Dual inhibition of glycolysis and autophagy as a therapeutic strategy in the treatment of Ehrlich ascites carcinoma

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Abstract
Cancer cells have extra biosynthetic demands to sustain cell growth and redox homeostasis. Glycolysis and autophagy are crucial to fuel and recycle these biosynthetic demands. This plasticity of cancer cell metabolism participates in therapy resistances. The current study was designed to assess the therapeutic efficacy of dual targeting of glycolysis and autophagy in cancer. Using 3-bromopyruvate (3-BP; antiglycolytic inhibitor) and hydroxychloroquine (HCQ; autophagy inhibitor), we demonstrate their antitumor activity in Ehrlich ascites carcinoma (EAC)–bearing mice. A combination of 3-BP and HCQ significantly decreases tumor ascitic volume and cell count as compared with the EAC group and individual treatment groups. The enhanced antitumor activity is accompanied by hexokinase inactivation, inhibition of cellular protective autophagy, elevated antioxidant activity, and reduced oxidative stress levels. Together, these results suggest targeting both pathways in cancer as an effective therapeutic strategy. Further studies are required to validate this strategy in different cancer models and preclinical trials.

KEYWORDS
3-bromopyruvate, autophagy, glycolysis, hexokinase 2, hydroxychloroquine

1 | INTRODUCTION

Metabolic reprogramming of cancer cells is one of their unique features. This reprogramming is crucial to fuel the biosynthetic demands of cell growth and division, and redox homeostasis maintenance.⁴ Regardless of the presence of cellular oxygen, cancer cells rely on aerobic glycolysis over oxidative phosphorylation to generate the required cellular energy.⁵ The relationship between energy production and tumorigenesis has been appreciated since Warburg first described aerobic glycolysis as the main source of energy for cancer cells in an effect termed "Warburg effect."⁶ This altered energy metabolism not only support cancer cells with energy but also generate essential metabolic intermediates. These metabolites are essential for cancer cell proliferation, invasiveness, and resistance to chemotherapy.⁷

Several preclinical and early clinical studies have shown a great effectiveness in targeting the glycolytic pathway using inhibitors for key metabolic enzymes, such as hexokinase 2 (HK2).⁸ HK2 is predominantly expressed in cancer cells to promote the Warburg effect and protect cancer cells from apoptosis induced by chemotherapy.⁹ For instance, the HK2 inhibitor 3-bromopyruvate (3-BP) dissociates HK2 from the mitochondrial complex leading to sensitivity to cancer therapeutics.¹⁰ Data from preclinical and early clinical studies demonstrated antiglycolytic inhibitors as effective therapeutics to suppress cancer progression.¹¹ Furthermore, cancer cell metabolism uses autophagy to recycle intracellular components in metabolic stress and during cancer therapies.¹²

Autophagy is a multistep lysosomal degradation pathway used essentially by cancer cells for nutrient recycling and metabolic adaptation.¹³ Several studies have shown that autophagy induction may limit tumor development.¹⁴,¹⁵ However, evidence from in vivo studies have shown that autophagy inhibition suppresses the growth of established tumors and makes cells more sensitive to cancer therapeutics.¹⁶ Moreover, the use of autophagy modulation strategies depends on cancer genotypes and should be used in a context-dependent manner.¹⁷ Of note, the only clinically
available autophagy inhibitors are chloroquine derivatives, such as hydroxychloroquine (HCQ). Mechanistically, HCQ inhibits autophagy via lysosomal acidification inhibition and alteration of autophagosome fusion and degradation. HCQ has a long history of use for the treatment of malaria and rheumatologic disorders and is currently repurposed in human clinical trials for cancer treatment.

In this study, we demonstrate that the combined treatment of 3-BP (glycolysis inhibitor) and HCQ (autophagy inhibitor) showed a synergistic effect on the growth inhibition of Ehrlich ascites carcinoma (EAC) cells. This inhibition is attributed to HCQ and 3-BP-mediated hexokinase inactivation, an increase of antioxidants activity, and inhibition of autophagy. Together, these results propose HCQ and 3-BP as possible drug combination treatment in the EAC model.

2 | MATERIALS AND METHODS

2.1 | Mice

BALB/c female mice with established tumors and normal mice (20-25 g) were purchased from the National Cancer Institute (Cairo University, Egypt). The mice were properly housed under optimum temperature (23°C-25°C, relative humidity 55%) with free access to drinking water and food. The mice were allowed acclimatization to laboratory conditions 1 week before the start of the experiment. All mice experiments were performed to comply with the ARRIVE guidelines and in accordance with the Scientific Procedures Act, 1986. The study plan was approved by the animal ethical committee based at the Faculty of Science, University of Tanta, Egypt.

2.2 | Experimental design

Mice were divided into 10 groups (10 mice each). The 10 groups were as follows: GP 1: naive (0.2 mL of saline; intraperitoneal [IP]; 10 days, 3 times/week), GP 2: EAC (0.5 × 10⁶ cells; IP), GP 3: naive + 3-BP (10 mg/kg; IP; 10 days, 3 times/week), GP 4: EAC + 3-BP (EAC cells [IP] + 3-BP, 10 mg/kg; IP; 10 days, 3 times/week), GP 5: naive + HCQ (60 mg/kg; IP; 10 days, 3 times/week), GP 6: EAC + HCQ (EAC cells [IP] + HCQ, 60 mg/kg; IP; 10 days, 3 times/week), GP 7: naive + cisplatin (CIS [40 mg/kg; IP]; 10 days, 3 times/week), GP 8: EAC + cisplatin + CIS [40 mg/kg; IP; 10 days, 3 times/week], GP 9: naïve+3-BP + HCQ (3-BP + HCQ, 10 days, 3 times/week), and GP 10: EAC + 3-BP + HCQ (EAC cells [IP] + 3-BP + HCQ, 10 days, 3 times/week). 3-BP, HCQ, and CIS were purchased from Sigma-Aldrich (St., Louis, MO). 3-BP and CIS are dissolved in ethanol and dimethyl sulfoxide DMSO respectively as stock solutions and then diluted to the appropriate doses in phosphate-buffer saline (PBS) before the experiment. HCQ is dissolved directly in PBS (pH 7.2) to the appropriate experimental dose. Chemicals are prepared fresh and not stored for more than 1 month.

2.3 | Trypan blue assay

After tumor establishment, EAC cells were collected from the peritoneal cavity of experimental mice, suspended in saline (sterile, isotonic), mixed with trypan blue staining (0.4%), and counted in a hemocytometer. The percentage of viable cancer cells was estimated according to total and dead cancer cells as described previously.

2.4 | Nuclear and cytosol extraction

To separate the nuclear extract from the cytoplasmic and membranous fraction, we used the nuclear/cytosol fractionation kit (BioVision Incorporated) as described previously. In brief, the cells were collected by centrifugation (600g) for 5 minutes at 4°C and mixed with cytosol extraction buffer A with DTT and protease inhibitors for 10 minutes on ice. Cytosol extraction buffer B was added, and the mixture was centrifuged to cytoplasmic extract fraction (supernatant). The pellet was resuspended in nuclear extraction buffer and we followed the same steps as before to obtain the nuclear extract.

2.5 | Hexokinase colorimetric assay

The activity of HK2 was measured in the membranous fractions of cancer cells using a hexokinase assay kit (BioVision Incorporated) and as described previously. In brief, 1 × 10⁶ cells were homogenized in ice-cold HK assay buffer, centrifuged (12 000 rpm, 5 minutes), and the supernatant containing membranous HK2 was collected. Enzymatic activity was calculated by the colorimetric assay (OD₄₅₀nm) to measure the amount of NADH generated per min at pH 8 at RT. The sample test was performed against the NADH standard curve.

2.6 | Computational molecular docking

The molecular docking analysis was carried out using a Docking server (https://www.dockingserver.com). Gasteiger partial charges were added to the ligand atoms, nonpolar hydrogen atoms were merged, and rotatable bonds were determined. The calculations were carried on 3-BP interacting with HK2 (https://www.rcsb.org/pdb/explore.do?structureId=2NZT). Essential hydrogen atoms and solvation parameters were added using AutoDock tools. Distance-dependent dielectric functions were used in the calculation of the Van der Waals and electrostatic energies. Lamarckian genetic algorithm and Solis and Wets methods were used for docking simulations. Each docking test was elucidated from 10 different runs set to terminate after 250 000 maximum energy evaluations.

2.7 | Flow cytometry analysis (autophagy assay)

Autophagosomes of the cells were analyzed using a flow cytometer. A proprietary fluorescent autophagosome marker
\( \lambda_{ex} = 333/\lambda_{em} = 518 \text{ nm} \) was used within the autophagy assay kit (Sigma-Aldrich). Cells were centrifuged, resuspended with the autophagosome detection working reagent for 15 to 60 minutes at cell culture incubator (humidity chamber: 37°C, 5% CO₂). Excess dye was removed using wash buffer and fluorescence intensity \( \lambda_{ex} = 360/\lambda_{em} = 520 \text{ nm} \) was measured using the Attune flow cytometer (Applied Biosystem).

2.8 | Histopathological investigation

Liver specimens were excised from animals after surgery and fixed in 4% paraformaldehyde solution at room temperature for 2 hours. Hepatic tissues were then washed (PBS) twice, dehydrated with serial dilutions of alcohol (methyl, ethyl, and absolute ethyl), cleared in xylene, and embedded in paraffin (56°C for 24 hours). Tissues embedded in paraffin wax blocks were cut using a microtome, collected on glass slides, deparaffinized and stained with hematoxylin & eosin stain, and investigated under light microscopy.

2.9 | Estimation of malondialdehyde and antioxidant enzymes in hepatic tissue

Liver tissues were excised during dissection, washed with ice-cold saline, homogenized (10% wt/vol) and the homogenate was used to estimate oxidative (malondialdehyde [MDA]) and antioxidants (catalase, total thiol, glutathione-s-transferase [GST], and total antioxidant capacity [TAC]) parameters as described previously.\[^{16-18}\]

2.10 | Statistical analysis

The results are represented as mean ± standard error (SE). Statistical analyses were performed by one-way analysis of variance and Tukey test using SPSS statistical version 16 software package (SPSS Inc). \(*P < .05\), \(**P < .01\), \(***P < .001\), \(****P < .0001\) compared with normal control group (GP 1), and \(*P < .05\), \(**P < 0.01\), \(***P < .001\), \(****P < .0001\) compared with EAC group (GP 2).

3 | RESULTS

3.1 | Mice experimental design

To have established tumors in mice, we used the EAC model which is a spontaneous murine adenocarcinoma. EAC-bearing mice were purchased from the National Cancer Institute (Cairo University, Egypt). This model is adapted to form ascites in mice and can be delivered to outbred mice by serial passages using IP injection. EAC cells were harvested from the ascitic fluid of BALB/c mice with established ascitic tumors (10 days).

![FIGURE 1](image-url)  
**FIGURE 1** Ehrlich ascites carcinoma experimental design. 3-BP, 3-Bromopyruvate; EAC, Ehrlich ascites carcinoma; HCQ, Hydroxychloroquine
One hundred female BALB/c mice were divided randomly into 10 experimental groups (Figure 1) as follows:

- **GP 1:** Naïve group where mice were injected (IP) with saline for 10 days, 3 times/week.
- **GP 2:** EAC group where the established tumor was performed by injecting (IP) 0.5 × 10^6 EAC cells into the peritoneal cavity of the animals.
- **GP 3:** Naïve + 3-BP group where mice were treated with 3-BP (10 mg/kg body weight; IP) for 10 days, 3 times/week.
- **GP 4:** EAC + 3-BP group where the established tumor was performed in mice, then mice were treated with 3-BP (10 mg/kg body weight; IP) for 10 days, 3 times/week.
- **GP 5:** Naïve + HCQ group where mice were treated with HCQ (60 mg/kg body weight; IP) for 10 days, 3 times/week.
- **GP 6:** EAC + HCQ group where the established tumor was performed in mice, then mice were treated with HCQ (60 mg/kg body weight; IP) for 10 days, 3 times/week.
- **GP 7:** Naïve + CIS group where mice were injected (IP) with CIS (40 mg/kg body weight) for 10 days, 3 times/week.
- **GP 8:** EAC + CIS group where the established tumor was performed in mice, then mice were injected (IP) with CIS (40 mg/kg body weight) for 10 days, 3 times/week.
- **GP 9:** Naïve + 3-BP + HCQ group where mice were treated with 3-BP (10 mg/kg body weight; IP) and HCQ (60 mg/kg body weight) for 10 days, 3 times/week.
- **GP 10:** EAC + 3-BP + HCQ group where the established tumor was performed in mice, then mice were treated with 3-BP (10 mg/kg body weight; IP) and HCQ (60 mg/kg body weight) for 10 days, 3 times/week.

The body weight of the mice was recorded regularly throughout the experiment. In the end, mice were culled and ascitic fluid was collected from the peritoneal cavity to evaluate the ascitic tumor volume (mL). Also, the viability of cancer cells was determined by a 0.4% trypan blue assay and counted accordingly.
3.2 | HCQ potentiates the antitumor effect of 3-BP

The mouse’s body weight was recorded and were within the normal range (20-25 g) except EAC, which showed an increase in body weight due to ascitic fluid formation (Figure 2A). The relative liver weight was also normal without apparent shrinkage or cell death throughout the experimental groups (Figure 2B). Administration of 3-BP had a strong antitumor effect on EAC cells as indicated by ascitic volume and live and total cancer cells (Figure 2C-E). The antitumor impact of 3-BP was quite similar to the effect of the well-known cancer drug CIS. More important, the addition of HCQ to 3-BP further enhanced the antitumor effect with a significant decrease in live cancer cells in EAC + 3-BP + HCQ group as compared with either CIS or each individual treatment group (Figure 2D).

3.3 | 3-BP inhibits glycolysis of EAC cells

To ensure the metabolic effect of 3-BP on EAC cells. We determined the activity of HK2 in the experimental group. As shown in Figure 3A,B, 3-BP treatment significantly decreased the activity of HK2 in cancer

![Graph](image1)

**FIGURE 3** Inhibition of hexokinase 2 activity by 3-bromopyruvate (A) hexokinase activity assay was performed by enzyme-linked immunosorbent assay in each experimental group. *P < .05, **P < .01 compared with EAC group (GP 2). B. The 3D modeling of the crystal structure of HK2 (PDB: 2NZT) with 3-bromopyruvate interacting with active residues of HK2. C. The estimated free energy of binding, vDW + H-bond + Desolv energy, electrostatic energy, and total intermolecular energies, and the inhibition constant (Ki) of 3-BP and HK2 docking. D. The 2D plot of 3-BP interacting with HK2 active residues through ligand, nonligand, and hydrogen bonding (HB). HB plot is also shown with active residues involved in interaction with 3-BP. 3-BP, 3-bromopyruvate; EAC, Ehrlich ascites carcinoma; HCQ, hydroxychloroquine.
cells as compared with the EAC group (GP 2). Notably, CIS treatment repressed HK2 activity (Figure 3A) as previously reported by our group.\textsuperscript{4} To confirm the molecular inhibition of HK2 by 3-BP, we performed in silico molecular docking analysis using the molecular docking server (https://www.dockingserver.com). We used the HK2 crystal structure (PDB: 2NZT; RCSB protein data bank) and 3-BP structure available at PubChem (https://pubchem.ncbi.nlm.nih.gov). As shown in Figure 3C, the estimated free energy of binding, vdW + H-bond + Desolv, electrostatic, and total intermolecular energies are $-2.68$, $-3.33$, $+0.14$, and $-3.19$ kcal/mol, respectively. Furthermore, the inhibition constant ($k_i$) is 10.83 mM. These binding energy values confirm the specific binding of 3-BP to HK2 active residues (LYS 173, ASP 209, ILE 229, ASN 235, GLU 260, GLN 291, and GLU 294) as shown in Figure 3D. Also, 3-BP interacts with HK2 active residues through ligand, nonligand, and hydrogen bonding (HB). HB plot shows HK2-active residues involved in the interaction with 3-BP (Figure 3D).

### 3.4 HCQ inhibits autophagy of EAC cells

To measure the autophagic flux of EAC cells, we further performed flow cytometry analysis of the autophagic marker (fluorescence intensity of autophagosome marker). As shown in Figure 4, the treatment of HCQ significantly inhibited cellular protective autophagy of EAC cells as
compared with the EAC group (Figure 4C). Interestingly, a combination of 3-BP and HCQ has a synergistic effect on autophagy of cancer cells (Figure 4D,E). These results are consistent with the enhanced antitumor effect of both drugs on EAC cells and cell death. Also, this explains why HCQ treatment (EAC + HCQ) significantly decreases live tumor cells and not total cell count (Figure 2D,E).

3.5 | Combination of 3-BP and HCQ alleviates liver and kidney functions and increase antioxidants level

To assess the safety of these drugs, we measured normal physiological processes in mice including liver and kidney function and oxidant/antioxidant balance. As shown in Table 1, combination therapy (GP 10: EAC + 3-BP + HCQ) alleviated liver (alanine aminotransferase activity) function and did not affect kidney functions (urea and creatinine) as compared with CIS-treated group (GP 8). More important, we measured oxidative stress (MDA) and several antioxidants (catalase, GST, total thiol, and TAC) in hepatic tissue. Interestingly, a combination of 3-BP and HCQ augmented antioxidant level and reduced oxidative stress in hepatic tissue as compared with the CIS-treated group (GP 8). These findings are indicative of less oxidative burden of these drugs as compared with CIS (Table 1).

3.6 | Combination of 3-BP and HCQ ameliorates hepatic histopathology

As shown in Figure 5, there was no apparent histopathological alteration in the naïve group, while neoplastic cells were embedded in the hepatic parenchyma of the EAC group. These neoplastic cells were associated with diffuse Kupffer cell proliferation between hepatocytes. In naïve + 3-BP (GP 3) and naïve + HCQ (GP 5), there was no histological change in the liver, indicating the safety of 3-BP and HCQ drugs on hepatocytes. In EAC + 3-BP (GP 4) and EAC + HCQ (GP 6), there was focal extravasation of red blood cells in the hepatic parenchyma with dilatation in the central and portal veins. These specimens also showed inflammatory cell infiltration and diffuse Kupffer cell proliferation between hepatocytes. In naïve + CIS group (GP 7), the hepatic parenchyma showed focal lymphoid cell aggregation with Kupffer cell proliferation. However, in the EAC + CIS group (GP 8), massive inflammatory cell aggregation was monitored in the portal area associated with Kupffer cell proliferation. Interestingly, in naïve + 3-BP + HCQ group (GP 9), there were no apparent histopathological alterations. In EAC + 3-BP + HCQ group (GP 10), diffuse Kupffer cell proliferation was observed in between degenerated hepatocytes (Figure 5).

4 | DISCUSSION

The glycolytic enzyme hexokinase has four isotypes (I-IV) in mammalian tissues. Specifically, HK2 is located on the outer mitochondrial membrane and is frequently overexpressed in malignant cells. Overexpression of HK2 and upregulated activity has been observed in several types of cancers including breast, colorectal, liver, and pancreatic malignancies. Specific inhibitors of HK2 and other metabolic regulators of cancer cells have been used to suppress tumor activity. 3-BP has shown high antitumor activity through its ability to inhibit ATP generation, by targeting HK2 in cancer. Therefore, we...
designed this study to assess the effect of targeting glycolysis and autophagy in a murine cancer model. The coadministered group of 3-BP and HCQ has shown enhanced antitumor activity and an increased level of antioxidants. These findings are consistent with the reported ability of 3-BP to increase the expression of antioxidant genes.\(^{24}\) Owing to increased growth rate, cancer cells have increased steady-state reactive oxygen species pool and less antioxidant capacity. In the Warburg effect, glucose is metabolized to lactate, which has no antioxidant potency. However, this increased intrinsic oxidative stress is nontoxic to cancer cells due to the activity of HK2 as antioxidant and NADPH\(^+\) produced by hexose monophosphate shunt. In normal cells, pyruvate is rather the product of glucose metabolism, which is further metabolized to acetyl CoA and Krebs cycle intermediates (citrate, malate, and oxaloacetate), which are potent antioxidants.\(^{25}\)

The complex relationship between glycolysis, autophagy, and energy metabolism have raised great interest as potential anticancer drug targets.\(^{21}\) Autophagy is a crucial catabolic process where both extra- and intracellular components are engulfed and degraded to simple biomolecules. These biomolecules are pivotal to produce ATP.

**FIGURE 5** Histopathological assessment for mice hepatic tissues in each experimental group. Naïve (GP 1) tissue exhibits the normal histological structure of the central vein (CV) surrounding hepatocytes (h) in the parenchyma. Hepatic tissue of mice in the EAC group (GP 2) shows a group of neoplastic cells (ne) embedded in the hepatic parenchyma at the portal area surrounding the dilated portal vein. Also, there is diffuse Kupffer cells (k) proliferation in between the hepatocytes. Naïve + 3-BP group (GP 3) shows no histopathological alteration. EAC + 3-BP group (GP 4) shows the focal extravasation of red blood cells (ex) detected in the hepatic parenchyma. Naïve + HCQ group (GP 5) shows unaltered histopathology. EAC + HCQ group (GP 6) shows inflammatory cells (m) infiltration detected in the portal area (pv) with dilatation in the portal and central veins as well as diffuse Kupffer (k) cells proliferation in between the hepatocytes. Naïve + CIS group (GP 7) shows hepatic parenchyma with focal lymphoid cells (L) aggregation associated with diffuse Kupffer (k) cell proliferation in between the hepatocytes. EAC + CIS group (GP 8) shows massive inflammatory cells (m) aggregation detected in the portal area. Naïve + 3-BP + HCQ group (GP 9) shows no histopathological alteration. EAC + 3-BP + HCQ group (GP 10) shows diffuse Kupffer (k) cell proliferation observed in between the degenerated hepatocytes (d) (scale bar = 50 µm). 3-BP, 3-bromopyruvate; EAC, Ehrlich ascites carcinoma; HCQ, hydroxychloroquine.
and provide building blocks for biosynthesis.[10] Autophagy has a controversial role in cancer development depending on tumor stages and genotypes. In normal cells, autophagy has been identified as an antitumorigenic mechanism by isolation of damaged organelles.[10] In the early stages, cancer cells require high basal levels of autophagy for increased cell proliferation. However, in an already established tumor, autophagy is associated with hypoxic regions where there is increased metabolic demands.[10] In advanced cancers, autophagy addiction appears to maintain energy balance, recycle intracellular components, and build up tumorigenic factors.[10]

This information has led to the development of autophagy inhibitors, such as HCQ. HCQ has been extensively used to treat malaria and autoimmune diseases and is currently being repurposed for cancer treatment.[22] The antitumor effect of HCQ has been tested in phase I and II clinical trials with chemotherapy (eg, oxaliplatin) and antiangiogenic agents (eg, bevacizumab).[13,14,27] HCQ suppresses cancer cell growth through induction of apoptosis and inhibition of mitochondrial oxygen consumption.[28] Therefore, HCQ treatment is associated with increased rates of glycolysis.[28] Hence, dual inhibition of autophagy and glycolysis is a strong therapeutic strategy to tackle metabolic reprogramming in cancer cells.

In summary, we demonstrate the synergistic effect of dual targeting of autophagy and glycolysis in cancer cells. Combination therapy of 3-BP (glycolysis inhibitor) and HCQ (autophagy inhibitor) have enhanced antitumor effect on EAC and alleviated oxidative stress in hepatic tissues. Further studies are essential to test this therapeutic strategy in different cancer models and in combination with conventional chemotherapy.

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CONFLICT OF INTERESTS
The authors declare that there are no conflict of interests.

DATA AVAILABILITY STATEMENT
The data supporting the findings of this study are available upon request.

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REFERENCES
[1] N. Y. L. Ngoi, J. Q. Eu, J. Hirpara, L. Wang, J. S. J. Lim, S. C. Lee, Y. C. Lim, S. Pervaiz, B. C. Goh, A. L. A. Wong, Antioxid. Redox Signaling 2019, 32, 285. https://doi.org/10.1089/ars.2019.7947
[2] A. Asai, M. Konno, J. Koseki, M. Taniguchi, A. Vecchione, H. Ishii, Cancer Lett. 2020, 470, 141. https://doi.org/10.1016/j.canlet.2019.11.023
[3] X. Chen, Y. Qian, S. Wu, Free Radicals Biol. Med. 2015, 79, 253. https://doi.org/10.1016/j.freeradbiomed.2014.08.027
[4] M. A. Mansour, W. M. Ibrahim, E. S. Shalaan, A. F. Salama, J. Biochem. Mol. Toxicol. 2019, 33, e22350. https://doi.org/10.1002/jbt.22350
[5] A. F. Abdel-Wahab, W. Mahmoud, R. M. Al-Harizy, Pharmacol. Res. 2019, 150, 104511. https://doi.org/10.1016/j.phrs.2019.104511
[6] S. Cassim, V. A. Raymond, L. Dehbidis-Assadzadeh, P. Lapierre, M. Blodeau, Cell Cycle 2018, 17, 903. https://doi.org/10.1080/15384101.2018.1460023
[7] S. Marrache, S. Dhar, Chem. Sci. 2015, 6, 1832. https://doi.org/10.1039/C4CS01963F
[8] F. Ferro, S. Servais, P. Besson, S. Roger, J. F. Dumas, L. Brisson, Semin. Cell Dev. Biol. 2019, 98, 129. https://doi.org/10.1016/j.semcdb.2019.05.029
[9] P. Boya, P. Codogno, N. Rodriguez-Muela, Development 2018, 145, dev146506. https://doi.org/10.1242/dev.146506.
[10] R. K. Amaravadi, A. C. Kimmelman, J. Debnath, Cancer Discovery 2019, 9, 1167. https://doi.org/10.1158/2159-8290.CD-19-0292
[11] Y. Ji, L. Li, Y. X. Ma, W. T. Li, L. Li, H. Z. Zhu, M. H. Wu, J. R. Zhou, J. Nutr. Biochem. 2019, 69, 108. https://doi.org/10.1016/j.jnutbio.2019.03.018
[12] Y. Guo, Y. Feng, X. Cui, Q. Wang, X. Pan, Cancer Immunol. Immunother. 2019, 68, 1909. https://doi.org/10.1007/s00262-019-04215-8
[13] J. Malhotra, S. Jabbour, M. Orlick, G. Riedlinger, Y. Guo, E. White, J. Aisner, Cancer Treat. Res. Commun. 2019, 21, 100158. https://doi.org/10.1016/j.ctarc.2019.100158
[14] L. Q. Liu, S. B. Wang, Y. F. Shao, J. N. Shi, W. Wang, W. Y. Chen, Z. Q. Ye, J. Y. Jiang, Q. X. Fang, G. B. Zhang, Z. X. Xuan, Biomed. Pharmacother. 2019, 118, 109339. https://doi.org/10.1016/j.biopha.2019.109339
[15] B. M. Wolpin, D. A. Rubinson, X. Wang, J. A. Chan, J. M. Cleary, P. C. Enzinger, C. S. Fuchs, N. J. Mc Cleary, J. A. Meyerhardt, K. Ng, D. Schrag, A. L. Sikora, B. A. Spicer, L. Killion, H. Mamon, A. C. Kimmelman, Oncologist 2014, 19, 637. https://doi.org/10.1634/theoncologist.2014-0086
[16] W. Ibrahim, E. Tousson, E. M. Ali, M. A. Mansour, Gen. Comp. Endocrinol. 2011, 174, 143. https://doi.org/10.1016/j.ygcen.2011.08.012
[17] E. Tousson, E. M. Ali, W. Ibrahim, M. A. Mansour, Reprod. Sci. 2011, 18, 679. https://doi.org/10.1177/1933719110395401
[18] E. Tousson, E. M. Ali, W. Ibrahim, M. A. Mansour, Toxicol. Ind. Health 2012, 28, 566. https://doi.org/10.1177/0748233711420469
[19] J. J. Gu, A. Singh, K. Xue, C. Mavis, M. Barth, V. Yanamadala, P. Lenz, M. Grau, G. Lenz, M. S. Czuczmar, F. J. Hernandez-Illizaliturri, Oncotarget 2018, 9, 4020. https://doi.org/10.18632/oncotarget.23425
[20] L. Hooft, A. A. van der Veldt, O. S. Hoeckstra, M. Boers, C. F. Molthoff, P. J. van Diest, Clin. Endocrinol. 2008, 68, 252. https://doi.org/10.1111/j.1365-2265.2007.03031.x
[21] X. B. Li, J. D. Gu, Q. H. Zhou, Thorac. Cancer. 2015, 6, 17. https://doi.org/10.1111/1759-7714.12148
[22] M. K. Y. Siu, Y. X. Jiang, J. J. Wang, T. H. Y. Leung, C. Y. Han, B. K. Tsang, A. N. Y. Cheung, H. Y. S. Ngan, K. K. L. Chan, Cancers 2019, 11, 813. https://doi.org/10.3390/cancers11060813
[23] H. H. Baghdadi, Saudi J. Med. Med. Sci. 2017, 5, 9. https://doi.org/10.4103/1658-631X.194253
[24] L. Pulaski, I. Jatczak-Pawlik, M. Sobalska-Kwapis, D. Strapagiel, G. Bartosz, I. Sadowska-Bartosz, Free Radical Res. 2019, 53, 170. https://doi.org/10.1080/10701576.2018.1541176
[25] S. M. El Sayed, A. A. Mahmoud, S. A. El Sawy, E. A. Abdelaal, A. M. Fouad, R. S. Younis, M. S. Hashim, S. B. Hemdan, Z. M. Kadry, M. A. Abdelmoaty, A. G. Gabr, F. M. Omran, M. M. Nabo, N. S. Ahmed, Med. Hypotheses 2013, 81, 866. https://doi.org/10.1016/j.mehy.2013.08.024
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