.** Monodansylcadaverine-labeled vacuoles in K562 cells. Autophagic vacuoles were labeled with 0.05 mmol/L monodansylcadaverine (MDC) in phosphatebuffered saline (PBS) at 37°C for 10 min. The fluorescent density and the MDC-labeled particles in K562 cells were higher in As2O3 treatment group than in control group. The number of MDC-labeled particles in As2O3 cells was significantly lower in combined 3-MA and As2O3 treatment group than in single As2O3 treatment group (>400 magnifications). A: Control; B: 2 µmol/L As2O3 for 24 h; C: 4 µmol/L As2O3 for 24 h; D: 2 µmol/L As2O3 for 48 h; E: 4 µmol/L As2O3 for 48 h; F: 2 µmol/L As2O3+3-MA for 24 h.
Figure 2. Monodansylcadaverine-labeled vacuoles in K562/ADM cells. Autophagic vacuoles were labeled with 0.05 mmol/L monodansylcadaverine (MDC) in phosphatebuffered saline (PBS) at 37°C for 10 min. The fluorescent density and the MDC-labeled particles in K562 cells were higher in As$_2$O$_3$ treatment group than in control group. The number of MDC-labeled particles in As$_2$O$_3$ cells was significantly lower in combined 3-MA and As$_2$O$_3$ treatment group than in single As$_2$O$_3$ treatment group (×400 magnifications). A: Control; B: 2 µmol/L As$_2$O$_3$ for 24 h; C: 4 µmol/L As$_2$O$_3$ for 24 h; D: 2 µmol/L As$_2$O$_3$ for 48 h; E: 4 µmol/L As$_2$O$_3$ for 48 h; F: 4 µmol/L As$_2$O$_3$+3-MA for 24 h.

Figure 3. Transmission electron microscopy showing normal morphology of cytoplasm, cell organelles, and nuclei of K562(A) and K562/ADM(C) cells not treated with As$_2$O$_3$. Characteristic ultrastructural morphology of autophagy, a large number of autophagic vacuoles and double-membrane of K562(B) and K562/ADM(D) cells treated with As$_2$O$_3$. Arrowheads represent double-membrane, autophagosomes, and autolysosomes. A: K562: Control; B: K562:2 µmol/L As$_2$O$_3$ for 48 h; C: K562/ADM: control; D: K562/ADM: 4 µmol/L As$_2$O$_3$ for 24 h.

Figure 4. Flow cytometry detected MDC staining of K562 cells treated with As$_2$O$_3$. A: Control; B: 2 µmol/L As$_2$O$_3$ for 24 h; C: 4 µmol/L As$_2$O$_3$ for 24 h; D: 2 µmol/L As$_2$O$_3$ for 48 h; E: 4 µmol/L As$_2$O$_3$ for 48 h.
demonstrated $\text{As}_2\text{O}_3$ could induce K562 and K562/ADM cells to generate autophagy, which was consistent with the vacuolization obtained by inverted phase contrast microscopy.

**The MDC fluorescent staining of $\text{As}_2\text{O}_3$-treated cells**

MDC staining assay showed $\text{As}_2\text{O}_3$ induced autophagy of K562 and K562/ADM cells (Figures 4–7). Flow cytometry showed the number of MDC-positive cells was 1% in K562 cells not treated with $\text{As}_2\text{O}_3$ and was 20.9% and 82.6% in K562 cells treated with $\text{As}_2\text{O}_3$ at the concentration of 2 µmol/L for 24 h and 48 h; was 48.7% and 50% in K562 cells treated with $\text{As}_2\text{O}_3$ at the concentration of 4 µmol/L for 24 h and 48 h (Figures 4 and 5). It showed the number of MDC-positive cells was 1.42% in K562/ADM cells not treated with $\text{As}_2\text{O}_3$, and was 10% and 33.2% in K562/ADM cells treated with $\text{As}_2\text{O}_3$ at the concentration of 2 µmol/L for 24 h and 48 h; was 49.1% and 40% in K562/ADM cells treated with $\text{As}_2\text{O}_3$ at the concentration of 4 µmol/L for 24 h and 48 h. The results showed the number of MDC positive K562 cells was obvious increased after 2 µmol/L $\text{As}_2\text{O}_3$ treated for 48 h; and the number of MDC positive K562/ADM cells was obvious increased after 4 µmol/L $\text{As}_2\text{O}_3$ treated for 24 h. $\text{As}_2\text{O}_3$-induced autophagy in K562/ADM cells occurred earlier than in K562 cells.

**As$_2$O$_3$ up-regulated mRNA expression of Beclin-1 and LC3 but down-regulated mRNA expression of Bcl-2 in K562 and K562/ADM cells**

In order to understand the molecular mechanism underlying the autophagy induced by $\text{As}_2\text{O}_3$, real-time quantitative RT-PCR was performed to evaluate the effect of $\text{As}_2\text{O}_3$ on mRNA expression of Beclin-1 and LC3, which play key roles in autophagy (Liang et al., 1999; Kabeya et al., 2000). Real-time quantitative RT-PCR showed $\text{As}_2\text{O}_3$ activated the Beclin-1 and LC3 gene expression (Figure 8). At the same time, the mRNA expression of Bcl-2 decreased (Figure 9).

**Up-regulation of Beclin-1 protein contribute to autophagic cell death induced by $\text{As}_2\text{O}_3$**

To examine the mechanism of $\text{As}_2\text{O}_3$-induced autophagic cell death, we next performed western blot assay to evaluate the effect of $\text{As}_2\text{O}_3$ on expression of Beclin-1 protein. The results showed $\text{As}_2\text{O}_3$ induced increase in Beclin-1 protein expression of K562 cells, especially after 2 µmol/L $\text{As}_2\text{O}_3$ treated for 48 h (Figure 10), and an obvious increase in Beclin-1 protein expression of K562/ADM cells after 4 µmol/L $\text{As}_2\text{O}_3$ treated for 24 h (Figure 11), consistent with RT-PCR gene expression profile. The results showed K562 cells treated with $\text{As}_2\text{O}_3$ at the concentration of 2 µmol/L for 48 h occurred the most apparent autophagic variability changes, and then K562/ADM cells treated with $\text{As}_2\text{O}_3$ at the concentration of
4 µmol/L for 24 h resulted in the most apparent autophagic variability changes compared to control groups.

**Discussion**

We have demonstrated As$_2$O$_3$ inhibited both proliferation and viability of K562 and K562/ADM cells in a dose- and time-dependent manner, in which apoptosis is identified as the main mechanism, and the drug-resistant K562/ADM cells were more sensitive to As$_2$O$_3$, previously (Jing et al., 2009; Wang et al., 2005). Although much is known on the mechanism of arsenic trioxide induction of apoptosis, very little is known on the mechanism of
autophagy induction by arsenic trioxide. In this study, we directly examined the ability of As$_2$O$_3$ to induce autophagic cell death in leukemia cell lines.

Our results demonstrated autophagy was activated with As$_2$O$_3$ induced death of K562 and K562/ADM cells, revealing that the mechanism underlying the cytocidal action of As$_2$O$_3$ is more complex.

While under normal conditions, autophagy contributes to the turnover of long-lived proteins and elimination of damaged or aged organelles to maintain cell homeostasis (Yang et al., 2008). Further more, autophagy is implicated in multiple pathological processes, including infection by pathogens, inflammatory bowel disease, neurodegeneration and cancer (Morselli et al., 2009). On the one hand the hypoxia-induced autophagy leads to chemo-resistance of hepatocellular carcinoma cells (Song et al., 2009), whereas there is evidence that inhibition of autophagy appears to enhance the effects of BCR-ABL kinase inhibitors on Ph(+) primary chronic myelogenous leukemia stem cells (Bellodi et al., 2009). All of them indicated autophagy may act as a protective mechanism for malignant cells. On the other hand, more and more recent evidences showed autophagy plays an important role in tumor formation and suppression. Improper autophagy can result in cell death (Yang et al., 2008; Morselli et al., 2009).

Beclin-1, which maps to a tumor susceptibility locus on chromosome 17q21, is a mammalian orthologue of the yeast Apg6/Vps30 gene, is frequently monoallelically deleted in human breast, ovarian, and prostate cancers (Aita et al., 1999). Beclin-1 functions in autophagy as part of class III phosphatidylinositol 3-kinase (PI3k) complex, it binds to PI3KC3 or Bcl-X(L) to form complexes of Beclin-1-PI3KC3 or Beclin-1-Bcl-X(L) (Kihara et al., 1999). Beclin-1 gene transfer increases basal levels of autophagy and nutrient deprivation-induced autophagy in human breast carcinoma cells that lack detectable levels of endogenous Beclin-1 protein (Liang et al., 2001). Beclin-1 could be linked with HIF1a expression and poor survival of nasopharyngeal cancer patients undergoing radiotherapy and chemotherapy (Kim et al., 2008). Blocked autophagy also sensitizes cancer cells to radiation (Apel et al., 2008). Conversely, tumours with repressed Beclin-1 expression seem to bear increased inherent radio resistance because of increased anti-apoptotic potential (Anai et al., 2007). In our study, the mRNA expression level of Beclin-1 and the expression of Beclin-1 proteins in K562 and K562/ADM cells was measured. As$_2$O$_3$ treatment increased the expression of Beclin-1 gene (Figure 8A and 8B) and Beclin-1 protein in K562 and K562/ADM cells (Figures 10 and 11), especially in K562 cells treated with 2 µmol/L As$_2$O$_3$ for 48 h, in K562/ADM cells treated with 4 µmol/L As$_2$O$_3$ for 24 h. Indicating that up-regulation of Beclin-1 may contribute to As$_2$O$_3$- induced autophagy and play an important role in the non-apoptotic cell death induced by As$_2$O$_3$.

As we know, LC3, an autophagosomal marker. During autophagy, autophagosomes engulf cytoplasmic components, including cytosolic proteins and organelles. Concomitantly, a cytosolic form of LC3 (LC3-I) is conjugated to phosphatidylethanolamine to form LC3-phosphatidylethanolamine conjugate (LC3-II), which is recruited to autophagosomal membranes (Tanida et al., 2004). Thus detecting LC3 gene by RT-PCR and LC3 protein by immunoblotting or immunofluorescence has become reliable methods for monitoring autophagy and autophagy-related processes, including autophagic cell death.

We detected the mRNA expression of LC3 gene (Figure 8C and 8D), including LC3I and LC3II, suggesting that arsenic trioxide activates expression of the LC3 gene, which acts later to complete the autophagosome formation. Prompting that not only LC3 protein involves in the process of autophagy, but also the LC3 gene.

The anti-apoptotic protein, Bcl-2, interacts with the evolutionarily conserved autophagy protein, Beclin-1, which not only regulates apoptosis, but also controls non-apoptotic programmed cell death that depends on the autophagy genes (Shimizu et al., 2004). Down-regulation of Bcl-2 in Molt-4 cells exposed to As$_2$O$_3$ suggesting Bcl-2 may contribute to autophagy induced by it (Qian et al., 2007). The interaction between Bcl-2 and Beclin-1 in the endoplasmic reticulum blocks a signal that is essential for the formation of the Beclin 1-Vps34 autophagy-promoting complex (Pattinirge and Levine, 2006). In this study, we found when autophagy is induced, the mRNA expression of Bcl-2 decreased both in K562 and K562/ADM cells (Figure 9).

We found that the obvious autophagic changes induced by As$_2$O$_3$ in K562 and K562/ADM cells occurred at different dose and time. and the autophagy of K562/ADM cells displayed earlier than that of K562 cells. These may partly explain why K562/ADM cells are more sensitive to arsenic trioxide-induced cell death (Jing et al., 2009; Wang et al., 2005).

In conclusion, As$_2$O$_3$ is a potent antitumor agent by inducing cell autophagy besides apoptosis. The relationship between apoptosis and autophagy induced by arsenic trioxide remains unclear, and further study is required to understand the difference of molecular interactions in arsenic trioxide-induced autophagy between drug-sensitive and -resistant leukemia cells, and the relevance of arsenic trioxide-induced autophagy and apoptosis in leukemia.

**Declaration of interest**

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**References**

Chen GQ, Shi XG, Tang W, Xiong SM, Zhu J, Cai X, Han ZG, Ni JH, Shi GY, Jia PM, Liu MM, He KL, Niu C, Ma J, Zhang P, Zhang TD, Paul P,
Soignet SL, Miller W, Waxman S, Wang ZY, de The H, Chen SJ, Chen Z. 1997. Use of arsenic trioxide (As2O3) in the treatment of acute promyelocytic leukemia (APL): I. As2O3 exerts dose-dependent dual effects on APL cells. Blood 89:3345–3353.

Shen ZX, Chen GQ, Ni JH, Li XS, Xiong SM, Qiu QY, Zhu J, Tang W, Sun GL, Yang KQ, Chen Y, Zhou L, Fang ZW, Wang YT, Ma J, Zhang P, Zhang TD, Chen SJ, Chen Z, Wang ZY. 1997. Use of arsenic trioxide (As2O3) in the treatment of acute promyelocytic leukemia (APL): II. Clinical efficacy and pharmacokinetics in relapsed patients. Blood 89:3354–3360.

Soignet SL, Maskal P, Wang ZG, Jhanwar S, Calleja E, Dardashti LJ, Corso DB, Munafò DB, Colombo MI. 2001. A novel assay to study autophagy: regulation of autophagosome vacuole size by amino acid deprivation. J Cell Sci 4:3619–3629.

Zhang QJ, Li YM, Liu T, He WT, Chen YT, Chen XH, Li X, Zhou WC, Yi JR, Ren ZJ et al. 2010. Antitumor effect of matrine in human hepatoma G2 cells by inducing apoptosis and autophagy. World J Gastroenterol 16:4281–4290.

Mizushima N. 2004. Methods for monitoring autophagy. Int J Biochem Cell Biol 36:2491–2502.

Liang XH, Jackson S, Seaman M, Brown K, Kemptes K, Hibihoosh H, Levine B. 1999. Induction of autophagy and inhibition of tumorigenesis by beclin 1. Nature 402:672–676.

Kabeya Y, Mizushima N, Ueno T, Yamamoto A, Kirisako T, Noda T, Kominami E, Ohsumi Y, Yoshimori T. 2000. LC3, a mammalian homologue of yeast Apg8p, is localized in autophagosome membranes after processing [I]. EMBO J 19:5720–5728.

Kroemer G. 2009. Anti- and pro-tumor functions of autophagy. Biochim Biophys Acta 1793:1524–1532.

Morselli E, Galluzzi L, Kepp O, Vincenzi JM, Criollo A, Maiuri MC, Kroemer G. 2009. Hypoxia-induced autophagy contributes to the chemoresistance of hepatocellular carcinoma cells. Autophagy 5:1131–1144.

Bellodi C, Lidonnici MR, Hamilton A, Helgason GV, Soliera AR, Ronchetti M, Galavotti S, Young KW, Selmi T, Yacobi R, Van Etten RA, Donato N, Hunter A, Dinsdale D, Tirrò E, Vigneri P, Nicotera P, Dyer MJ, Holynke T, Salomoni P, Calabretta B. 2009. Targeting autophagy potentiates tyrosine kinase inhibitor-induced cell death in Philadelphia chromosome-positive cells, including primary CML stem cells. J Clin Invest 119:1109–1123.

Aita VM, Liang XH, Murty VV, Pincus DL, Yu W, Cayanis E, Kalachikov S, Gilliam TC, Levine B. 1999. Cloning and genomic organization of beclin 1, a candidate tumor suppressor gene on chromosome 17q21. Genomics 59:59–65.

Liang XH, Yu J, Brown K, Levine B. 2001. Beclin 1 contains a leucine-rich nuclear export signal that is required for its autophagy and tumor suppressor function. Cancer Res 61:3443–3449.

Kim KW, Hwang M, Moretti L, Jabooin J, Cha YJ, Lu B. 2008. Autophagy upregulation by inhibitors of caspase-3 and mTOR enhances radiotherapy in a mouse model of lung cancer. Autophagy 4: 659–668.

Apel A, Herr I, Schwarz H, Rodeffmann HP, Mayer A. 2008. Blocked autophagy sensitizes resistant carcinoma cells to radiation therapy. Cancer Res 68:1485–1494.

Anai S, Goodison S, Shiverick K, Hirao Y, Brown BD, Rossor CJ. 2007. Knock-down of Becl-2 by antisense oligodeoxynucleotides induces radiosensitization and inhibition of angiogenesis in human PC-3 prostate xenografts. Mol Cancer Ther 6:101–111.

Kihara A, Kabeya Y, Ohsumi Y, Yoshimori T. 2001. Beclin-1 and autophagic vacuole formation. Cell 102:1055–1060.

Ronchi S, Trevisan M, Beretta S, Zucali R, Ielpo L. 2011. Induction and regulation of autophagy in human glioma cells by upregulation of mitochondrial cell death protein BNIP3. Oncogene 30:477–479.

Munafò DB, Colombo MI. 2001. A novel assay to study autophagy: regulation of autophagosome vacuole size by amino acid deprivation. J Cell Sci 4:3619–3629.