Combination of arsenic trioxide and cisplatin synergistically inhibits both hexokinase activity and viability of Ehrlich ascites carcinoma cells

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Abstract
Hexokinase-2 is overexpressed in several carcinomas including breast cancer to sustain energy for rapidly dividing cells and associates with chemoresistance. However, the impact of chemo drugs (alone or in combination) on hexokinase activity and autophagic cell death is unclear. In this report, we used an in vivo murine adenocarcinoma model to validate the effects of As2O3 and cisplatin on hexokinase activity and autophagic cancer cell death. We found that the two drugs inhibit hexokinase activity and induce autophagic marker, beclin 1 expression. Interestingly, combining As2O3 with cisplatin synergistically enhanced these effects and alleviated oxidative stress often encountered in As2O3 treatment. Altogether, our data provide direct evidence that inhibition of hexokinase activity and induction of autophagic cell death are mediating the antineoplastic effects of As2O3 and cisplatin. Our findings raise the potential of combining As2O3 with cisplatin as an approach to augment cisplatin-induced cell death and combat cisplatin chemoresistance in cancer.

Keywords
arsenic trioxide, autophagy, beclin 1, cancer, cisplatin, Ehrlich ascites carcinoma, hexokinase

1 INTRODUCTION
Autophagy is an evolutionarily conserved intracellular self-eating pathway in all eukaryotic cells. It involves the maintenance of cellular homeostasis and quality control of cellular components by the catabolic degradation of damaged proteins, cellular organelles, and other cytoplasmic components. In this pathway, the damaged components are engulfed in autophagosomes which are double-membrane vesicles and then carried to lysosomes for final degradation and recycling of degradative products back into the cytoplasm. Due to the crucial biological function of this conserved process, its dysregulation leads to various disorders such as neurodegeneration, microbial infection and most importantly cancer. In the case of cancer, extensive and elaborate research has been conducted to understand the paradoxical roles of autophagy in cancer which seems to either promote or suppress tumorigenesis in a context-dependent manner.

Ehrlich ascites carcinoma (EAC) is an in vivo model of spontaneous murine mammary adenocarcinoma. The advantage of this cancer model is that its cells can be carried in outbred mice by serial intraperitoneal (I.P.) passages. The reason behind its rapid proliferation in any mouse host is the lack of H-2 histocompatibility antigens. This made this model useful for investigations of biochemical alterations in cancer cells after chemotherapy such as hexokinases (HKs) activity. HK is a class of enzymes responsible for phosphorylation of glucose to glucose-6-phosphate to catalyze the first step of glucose metabolism. Hexokinase-2 (HK2), also known as an oncogenic kinase is upregulated in several carcinomas including breast, colorectal, glioma, liver, and ovarian cancers which are associated with enhanced aerobic glycolysis (Warburg effect). Its overexpression helps to sustain cancer cell growth through maintenance of growth factor-independent glucose metabolism after depletion of growth factors. Accumulating evidence indicates the dual role of HK2 in glycolysis and autophagy. Although there is an
increase of the understanding of both processes in cancer, the interrelationship between both pathways in cancer has not been fully elucidated. In efforts to search for novel HK2 inhibitors by in silico molecular docking analysis, we found the antileukemic drug, arsenic trioxide (ATO), to inhibit HK2 activity.

Arsenic trioxide (As$_2$O$_3$) (ATO) is a metalloid with potent antineoplastic effects in several types of cancer especially acute promyelocytic leukemia and breast cancer.$^{[12,13]}$ ATO-dependent effect is thought to include the generation of reactive oxygen species (ROS) leading to the activation of proapoptotic pathways in different cancer cells.$^{[14]}$ Also, ATO is reported to activate the c-Jun N-terminal kinase (JNK) signaling and inhibit the AKT activity in cancer cells.$^{[15,16]}$ Due to the cytotoxicity and low efficacy of ATO when it is used alone, a combination of ATO with other chemotherapeutic drugs may provide a rational basis for novel therapeutic combinations. Despite these efforts, the specific cellular events that account for differential effects of ATO on cancer cells’ viability and the possible therapeutic combinations remain to be well-defined.

In this study, we provide evidence that ATO and cisplatin have a synergistic effect on the induction of autophagy in EAC cells. Based on the in silico molecular docking analysis, cisplatin and arsenic trioxide was found to inhibit the activity of HK2 and induce expression of the autophagic marker beclin 1 (BECN1) to suppress tumorigenesis. Our data demonstrate the synergistic antitumor effect of arsenic trioxide with cisplatin in EAC, which may have clinical implications in future studies.

2 | MATERIALS AND METHODS

2.1 | Animals

Female BALB/c mice, weighing 20-25 g purchased from National Cancer Institute (Cairo University, Egypt), were housed at the animal facility at Medical Biochemistry Department, Faculty of Medicine, Tanta University, Egypt. Mice were maintained on laboratory standard experimental conditions (temperature 23°C ± 2°C, relative humidity 55%, balanced diet and free access to water). The mice were kept for 1 week before the start of the experiment for acclimatization. All animal experiments were performed in accordance with the ARRIVE guidelines and in accordance with the UK Animals (Scientific Procedures) Act, 1986 and associated guidelines. The research plan was approved by the Ethical Committee of Faculty of Science, Tanta University, Egypt.

2.2 | Experimental design

Mice were divided into eight groups (10 mice each). The eight groups were as follows: GP I: Naïve (0.2 mL of saline; I.P.), GP II: EAC (0.5 × 10$^6$ cells; I.P.), GP III: CIS group (0.2 mL of saline; I.P. + CIS (40 µg/mouse), GP IV: ATO group (0.2 mL of saline; I.P.) + ATO (5 mg/kg), GP V: ATO & CIS group (0.2 mL of saline; I.P.) + ATO (5 mg/kg) + CIS (10 µg/mouse). Arsenic trioxide and cisplatin were purchased from Sigma-Aldrich (St Louis, MO). The drugs were dissolved in phosphate buffer saline (PBS) with the appropriate doses.

2.3 | Hematological analyses

Estimation of hemoglobin (Hb) content and count of platelets, WBCs, lymphocytes, and monocytes were performed using standard automated procedures as described elsewhere.

2.4 | Trypan blue test

Two weeks after tumor inoculation, EAC cells were isolated from the peritoneal cavity of mice from each group, suspended in sterile isotonic saline, mixed with a 0.4% trypan blue staining and counted in a hemocytometer. Percentage of viability (%) = (number of viable cells x 100)/total number of cells. Also, the ascitic volume was measured accordingly.

2.5 | Nuclear/cytosol fractionation

The Nuclear/cytosol fractionation kit (Biovision Incorporated, Milpitas, CA) was used to provide the separation of the nuclear extract from the cytoplasmic and membranous fractions. Briefly, 0.2 mL of Cytosol Extraction Buffer A-Mix was added to a volume of EAC cells (2 × 10$^6$), then vortexed and 11 µL of ice-cold Cytosol Extraction Buffer-B was added. The samples were centrifuged in a microcentrifuge (16,000×g). The supernatant was the cytoplasmic extract and 100 µL of ice-cold nuclear extraction buffer mix was added to the pellet then vortexed every 10 minutes for a total 40 minutes. The samples were further centrifuged in a microcentrifuge (16,000×g) and the supernatant was the nuclear extract whereas the pellet was the membranous extract.

2.6 | HK colorimetric assay

In the hexokinase assay kit (Biovision Incorporated), glucose is converted to glucose-6-phosphate by HK, then glucose-6-phosphate is oxidized by a glucose-6-phosphate dehydrogenase to form nicotinamide adenine dinucleotide, which reduces a colorless probe to a colored product with a strong absorbance at 450 nm. Briefly, 200 µL of HK assay buffer was added to the membranous extract, and then centrifuged at 13,000×g for 10 minutes in a microcentrifuge. A total of 50 µL was taken from the supernatant and mixed with 50 µL of the reaction mix. The activity of HK in the membranous fractions of the samples was measured accordingly.

2.7 | Mouse BECN1 determination by enzyme-linked immunosorbent assay

The mouse beclin 1 Elisa kit was purchased from Sunredbio, Shanghai, China. The assay uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assess the level of
mouse BECN1 in the cytosolic fractions of the samples. BECN1 containing samples were added to wells pre-coated with mouse BECN1 monoclonal antibody, then, BECN1 antibody labeled with biotin was added, and combined with Streptavidin-HRP to form the immune complex. Chromogen Solutions A & B were added and the concentration of BECN1 was calculated accordingly.

2.8 | Estimation of malondialdehyde concentration

The liver specimens were dissected, thoroughly washed with ice-cold 0.9% NaCl and homogenized (10% w/v) using phosphate buffer saline (pH 7.0). The liver tissue homogenate was used for the estimation of hepatic concentrations of the lipid peroxidation biomarker malondialdehyde (MDA) and the total antioxidant capacity (TAC). The MDA is one of the terminal products formed at the time of the decomposition of polyunsaturated fatty acids caused by free radicals. For MDA, 0.5 mL of trichloroacetic acid (20%) and 1 mL of thiobarbituric acid (0.67%) were mixed with 0.5 mL of the liver homogenate. The optical density of the supernatant was measured using Shimadzu UV-visible spectrophotometer at 530 nm.

2.9 | Ferric reducing antioxidant capacity

The method measures the ferric reducing ability (FRAP) of the samples which correlates with the antioxidant (enzymatic & non-enzymatic) status. At low pH, when a ferric tripyridyltriazine (FeIII-TPTZ) complex is reduced to the ferrous form (FeII), an intense blue color with an absorption maximum at 593 nm develops. The rate-limiting factor of (FeII-TPTZ), and hence color formation is the reducing ability of the sample.

2.10 | Histopathological investigation

Hepatic tissues were excised from the mice after surgery and fixed in 10% paraformaldehyde saline for 24 hours. Then, the specimens were washed in tap water, dehydrated with serial dilutions of alcohols (methyl, ethyl, and absolute ethyl), cleared in xylene and embedded in paraffin at 56°C in a hot air oven for 24 hours. Sectioning of the paraffin wax tissue blocks was performed at 4 μm by the microtome. The sections were collected on glass slides, deparaffinized and stained by hematoxylin and eosin stain for routine examination under an electric microscope.

2.11 | In silico molecular docking

The molecular docking software, HEX 8.0.0, was used to study the arsenic trioxide or cisplatin–HK2 interactions. HEX is a molecular interactive graphics program used for calculating and displaying feasible docking poses of these chemicals in the active pocket of HK2. The crystal structure of the human HK2 (PDB ID: 2NZT) was downloaded from the Protein Data Bank (https://www.rcsb.org/pdb/explore.do?structureId=2NZT) and the Mol2 files of arsenic trioxide and cisplatin were obtained from http://pubchem.ncbi.nlm.nih.gov/. The Mol2 files were further converted into PDB format using the University of California San Francisco (UCSF) chimera software (San Francisco, CA). Preparation of the docking analysis was done by UCSF Chimera. The HEX 8.0 software performs in silico docking using spherical polar Fourier correlations. It requires the ligand (ATO or CIS) and the receptor (HK2) as input in PDB format. The parameters that were used for docking include fast Fourier transform mode—3D, correlation type—shape only, grid dimension—0.6, ligand range—180, receptor range—180, distance range—40, and twist range—360. Visualization of the docked poses was performed by the UCSF Chimera.

2.12 | Statistical analysis

The results were presented as the mean ± standard error (SE). Statistical analyses were performed by unpaired t test using SPSS statistical version 16 software package (SPSS Inc., Armonk, NY). P values (*P < 0.05, **P < 0.01, and ***P < 0.001) were considered statistically significant.

3 | RESULTS

3.1 | EAC in vivo model

EAC is an in vivo mouse model with a spontaneous murine adenocarcinoma. The advantages of this cancer model are various since it is adapted to ascites form and can be transferred in outbred mice using serial I.P.
passages. In the current study, EAC cells were collected from the ascitic fluid of BALB/c mice with 10 days old ascitic tumor. A total of 80 BALB/c female mice were randomly divided into eight groups with 10 mice each as shown in Figure 1. In EAC groups, $0.5 \times 10^6$ cells were injected I.P. in the mice (day 0) and after 24 hours the mice were treated with cisplatin (CIS; 40 µg/mouse), arsenic trioxide (5 mg/kg) or both CIS (10 µg/mouse) and ATO (5 mg/kg) I.P. consecutively for 12 days (Figure 1). The eight groups are as follows:

GP I: Naïve (0.2 mL of saline; I.P.)
GP II: EAC (0.5 x $10^6$ cells; I.P.)
GP III: CIS group ([0.2 mL of saline; I.P.] + CIS [40 µg/mouse])
GP IV: ATO group ([0.2 mL of saline; I.P.] + ATO [5 mg/kg])
GP V: ATO & CIS group ([0.2 mL of saline; I.P.] + ATO [5 mg/kg] + CIS [10 µg/mouse])
GP VI: CIS-treated EAC ([0.5 x $10^6$ cells; I.P.] + CIS [40 µg/mouse])
GP VII: ATO-treated EAC ([0.5 x $10^6$ cells; I.P.] + ATO [5 mg/kg])
GP VIII: ATO & CIS-treated EAC ([0.5 x $10^6$ cells; I.P.] + ATO [5 mg/kg] + CIS [10 µg/mouse])

Notably, we used the higher dose of CIS (40 µg/mouse) when it is administered alone and the lower dose (10 µg/mouse) when it is administered with ATO (5 mg/kg). After 12 days of drug administration (I.P.), the mice were euthanized and the ascitic fluid was collected from the peritoneal cavity of each mouse to evaluate its volume and the viability of cancer cells by trypan blue assay.

The total body weights of the mice were recorded regularly on predetermined days. The final bodyweight (day 12) was increased as compared to the initial day in all the experimental groups except in case of CIS, ATO, and ATO and CIS groups where they showed a decrease (Figure 2A). Interestingly, the impact of these chemicals on the body weights of EAC mice was improved as compared to control mice (Figure 2A and 2B). Moreover, the cytotoxicity of the drugs on control and EAC mice was monitored by performing the complete blood count. As shown in Figure 2C, Hb levels (g/dL) were within the normal range (10.2-16.6 g/dL) except in case of ATO and CIS-treated EAC mice with Hb level 6.39 g/dL. The count of platelets, WBCs, lymphocytes, and monocytes was within the normal range of the laboratory animal values.
Combination of arsenic trioxide and cisplatin reduces cancer cell viability

To evaluate the antitumor efficacy of CIS, ATO or the combination against EAC, we can either examine the cells cytologically after treatment or measure the volume of an ascitic fluid formed after therapy. As shown in Figure 3A, the viability of cancer cells was significantly reduced in CIS- and ATO-treated EAC cells and a more significant decrease is observed in the combination group (ATO and CIS-treated EAC). Figure 3B further exhibited a significant decrease in the ascitic fluid volume collected after therapy in the EAC groups treated with CIS, ATO or the combination.

To investigate ROS generation in these groups, measured the oxidative stress marker MDA and the TAC in liver tissues. As shown in Figure 3C and 3D, there is a significant increase of MDA and a significant decrease in TAC in ATO- and CIS-treated EAC groups as compared to EAC group. Interestingly, combining both ATO and CIS in ATO and CIS-treated EAC decreased MDA level and augmented the level of TAC. Together, these findings indicate that treatment of ATO with CIS enhances the antineoplastic effect of CIS and ameliorates the ROS damage caused by each drug alone.

Histopathologically, in the control naïve mice, there was no histopathological alteration and the histological architecture of the

![Graphs showing viability of cancer cells, ascitic volume, MDA, and TAC levels in different treatment groups.](image-url)
central vein and hepatocytes in the parenchyma was normal (Figure 4A). However, in EAC group, a group of neoplastic cells were embedded in the hepatic parenchyma at the portal area surrounding the dilated portal vein, associated with diffuse Kupffer cells proliferation in between the hepatocytes (Figure 4B). Moreover, vascular degeneration was observed in the hepatocytes all over the parenchyma. In CIS-treated EAC group, there is diffuse Kupffer cells proliferation in between the hepatocytes (Figure 4C). In ATO-treated EAC group, massive inflammatory cells aggregation was detected in the portal area, and vascular degeneration was observed in the hepatocytes all over the parenchyma (Figure 4D). In ATO- and CIS-treated EAC group, the hepatic parenchyma showed the focal area of necrosis with inflammatory cells infiltration (Figure 4E). The combination therapy is thought to replenish the hepatic tissue with the normal TAC/ROS balance to reduce the aberrations found in the EAC group. The detailed histopathological assessment of these groups is shown in Table 1.

### 3.3 Arsenic trioxide and cisplatin treatments inhibit HK activity and induce autophagy

To get into more mechanistic insight, we sought to determine whether treatment of cells with cisplatin, arsenic trioxide, or the combination results in a change of the activity of the metabolic key enzyme HK and/or induction of autophagy. For that purpose, after collecting the cells, nuclear/cytosol fractionation protocol was performed to provide separation of the cytosolic fraction from the nuclear and membranous fraction. Due to the subcellular localization of HK in the mitochondria, we assayed its activity in the membranous fraction. As shown in Figure 5A,

### Table 1 The severity of histopathological alterations in the liver of different experimental groups

| Groups                        | Histopathological Alteration | Normal group | EAC group | CIS-treated EAC group | ATO-treated EAC group | ATO and CIS-treated EAC group |
|-------------------------------|------------------------------|--------------|-----------|-----------------------|-----------------------|-----------------------------|
|                               | Vacuolar degeneration in hepatocytes | -            | +++       | -                     | -                     | +++                         |
|                               | Focal necrosis in parenchyma   | -            | -         | -                     | -                     | -                           |
|                               | Portal inflammatory reaction   | -            | -         | +++                   | -                     | -                           |
|                               | Neoplastic cells in the parenchyma | -            | ++        | -                     | -                     | -                           |
|                               | Kupffer cells proliferation    | -            | +         | +++                   | -                     | -                           |
|                               | Congestion in central vein     | -            | -         | +                     | -                     | -                           |
|                               | Congestion in portal vein      | -            | -         | -                     | -                     | -                           |
|                               | Extravasation of red blood cell| -            | -         | -                     | -                     | +                           |
|                               | Focal inflammatory cell aggregation in portal vein | - | - | - | - | - |

Abbreviations: ATO, arsenic trioxide; CIS, cisplatin; EAC, Ehrlich ascites carcinoma.
treatment of EAC mice with CIS or ATO significantly decreased the activity of HK. Combination treatment with CIS and ATO further reduced the activity of the enzyme as compared to CIS or ATO treatment alone.

To assess the impact of these drugs on the autophagic flux of cancer cells, we measured the expression level of the autophagic marker beclin 1 in the cytosol fraction by ELISA. Despite its subcellular location in endosomes, nucleus, golgi apparatus, and mitochondria, beclin 1-VPS34-VPS15 forms a proautophagic complex with Atg14L or UV radiation resistance-associated gene (UVRAG) in the cytosol. Figure 5B exhibits induction of the BECN1 expression and stability by treatment with either CIS or ATO with a further induction by the combination treatment.

To assess whether there is an association between the inhibition of HK activity and the induction of autophagy in EAC cells, we analyzed the correlation between the mRNA levels of HK2 and BECN1 genes. ATO, arsenic trioxide; BECN1, beclin 1; CIS, cisplatin; EAC, Ehrlich ascites carcinoma; ELISA, enzyme-linked immunosorbent assay; HK, hexokinase; HK2, hexokinase-2; mRNA, messenger RNA; TCGA, The Cancer Genome Atlas.

FIGURE 5 Combination of arsenic trioxide and cisplatin inhibits HK activity and upregulates beclin 1 expression. A, Hexokinase activity assay was performed by ELISA and the graph indicates the average hexokinase activity in each group (*P < 0.05, **P < 0.01, ***P < 0.001). B, The expression of beclin 1 in the cytosolic fraction was estimated by ELISA and the graph indicates the average beclin 1 level in each group (*P < 0.05, **P < 0.01, ***P < 0.001). C, Analysis of TCGA breast cancer database using cBioPortal showing the correlation between HK2 and BECN1 mRNA levels. D, Genetic alterations of HK2 and BECN1 in patients with breast cancer in TCGA database were analyzed for HK2 and BECN1 genes. ATO, arsenic trioxide; BECN1, beclin 1; CIS, cisplatin; EAC, Ehrlich ascites carcinoma; ELISA, enzyme-linked immunosorbent assay; HK, hexokinase; HK2, hexokinase-2; mRNA, messenger RNA; TCGA, The Cancer Genome Atlas.
a nonsignificant correlation between HK2 and BECN1 mRNA levels indicating that the two proteins might work independently. Furthermore, the genetic alteration landscape of HK2 and BECN1 was analyzed by TCGA in the same data set. Interestingly, HK2 alterations were found in 4% of patients with overall mRNA upregulation and poor prognosis. However, BECN1 showed genetic aberration in 8% of patients of the same data set with overall mRNA downregulation in cancer. These analyses underscore the cancer-promoting and tumor-suppressing functions of HK2 and BECN1, respectively.

3.4 | Arsenic trioxide and cisplatin bind to HK2 in silico

To understand the molecular mechanism of the inhibitory effect of arsenic trioxide on the activity of HK2, we performed an in silico molecular docking analysis using the molecular interactive docking software HEX 8.00. HEX calculates and displays the feasible docking modes of protein-ligand interaction. This allows a molecular insight into the interaction between arsenic trioxide 3D structure imported from PubChem database and the HK2 crystal structure (PDB: 2NZT) imported from the RCSB protein data bank. Thus, we could identify the specific amino acids residues involved directly in the arsenic-HK2 interaction. Since arsenic trioxide is known to bind preferentially to cysteine residues, Figure 6D and 7B exhibit arsenic to bind to Cys256, Cys704, and Cys717 with a total binding energy -95.24 kcal/mol (Figure 6F).

Dissection of the crystal structure of HK2 (PDB: 2NZT) reveals that the protein is composed of C-terminus catalytic activity and N-terminus regulatory function as shown in Figure 6A. Asp205, Lys169, Asn204, Glu256, and Thr168 constitute the active site residues of HK2 and are positioned in the cleft at the interface between HK2 lobes. Moreover, the cysteine residues are in close proximity (10 Å) to one of the active sites of HK2 especially Cys256 and Cys704. However, Cys717 is >15 Å far from the active site. Hence, the arsenic binding to these residues is thought to be responsible for the inhibitory effect of this metalloid on HK2 activity. On the other hand, cisplatin binding takes place exclusively at the level of Arg69 (1.47 Å), Lys162 (1.5 Å), Leu163 (1.5 Å). These interactions are expected to confer the mechanism by which cisplatin inhibits the activity of HK2. The total binding energy of cisplatin with HK2 residues is -136.82 kcal/mol as shown in Figures 6E, 6F, and 7A.

![FIGURE 6 Arsenic trioxide and cisplatin have direct interaction with HK2.](image-url) A, The 3D modeling of the two chains of HK2 with the ribbon structure form. B, The 3D modeling of As$_2$O$_3$. C, The 3D modeling of cisplatin. D, The 3D modeling of As$_2$O$_3$ binding to HK2. E, The 3D modeling of cisplatin binding to HK2. F, The total binding energy of As$_2$O$_3$ or cisplatin binding to HK2 was calculated by HEX 8.00 and tabulated. 3D, three dimension; ATO, As$_2$O$_3$, arsenic trioxide; HK2, hexokinase-2.

| Ligand        | Receptor   | Total Energy (kcal/mol) (lowest) | Total Energy (kcal/mol) (highest) |
|---------------|------------|----------------------------------|-----------------------------------|
| Cisplatin     | Hexokinase 2 | -95.45                           | -136.82                           |
| Arsenic trioxide | Hexokinase 2 | -70.75                           | -95.24                            |
4 | DISCUSSION

In normal instances, autophagy removes misfolded proteins, damaged organelles, and oxidative stress, and reduces genomic instability, thereby acts as a tumor suppressor mechanism.[2] This function is obvious in the early stages of cancer, however, when cancer cells start to metastasize, it utilizes autophagy to sustain the enhanced growth rate which results in ROS generation. Hence, autophagy has a tumor-promoting function in advanced stages of cancer.[18,19] In the current study, the upregulation of the autophagy-related protein, BECN1, expression is associated with suppression of tumorigenesis supporting the nature of the tumor-suppressing function of autophagy in EAC. Similarly, evidence suggests the tumor suppressor role of BECN1 since monoallelic deletion of BECN1 has been observed in prostate, ovarian and breast cancers.[20-22]

Mechanistically, BECN1 induces autophagy by binding and activating Vps34 via a conserved domain required for its tumor-suppressing activity.[23] Also, evidence suggests that the loss of BECN1-positive regulators, UVRAG and Bif-1, reduces binding of BECN1 with Vps34, hence decreases autophagy and promotes tumorigenesis.[24] These evidence suggest the tumor-suppressing effect of autophagy especially in the early stages of cancer.

Our findings also showed that arsenic trioxide worked synergistically with cisplatin to induce the expression of the autophagic marker BECN1. Moreover, the combination therapy (CIS + ATO) alleviated ATO-mediated oxidative stress generation. Goussetis et al.[25] showed that arsenic trioxide has an antileukemic effect via induction of apoptosis and autophagy in cancer cells. As an apoptotic inducer, ATO increases the generation of ROS, hence induces caspase-dependent proapoptotic signals by
targeting the thioredoxin system.[26] Evidence has also shown that activation of JNK/MAPK is crucial for apoptotic cascade induction in leukemia.[27] On the other hand, arsenic trioxide induces cancer cell death by induction of autophagy in a caspase-independent manner.[28] Such induction is speculated to be dependent on the activation of MEK/ERK cascade but unrelated to JNK pathway.[29]

Similarly, cisplatin activates autophagy in cancer cells via inhibition of PI3K/Akt/mTOR and activation of ERK pathways via generation of ROS and DNA damage.[11]

Here, we found that both cisplatin and arsenic trioxide can inhibit the activity and directly bind to HK2 in cancer cells. This effect is synergistically enhanced when arsenic trioxide is given at a lower dose of cisplatin in combination. These observations suggest the favorable impact of ATO to increase the efficacy of cisplatin in vivo by abrogation of HK activity responsible for cisplatin resistance in cancer cells. Further, we propose that autophagy and HK activity work independently as two axes mediating the antineoplastic effects of the combination therapy with cisplatin and arsenic trioxide. Our findings clearly indicate that combining the two drugs could enhance the antitumor role of cisplatin and arsenic trioxide. Ou r findings clearly indicate that ATO with CIS may be a new therapeutic strategy to combat cisplatin chemoresistance in cancer.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

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REFERENCES

[1] Y. Kondo, T. Kanzawa, R. Sawaya, S. Kondo, Nat. Rev. Cancer 2005, 5(9), 726.
[2] P. Codogno, M. Mehrpour, T. Proikas-cezanne, Nat. Rev. Mol. Cell Biol. 2011, 13(1), 7.
[3] E. White, J. M. Mehnert, C. S. Chan, Clin. Cancer Res. 2015, 21(22), 5037.
[4] M. E. Papandreou, N. Tavernarakis, Biotechnol. J. 2017, 12(1), 1600175.
[5] L. Chen, J. F. Watkins, Nature 1970, 225(5234), 734.
[6] M. J. Birnbaum, Dev. Cell. 2004, 7(6), 781.
[7] Y. Gao, Y. Yang, F. Yuan, J. Huang, W. Xu, B. Mao, Z. Yuan, W. Bi, Oncogene 2017, 6(9), e383.
[8] A. V. Kudryavtseva, M. S. Fedorova, A. Zhavoronkov et al., BMC Genet. 2016, 17(Suppl 3), 156.
[9] H. Liu, N. Liu, Y. Cheng, W. Jin, P. Zhang, X. Wang, H. Yang, X. Xu, Z. Wang, Y. Tu, PLOS One. 2017, 12(12), e0189353.
[10] L. Jiao, H. L. Zhang, D. D. Li et al., Autophagy 2017, 1.
[11] X. Y. Zhang, M. Zhang, Q. Cong, M. X. Zhang, M. Y. Zhang, Y. Y. Lu, C. J. Xu, Int. J. Biochem. Cell Biol. 2017, 95, 9.
[12] H. H. Zhu, Z. P. Guo, J. S. Jia, Q. Jiang, H. Xiang, J. X. Huang, Leuk. Res. 2017, 65, 14.
[13] Y. Shi, T. Cao, H. Huang, C. Lian, Y. Yang, Z. Wang, J. Ma, J. Xia, Cell Cycle. 2017, 16(24), 2396.
[14] J. Chayapong, H. Madhyastha, R. Madhyastha, Q. I. Nurrahmah, Y. Nakajima, N. Choijookhuu, Y. Hishikawa, M. Maruyama, Environ. Sci. Pollut. Res. Int. 2017, 24(6), 5316.
[15] N. Gafis, E. Katsoulidis, A. Sassano, M. S. Tallman, L. S. Higgins, A. R. Nebreda, R. J. Davis, L. C. Platanias, Cancer Res. 2006, 66(13), 6763.
[16] K. K. Mann, M. Colombo, W. H. Miller, Mol. Cancer Ther. 2008, 7(6), 1680.
[17] D. C. Koboldt, R. S. Fulton, M. D. McLellan, H. Schmidt, J. Kalicki-Veizer, J. F. Michaelson, L. L. Fulton, D. J. Dooling, L. Ding, E. R. Mardis, R. K. Wilson, A. Arly, M. Balasundaram, Y. Butterfield, R. Carlsen, C. Carter, A. Chu, E. Chua, H. E. Chiu, D. Coope, N. Dhalla, R. Guin, C. Hirst, M. Hirst, R. A. Holt, D. Lee, H. I. Li, M. Mayo, R. A. Moore, A. J. Mungall, E. Pleasance, A. Gordon Robertson, J. E. Schein, A. Shafiei, P. Pipahimalani, J. R. Slobodan, D. Stoll, A. Tam, N. Thiesen, R. J. Varhol, N. Wye, T. Zeng, Y. Zhao, I. Birol, S. Jones, M. A. Marra, A. D. Cherniak, G. Sakasena, R. C. Onofrio, N. H. Pho, S. L. Carter, S. E. Schumacher, B. Tabak, B. Hernandez, J. Gentry, H. Nguyen, A. Crenshaw, K. Ardlie, R. Beroukhim, W. Winckler, G. Getz, S. B. Gabriel, M. Meyerson, L. Chin, P. J. Park, R. Kucherlapati, K. A. Hoadley, J. Todd Auman, C. Fan, Y. J. Turman, Y. Shi, L. Li, M. D. Topal, X. He, H. H. Chao, A. Prat, G. O. Silva, M. D. Iglesia, W. Zhao, J. Usary, J. S. Berg, M. Adams, J. Booker, J. Wu, A. Gulabani, T. Bodenheimer, A. P. Hoyle, J. V. Simons, M. G. Soloway, L. E. Mose, S. R. Jefferys, S. Balu, J. S. Parker, D. Neil Hayes, C. M. Perou, S. Malik, S. Mahurkar, H. Shen, D. J. Weisenberger, T. Triche Jr, P. H. Lai, M. S. Bootwalla, D. T. Maglinte, B. Berman, D. J. Van Den Berg, S. B. Baylin, P. W. Laird, C. J. Creighton, L. A. Donehower, G. Getz, M. Noble, D. Voet, G. Sakasena, N. Gehlenborg, D. DiCara, J. Zhang, H. Zhang, C. J. Wu, S. Yingchun Liu, M. S. Lawrence, L. Zou, A. Sivachenko, P. Lin, P. Stoianov, R. Jing, J. Cho, R. Sinha, R. W. Park, M. D. Nazaire, J. Robinson, H. Thorvaldsdottir, J. Mesirov, P. J. Park, L. Chin, S. Reynolds, R. B. Kreisberg, B. Bernard, R. Bressler, T. Erkkila, J. Lin, V. Thorsson, W. Zhang, I. Shmulevich, G. Ciriello, N. Weinhold, N. Schultz, J. Gao, E. Cerami, B. Gross, A. Jacobsen, R. Sinha, B. Arman Aksoy, Y. Antipin, B. Reva, R. Shen, B. S. Taylor, M. Ladanyi, C. Sander, P. Anur, P. T. Spellman, Y. Lu, W. Liu, R. Verhaak, G. B. Mills, R. Akbani, N. Zhang, B. M. Broom, T. D. Casasent, C. Wakefield, A. K. Uhrro, K. Baggery, K. Coombes, J. N. Weinstein, D. Haussler, C. C. Benz, J. M. Stuart, S. C. Benz, J. Zhu, C. C. Szeto, G. K. Scott, C. Yau, E. O. Paull, D. Carlin, C. Wong, A. Sokolov, J. Thusberg, S. Mooney, N. Ng, T. C. Goldstein, K. Ellrott, M. Grifford, C. Wilks, S. Ma, B. Craft, C. Yan, Y. Hu, D. Meerzaman, J. M. Gastier-Foster, J. Bowen, N. C. Ramirez, A. D. Black, R. E. XPATH ERROR: unknown variable “tname”.], P. White, J. E. Zmuda, J. Frick, T. M. Lichtenberg, R. Brookens, M. M. George, M. A. Gerken, H. A. Harper, K. M. Leraas, L. J. Wise, T. R. Tabler, C. McAllister, T. Barr, M. Hart-Kothari, K.
Tarvin, C. Saller, G. Sandusky, C. Mitchell, M. V. Iacocca, J. Brown, B. Rabeno, C. Czerwinski, N. Petrelli, O. Dolzhansky, M. Abramov, O. Voronina, O. Potapova, J. R. Marks, W. M. Suchorska, D. Murawa, W. Kycler, M. Ibbs, K. Korski, A. Spychała, P. Murawa, J. J. Brzeziński, H. Perz, R. Łaźniak, M. Teresiak, H. Tatka, E. Leporowska, M. Bougusz-Czerniewicz, J. Malicki, A. Mackiewicz, M. Wiznerowicz, X. Van Le, B. Kohl, N. Viet Tien, R. Thorp, N. Van Bang, H. Sussman, B. Duc Phu, R. Hajek, N. Phil Hung, T. Viet The Phuong, H. Quyet Thang, K. Zaki Khan, R. Penny, D. Mallery, E. Curley, C. Shelton, P. Yena, J. N. Ingle, F. J. Couch, W. L. Lingle, T. A. King, A. Maria Gonzalez-Angulo, G. B. Mills, M. D. Dyer, S. Liu, X. Meng, M. Patangan, F. Waldman, H. Stöppler, W. Kimryn Rathmell, L. Thorne, M. Huang, L. Boice, A. Hill, C. Morrison, C. Gaudio, W. Bshara, K. Daily, S. C. Egea, M. D. Pegram, C. Gomez-Fernandez, R. Dhir, R. Bhargava, A. Brufsky, C. D. Shriver, J. A. Hooke, J. Leigh Campbell, R. J. Mural, H. Hu, S. Somiari, C. Larson, B. Deyrmin, L. Kvecher, A. J. Kovatchich, M. J. Ellis, T. A. King, H. Hu, F. J. Couch, R. J. Mural, T. Stricker, K. White, O. Olopade, J. N. Ingle, C. Luo, Y. Chen, J. R. Marks, F. Waldman, M. Wiznerowicz, R. Bose, L. W. Chang, A. H. Beck, A. Maria Gonzalez-Angulo, T. Pihl, M. Jensen, R. Sfeir, A. Kahn, A. Chu, P. Kothiyal, Z. Wang, E. Snyder, J. Pontius, B. Ayala, M. Backus, J. Walton, J. Baboud, D. Berton, M. Nicholls, D. Srinivasan, R. Raman, S. Girshik, P. Kigonya, S. Alonso, R. Sanbhadti, S. Barletta, D. Pot, M. Sheth, J. A. Demchok, K. R. Mills Shaw, L. Yang, G. Eley, M. L. Ferguson, R. W. Tarnuzzer, J. Zhang, L. Dillon, K. Bueto, P. Fielding, B. A. Ozenberger, M. S. Guyer, H. J. Sofia, J. D. Palchik, Nature 2012, 490(7418), 61.

[18] E. White, Nat. Rev. Cancer 2012, 12(6), 401.

[19] Z. J. Yang, C. E. Chee, S. Huang, F. A. Sinicrope, Mol. Cancer Ther. 2011, 10(9), 1533.

[20] V. M. Alita, X. H. Liang, V. V. S. Murty, D. L. Pincus, W. Yu, E. Cayanis, S. Kalachikov, T. Gilliam, B. Levine, Genomics. 1999, 59(1), 59.

[21] X. H. Liang, S. Jackson, M. Seaman, K. Brown, B. Kempkes, H. Hibshoosh, B. Levine, Nature 1999, 402(6762), 672.

[22] Y. Shen, D. D. Li, L. L. Wang, R. Deng, X. F. Zhu, Autophagy 2008, 4(8), 1067.

[23] N. Furuya, J. Yu, M. Byfield, S. Pattingre, B. Levine, Autophagy 2005, 1(1), 46.

[24] Y. Takahashi, D. Coppola, N. Matsushita, H. D. Cualing, M. Sun, Y. Sato, C. Liang, J. U. Jung, J. Q. Cheng, J. J. Mul, W. J. Pledger, H. G. Wang, Nat. Cell Biol. 2007, 9(10), 1142.

[25] D. J. Goussetis, J. K. Altman, H. Glaser, J. L. Mcneer, M. S. Tallman, L. C. Platanias, J. Biol. Chem. 2010, 285(39), 29989.

[26] Y. Kumagai, D. Sumi, Annu. Rev. Pharmacol. Toxicol. 2007, 47, 243.

[27] K. Davison, K. K. Mann, S. Waxman, W. H. Miller, Blood 2004, 103(9), 3496.

[28] J. Mccafferty-grad, N. J. Bahlis, N. Krett et al., Mol. Cancer Ther. 2003, 2(11), 1155.

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