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Research Article
Dihydroartemisinin Increases the Sensitivity of Acute Myeloid Leukemia Cells to Cytarabine via the Nrf2/HO-1 Anti-Oxidant Signaling Pathway

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Abstract

Background and Objective: In most cases of acute myeloid leukemia (AML), relapse frequently occurs due to chemoresistance. Modulating intracellular reactive oxygen species (ROS) levels may be a promising strategy to address the chemoresistance in AML. This study aimed to evaluate the potential of dihydroartemisinin (DHA) and its mechanism in improving the efficiency of cytarabine (Ara-C) in AML cells. **Materials and Methods:** After exposing HL-60 cells to Ara-C, DHA or their combination, the following analyses were carried out: CCK-8 assay for cell viability, the SynergyFinder tool for analyzing synergistic effect based CCK8 assay result, fluorometric assay with 2,7 -dichlorodihydrofluorescein for intracellular ROS levels, the flow cytometry for FITC/propidium iodide double staining and CD11b staining to investigate cell apoptosis and differentiation, western blot for the expression of Bax, Bcl-2, nuclear Nrf2 and HO-1 and Autodock tool assay for predicting the binding site of DHA. **Results:** The combination of Ara-C and DHA synergistically promoted the apoptosis and differentiation of HL-60 cells. Mechanistically, synergistic cytotoxic effects of Ara-C/DHA on HL-60 cells may be mediated by decreasing intracellular ROS levels. Combined with DHA blocked the activation of Nrf2/HO-1 anti-oxidant signaling caused by Ara-C. However, DHA only caused the down-regulation of HO-1, whereas the expression level of nuclear Nrf2 was unaffected. Molecular docking and Nrf2 transcriptional activity analysis revealed the effect of DHA is mediated by its suppression of Nrf2 transcriptional activity. **Conclusion:** The DHA can serve as an effective alternative in AML treatment, especially for patients exhibiting Ara-C resistance.

Key words: Dihydroartemisinin, acute myeloid leukemia cells, cytarabine, drug synergism, Nrf2

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

As an uncommon but aggressive hematopoietic malignancy, acute myeloid leukemia (AML) is characterized by uncontrolled clonal proliferation and the blockage of myeloid differentiation with historically high mortality rates[1]. To date, AML's initial treatment remains the anchor pro-drug Cytarabine (Ara-C), which is basically unchanged as the gold standard for over 30 years[2]. Generally, for newly diagnosed AML patients under the age of 60, the complete remission after this treatment is ~65%[3]. However, more than 60% of them relapse due to the development of Ara-C resistance and there is no salvage regimen at present[4]. Therefore, it's of significance to search for strategies for preventing or overcoming Ara-C resistance in order to improve the prognosis of AML patients. Much effort made to understand the mechanisms behind Ara-C resistance in AML, but only a little progress has been made in the past two decades[5].

One of the mechanisms that have been found to participate in the occurrence and development of Ara-C resistance in AML is reactive oxygen species (ROS) signaling[6]. Multiple compelling studies demonstrated that targeting redox homeostasis, a process involving the balance of ROS production and elimination, might be a potential strategy for sensitizing resistant cells to chemotherapy[7,8]. It's known that ROS is mainly produced from the endoplasmic reticulum oxidase, mitochondrial electron transport chain, as well as NADPH oxidase[9], while the elimination of ROS is associated with high abundance redox proteins, antioxidant enzymes and Nuclear Factor Erythroid 2-related Factor 2 (Nrf2)[10].

The Nrf2, a critical transcription factor related to cell homeostasis, plays an important role in modulating various physiological processes, such as redox homeostasis, proteasome degradation and energy metabolism[11]. Increasing reports supported that targeting Nrf2 signaling to regulate redox homeostasis may help improve the outcomes of chemotherapy for drug-resistant patients in many types of cancers[12], including AML[13]. For example, inhibition of Nrf2 by brusatol was shown to effectively sensitize AML to Ara-C[14]. Recently, a report emphasizes the contribution of Nrf2 in Ara-C resistance during AML treatment and proposes a potential therapeutic strategy by targeting Nrf2 for AML resistance[15].

As the first-generation derivative of artemisinin, dihydroartemisinin (DHA) is a widely used drug for patients with malaria, which has been proven to inhibit malignant tumors in many cancers[16]. The DHA exerts many anticancer effects, which include inhibiting proliferation, tumor metastasis and angiogenesis, inducing apoptosis and autophagy and promoting immune function[17]. The potential value and the mechanism of DHA for patients with AML have been reported in the last decade[18,19]. Meanwhile, multiple research reported that DHA regulates ROS signaling to potentiate the efficacy of chemotherapy in drug-resistant tumors, including colorectal cancer[20] and ovarian cancer[21]. However, whether DHA has the capacity of enhancing the sensitivity of AML cells to Ara-C remains not yet investigated.

Based on these, the present study investigated whether DHA could target the ROS signaling to sensitize AML cells to Ara-C with an attempt to uncover a novel alternative therapy for preventing or reversing Ara-C resistance in AML treatment.

MATERIALS AND METHODS

Study area: The whole study was conducted at Zunyi Medical and Pharmaceutical College, Affiliated Hospital of Zunyi Medical University from January, 2022 to November, 2022.

Cell culture and treatment: Human AML cell line (HL-60) supplied by Procell (cat.no CL-0110) was grown in complete RPMI 1640 medium, which contained 10% FBS (10%, v/v) and penicillin/streptomycin (100 U mL^{-1}).

The HL-60 cells were seeded in the 96-well or six-well plates at a density of 1.5×10^4 or 2×10^5 cells per well respectively, prior to the treatment 24 hrs. For experiments with DHA or/and HL-60 cells were incubated with diverse concentrations of DHA (0.1, 0.2, 0.4, 0.6, 0.8 and 1 μM for CCK-8 assay, 0.6 μM for other assays) combined with or without Ara-C (0.01, 0.02, 0.04, 0.06, 0.08 and 0.1 μM for CCK-8 assay, 0.04 μM for other assays) at 37°C. Untreated HL-60 cells served as the experimental control.

Cell viability detection: The CCK-8 kit purchased from Dojindo (Kumamoto, Japan) was applied to detect the viability of HL-60 cells. Briefly, after finishing 48 hrs treatment, the media containing the drug was replaced by media containing 10% CCK-8 solution (v/v) for another 1.5 hrs cultivation. Afterward, by using a Microplate reader (Bio-Rad, California, USA), the absorbance at 450 nm was measured for the calculation of cell viability. Finally, the synergy effects between DHA and Ara-C were analyzed by SynergyFinder[22] based on the result of the CCK-8 assay.

Cell apoptosis analysis: The apoptotic HL-60 cells were investigated after 48 hrs treatment by Annexin V-FITC/propidium iodide (PI) double staining and analyzing the expression of apoptosis-related proteins (Bax and Bcl-2).

Table 1: Antibodies used in this research

Antibody	Manufacturer	Cat. No
Bax	Bioss Inc.	bsm-52316R
Bcl-2	GeneTex	GTX100064
Nuclear Nrf2	GeneTex	GTX103322
HO-1	Biorbyt	orb650009
Lamin B	Bioss Inc.	bs-24328R
β-actin	GeneTex	GTX109639

For Annexin V-FITC/PI double staining, the HL-60 cells were collected and washed twice with phosphate-buffered saline (PBS). Then, 5 μL of Annexin-V dye solution and 10 μL of PI dye solution (Sigma-Aldrich, MO, USA) were added to stain HL-60 cells for 30 min at 4°C in the dark. The apoptosis rate was checked by using flow cytometry (BD Biosciences, New Jersey, USA).

The detection of Bax and Bcl-2 expression was described in the western blot analysis part.

AML cell differentiation analysis: After 48 hrs treatment, HL-60 cells were collected and washed with phosphate-buffered saline (PBS). Then, HL-60 cells were resuspended in FACS buffer prior to incubation with antibodies specific for CD11b (a well-known differentiation marker) for 1 hr at 37°C in the dark. Finally, the cells were subjected to a flow cytometer for analyzing CD11b positive cells.

ROS measurement: Intracellular ROS of HL-60 cells with diverse treatment was detected by fluorometric assay with 2,7-Dichlorodihydrofluorescein Diacetate (DCFDA). Briefly, after treating with HL-60 cells for 48 hrs, DCFDA fluorescent dye (Sigma-Aldrich) was added to cells at a concentration of 25 μM per well for 20 min. The production of ROS was observed and analyzed by a BX51 microscope (Olympus, Tokyo, Japan) and flow cytometer, respectively.

Western blot analysis: After isolating total protein from HL-60 cells with Radio-Immunoprecipitation Assay (RIPA) buffer (10X) (Cell Signaling, Massachusetts, USA), protein quantification was performed with a bicinchoninic acid (BCA) assay kit (Sigma-Aldrich). In the meantime, the nuclear extraction kit (Thermo Scientific, Massachusetts, USA) was applied to the purification of the nuclear proteins. The protein was subjected to separation using SDS-PAGE gel before being transferred onto Polyvinylidene Difluoride (PVDF) membranes. Afterward, membranes were blocked with 5% skim milk and subsequently incubated with primary antibodies specific for Bax, Bcl-2, Nrf2, Lamin B, HO-1 and Glyceraldehyde 3-phosphate dehydrogenase (GADPH) overnight. Next, membranes were washed thrice before the incubation with HRP-conjugated secondary antibody for 2 hrs. Finally, an ECL detection reagent (Sigma-Aldrich, Missouri, USA) was used to visualize protein bands. The information on antibodies used in this study was described in Table 1.

Molecular docking: The crystal structure of Nrf2 (Identifier: AF-Q60795-F1) and the molecular structure of DHA (Compound CID: 3000518) were downloaded from UniProt and PubChem, respectively. Autodock tools were exploited for docking simulation of DHA and predicting its binding affinity with the Nrf2. The results were visualized by the PyMol tool (Version 1.3, Schrödinger, LLC, New York, USA).

Nrf2 transcriptional activity analysis: In order to assess Nrf2 transcriptional activity, the antioxidant response element (ARE) luciferase reporter assay was performed with the Dual-Glo luciferase assay kit (Promega, Germany). In brief, cells were transfected with a pGL3-basic luciferase reporter plasmid containing eight copies of AREs (5-GTGACAAAGCA-3). Meanwhile, the same amount of *Renilla* included in each transfection was utilized to standardize transfection efficiency. Treated or untreated HL-60 cells were collected with RIPA buffer and subsequently incubated with the Dual-Glo luciferase assay reagent for 10 min. After detecting the firefly luminescence activities, Dual-Glo Stop and Glo reagent was added for another 10 min incubation. Finally, the *Renilla* luminescence activities were detected and further normalized by the *Renilla* activities.

Statistical analysis: All data were expressed as Mean±Standard error of the mean. The Student's t-test was used for comparison between the two groups. For multiple-group conditions, one-way ANOVA was performed with Bonferroni's method. The $p<0.05$ were considered statistically significant.

RESULTS

Synergistically DHA and Ara-C have a suppressive effect on the progress of AML cells: The chemical molecular structure was shown in Fig. 1a. To determine whether the combination of Ara-C and DHA exerts a synergistic effect to inhibit the proliferation of AML cells, HL-60 cells exposed to DHA, Ara-C and the combination of them. In comparison with the single drug, the combined treatment remarkedly decreased the absolute number of viable HL-60 cells (Fig. 1b). To detect the synergistic efficacy, the synergy analysis was performed with the online Synergyfinder tools. The DHA were found to act synergistically with Ara-C in HL-60 cells (Synergy scores >10, Fig. 1c). These results revealed an obvious synergistic effect of the combination of Ara-C and DHA in the HL-60 cell line.

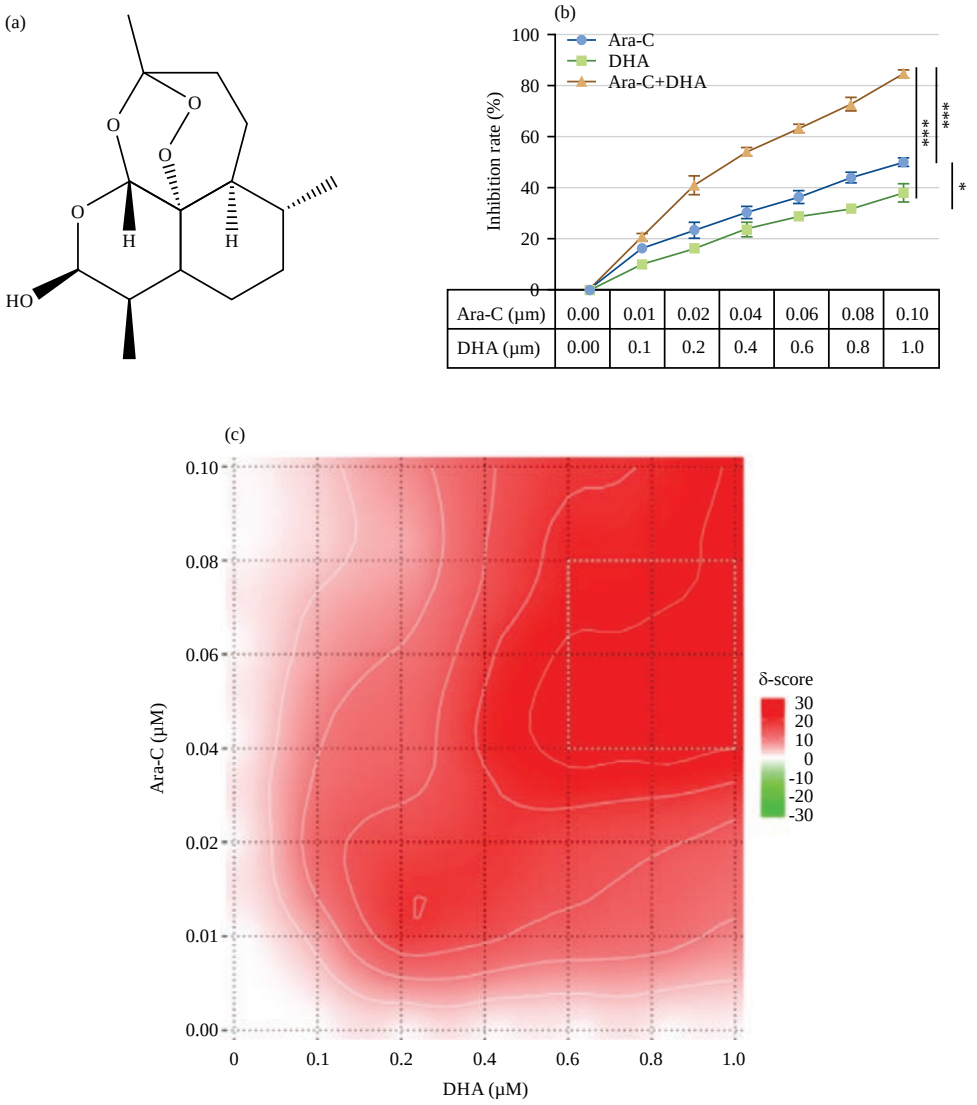

Fig. 1(a-c): Synergistically DHA and Ara-C have an inhibitory effect on the progress of HL-60 cells, (a) Chemical structure of DHA, (b) CCK8 assay was carried on to detect the cell viability of HL-60 cells after being treated with increasing concentrations of DHA (0.1, 0.2, 0.4, 0.6, 0.8 and 1 μM), Ara-C (0.01, 0.02, 0.04, 0.06, 0.08 and 0.10 μM) and their combination for 48 hrs and (c) HSA model heatmap was drawn by SynergyFinder

Synergistically DHA and Ara-C have a promotional effect on the apoptosis of AML cells: Since the major mechanism underlying most chemotherapeutic drug work is stimulating the apoptosis of cancer cells, flow cytometry was carried out on HL-60 cells stained with Annexin V-FITC/PI in order to investigate the effect of the combination of Ara-C and DHA on apoptosis. As expected, it was observed that the apoptosis of HL-60 cells was induced in response to treatments (Fig. 2a).

A 24 hrs exposure to Ara-C and DHA led to a significant increase in the apoptotic ratio of HL-60 cells (control: 3.87±1.19%, Ara-C: 11.27±1.50%, $p<0.05$, DHA: 11.00±0.41%, $p<0.05$) (Fig. 2b). Of note, the combination of Ara-C and DHA further aggravated the apoptosis in HL-60 cells (Ara-C+DHA: 20.54±1.91%, $p<0.05$) (Fig. 2b). Moreover, western blot analysis showed that the protein expression levels of Bax (a pro-apoptosis protein) and Bcl-2 (an anti-apoptosis protein) were significantly up-regulated and down-regulated, respectively, after all, treatments (Fig. 2c). Among the three types of treatments, the combination of Ara-C and DHA exerted the strongest role in apoptosis induction (Fig. 2c), demonstrating that the synergism between Ara-C and DHA is related to the aggravation of apoptosis.

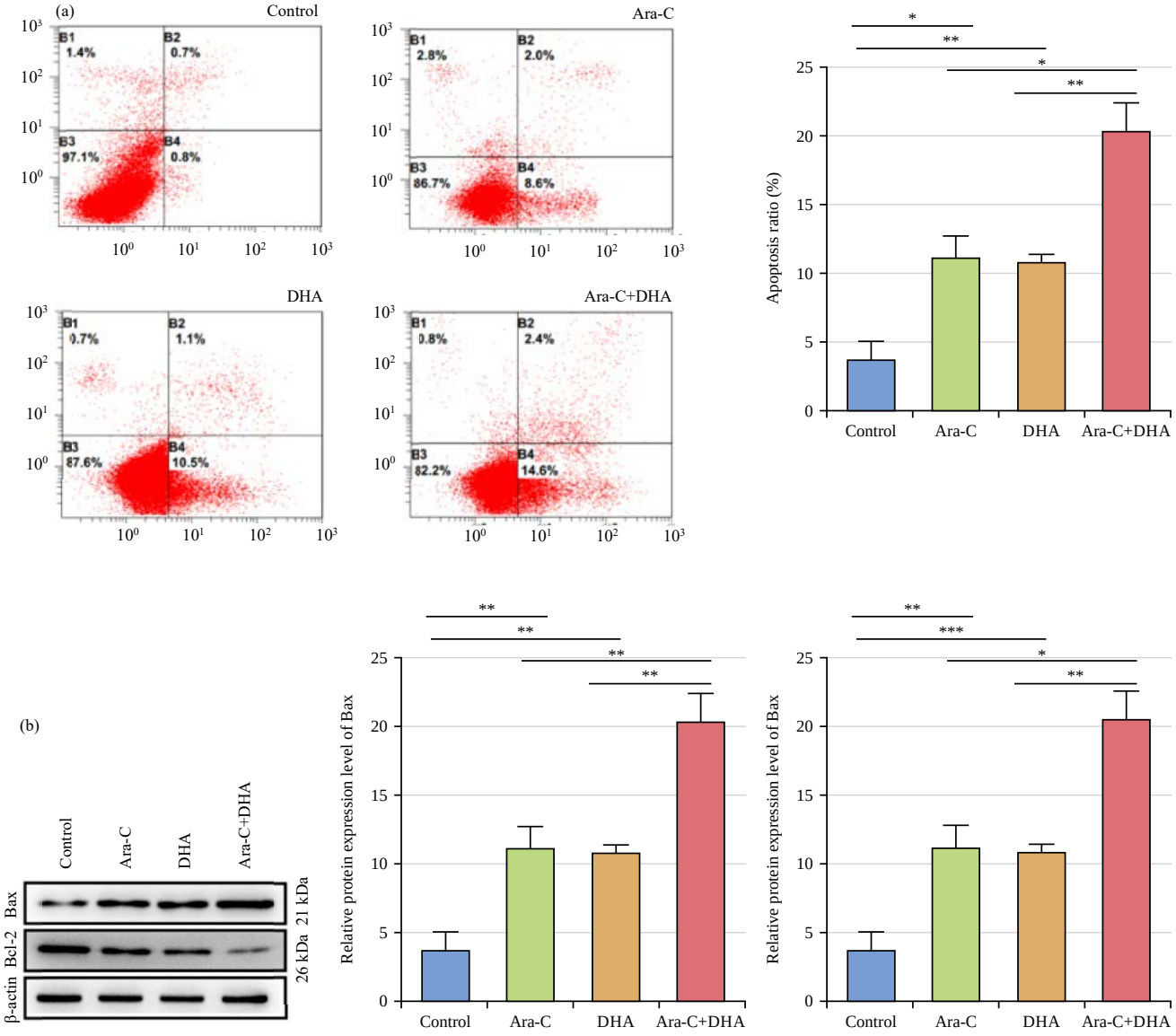

Fig. 2(a-b): Synergistically DHA and Ara-C have promotional effects on the apoptosis and differentiation of HL-60 cells. As HL-60 cells were treated with 0.06 μM of Ara-C, 0.4 μM of DHA and their combination for 48 hrs, (a) Annexin V-FITC/PI double staining was performed with flow cytometry to assess cell apoptosis and (b) Western blot analysis detected apoptosis-related proteins

*p<0.05, **p<0.01 and ***p<0.005

Synergistically DHA and Ara-C have a promotional effect on the differentiation of AML cells: As differentiation arrest is a well-known hallmark of AML clinically, forcing cancer cells to differentiate is one of the target strategies for AML treatment. After diverse treatments, the detection of the CD11b marker on the cell surface was carried out to investigate the effect of Ara-C and/or DHA on the differentiation of HL-60 cells (Fig. 3a). The result showed that all treatments can induce cell differentiation of HL-60 cells, as revealed by the percentage of CD11b-positive cells was significantly elevated from 2.03±0.28 to 20.23±1.45%, 9.82±0.72 and 40.04±1.24% after HL-60 cells being treated with Ara-C, DHA and Ara-C+DHA (Fig. 3b). Together, our findings indicate that synergistically DHA and Ara-C can enhance the differentiation of AML cells in culture.

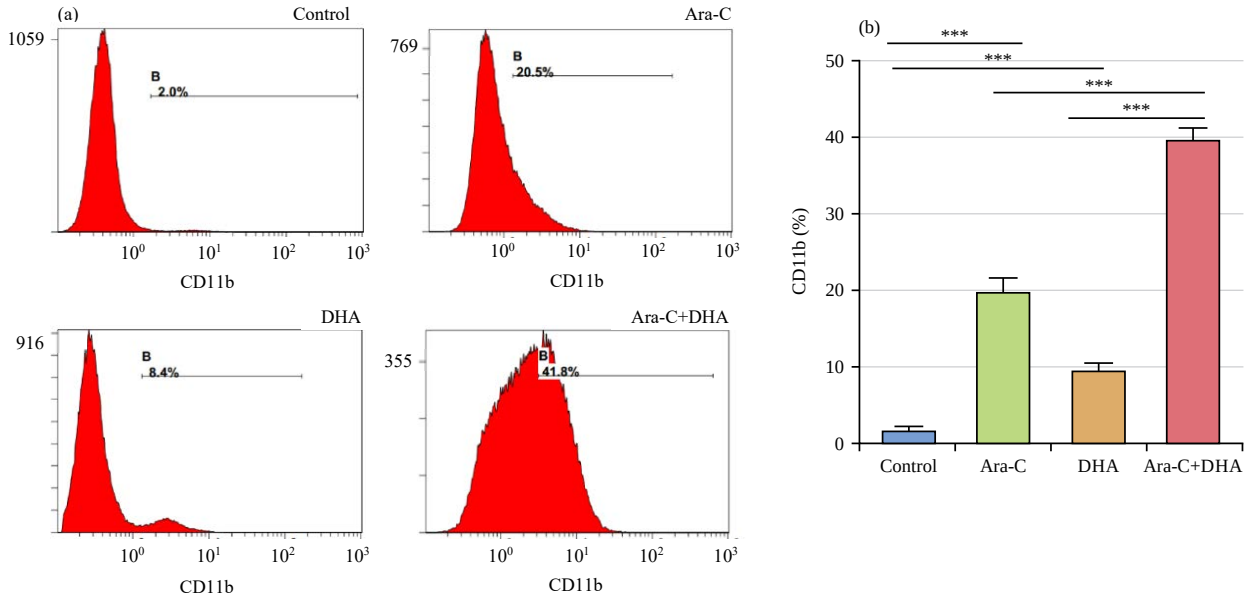

Fig. 3(a-b): Synergistically DHA and Ara-C have a promotional effect on the differentiation of HL-60 cells, (a) HL-60 cells were treated with 0.06 μM of Ara-C, 0.4 μM of DHA and their combination for 48 hrs. CD11b staining was performed with flow cytometry to investigate HL-60 cell differentiation and (b) Percentage of CD11b positive cells in each group
***p<0.005

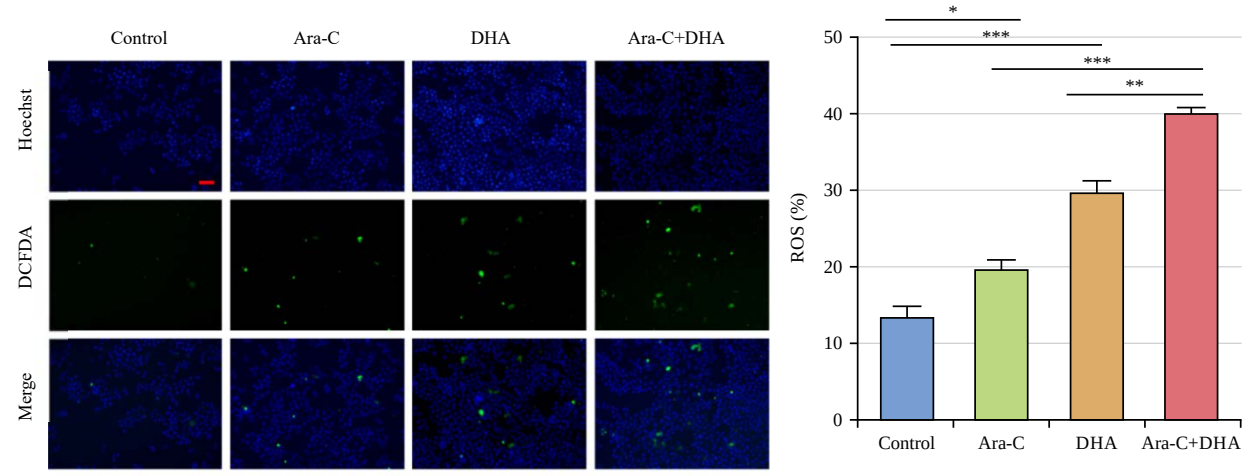

Fig. 4: Synergistically DHA and Ara-C elevate the ROS levels of HL-60 cells. HL-60 cells were treated with 0.06 μM of Ara-C, 0.4 μM of DHA and their combination for 48 hrs. DCFDA fluorometric assay was performed to detect ROS levels in HL-60 cells
*p<0.05, **p<0.01 and ***p<0.005

Synergistically DHA and Ara-C elevate the ROS levels of AML cells: Since the critical role of ROS has been implicated in triggering apoptosis and even drug resistance, the effects of Ara-C and/or DHA on the levels of ROS in HL-60 cells after treatment for 48 hrs were observed with DCFDA staining. Compared to the untreated HL-60 cells, the ROS levels in HL-60 cells with Ara-C were increased slightly, while those with DHA were elevated markedly, as expected (Fig. 4). Moreover, the HL-60 cells with the combined treatment triggered the strongest intracellular ROS levels, which suggested that the ROS induction role of Ara-C is strengthened in combination with DHA.

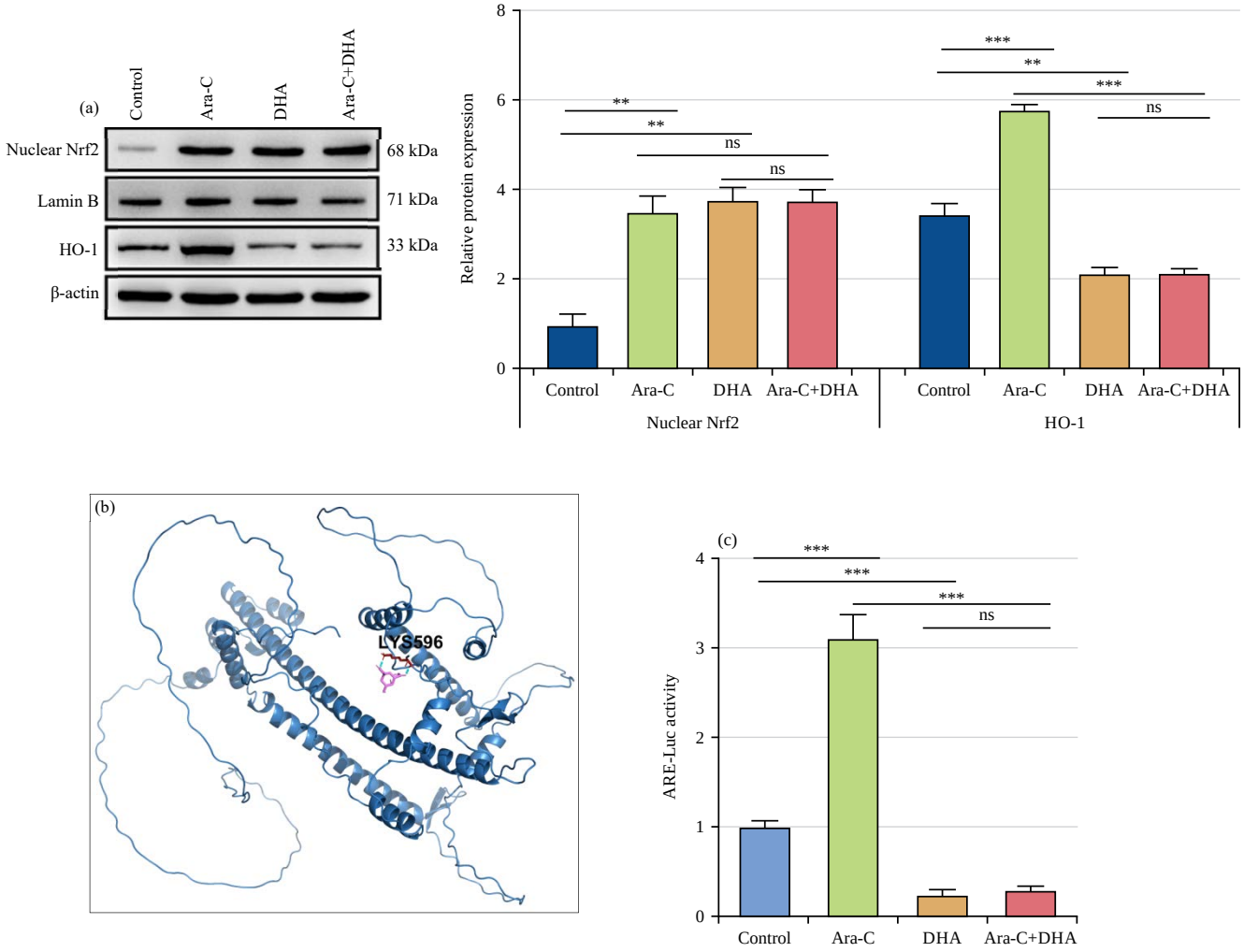

Fig. 5(a-c): DHA enhances the sensitivity of HL-60 cells to Ara-C by blocking the activation of the Nrf2/HO-1 signaling cascade, (a) Western blot analysis detected the nuclear NRF2 and HO-1 proteins in HL-60 cells after being treated with 0.06 μM of Ara-C, 0.4 μM of DHA and their combination for 48 hrs, (b) Molecular docking diagram of DHA with NRF2 and (c) ARE luciferase reporter assay analyzed the inhibition of NRF2 ARE activity in HL-60 cells after being treated with 0.06 μM of Ara-C, 0.4 μM of DHA and their combination for 48 hrs

**p<0.01, ***p<0.005 and ns: None significance

DHA enhances the sensitivity of AML cells to Ara-C by blocking the activation of the Nrf2/HO-1 signaling cascade: Accumulated data have highlighted the close relationship between the Nrf2/HO-system and ROS production over the last three decades. To protect cells against the damage of chemical agents caused by ROS overproduction, most cancer cells have endogenous defense strategies, one of which is the activation of the Nrf2/HO-1 signaling cascade. Hence, whether DHA is involved in regulating Nrf2/HO-system to promote ROS production, thereby enhancing the sensitivity of AML cells to Ara-C was further explored. Western blot analysis revealed whether a single drug or combination treatment led to the up-regulation of NRF2 in the nuclear HL-60 cells (Fig. 5a). However, there is no difference in nuclear NRF2 expression among the three types of treatment (Fig. 5a). Additionally, HO-1, the downstream protein of NRF2 with the strong ability to scavenge ROS, was markedly up-regulated in HL-60 cells after Ara-C treatment. Notably, the HO-1 up-regulation induced by Ara-C in HL-60 cells was blocked in combination with DHA (Fig. 5a).

In an attempt to uncover the mechanism behind the regulation of HO-1 expression by DHA, molecular docking analysis was performed. The analysis predicted a binding site of DHA in the Neh3 domain of NRF2, a domain responsible for transactivation (Fig. 5b). Finally, the NRF2-inducing transcriptional activity was analyzed by the luciferase reporter assay with an ARE. The Ara-C treatment strongly induced the transcriptional activity of NRF2, as revealed by the significant increase of ARE-luciferase activity in HL-60 cells (Fig. 5c). Compared with the controls, NRF2 transcriptional activity in HL-60 cells was reduced when exposed to DHA (Fig. 5c). Collectively, these results indicated that DHA enhances the sensitivity of AML cells to Ara-C by impairing NRF2 transcriptional activity.

DISCUSSION

Herein, this study, for the first time, proved the potential of DHA on improving Ara-C efficiency in AML therapy and preliminarily uncovered the underlying mechanism. The Ara-C is one of the key standard agents in AML therapy. Nevertheless, the application of Ara-C is still limited by acquired resistance. While approximately 65% of AML patients respond to Ara-C initially, most relapse as resistance to Ara-C develops[4]. An effective strategy for enhancing the sensitivity of AML to Ara-C or overcoming Ara-C resistance is therefore critical for patients with AML. In the last twenty years, the success of rational combination therapeutic strategy has been proven in other malignancies, suggesting a preferred efficacy over single-agent, which may be the approach to pursue in AML[23,24].

The value of natural compounds as clinical agents for patients to treat pathological conditions and enhance physiological functions has been proven over the last three decades[25]. For example, paclitaxel has been widely used in clinical therapy for multiple malignancies, including non-small cell lung cancer and ovarian cancer[26]. Several natural compounds exerted high potential in synergizing and reducing drug resistance in combination with chemotherapeutic drugs, thereby leading to tumor regression[27]. It has been reported that ginkgetin is capable of strengthening the antitumor effects of cisplatin by promoting ROS production and suppressing NRF2/HO-1[28].

As one of the most efficient anti-malarial analogs of artemisinin, DHA has been found to exert an antitumor effect on many cancer cells, including AML[29]. Evidence suggests that the therapeutic effect of DHA in several disorders relies on its regulation in ROS signaling[16,30]. A previous study reported that DHA improves the sensitivity of human glioma cells to radiation by inducing ROS production[31]. Moreover, DHA triggers ROS production to synergize the therapeutic efficacy of gemcitabine in ovarian cancer[32]. The potential of DHA in synergizing and even reversing drug resistance when combined with Ara-C in AML remains elusive. In this study, Ara-C alone exerted no more than 50% of inhibition on HL-60 cell viability at 0.1 μM. Based on SynergyFinder, the cell viability of treated HL-60 cells was assessed using matrices with increasing concentrations of Ara-C or/and DHA. By using the highest single-agent model, the synergistic effects of the potential could be analyzed. It means a potentially synergistic effect between the two drugs when a score >10[33]. The current result showed that the synergy score of DHA/Ara-C was 16.85 in HL-60 cells. The combination dose (0.06 μM of Ara-C with 0.4 μM of DHA) presented a potent synergistic effect among the combinations, which was therefore chosen in the subsequent experiment. The following *in vitro* experiment revealed that the combination of Ara-C with DHA displayed a significantly stronger antitumor effect than Ara-C alone, which suggested that it is promising to develop DHA as an adjuvant chemotherapy drug in AML.

Multiple mechanisms have been implicated in the process of chemoresistance, including enhancing DNA repairment, facilitating detoxification by metabolizing enzymes and inducing the activity of efflux transporter proteins[34,35]. The NRF2 has been suggested as a potent therapeutic target to enhance drug efficacy via the up-regulation of antioxidant-related enzymes, including HO-1[36]. Brusatol, an NRF2 inhibitor, improved the sensitivity of cancer cells to multiple chemotherapeutic drugs by suppressing NRF2 protein expression[37]. Tang *et al.*[38] found that, luteolin regulated NRF2 signaling to reduce cellular GSH levels, thereby enhancing the sensitivity of lung cancer cells to anticancer agents. In the current study, it was found that DHA could significantly inhibit the activity of NRF2 and HO-1 expression, which might through interacting with the Neh3 domain of NRF2 suppress its transactivation.

Taken together, the data of this study revealed the drug synergism of Ara-C/DHA *in vitro*. the current study findings may provide a novel adjuvant chemotherapy drug for AML treatment. However, several shortcomings exist in this study. Firstly, it remains to be validated the effects of the combination of Ara-C and DHA in the context of drug resistance. Further studies were therefore essential to exploit the function of DHA on Ara-C-resistant AML cell lines. Besides, additional *in vivo* experiment also needs to be further explored in order to substantiate current findings.

CONCLUSION

The results demonstrated that DHA effectively enhances the responsiveness of AML cells to Ara-C, which may rely on the regulation of the Nrf2/HO-1 anti-oxidant signaling. Current findings indicated the potential application of DHA as a promising new target to further enhance the efficacy of Ara-C chemotherapy. Future studies are also needed to assess the potential of DHA on Ara-C-resistant AML cell lines.

SIGNIFICANCE STATEMENT

The initial treatment of AML remains the anchor pro-drug Ara-C, while relapse frequently occurs in most cases of AML due to chemoresistance. An effective strategy for enhancing the sensitivity of AML to Ara-C or overcoming Ara-C resistance is therefore critical for patients with AML. This study, for the first time, demonstrated the potential of DHA in enhancing the responsiveness of AML cells to Ara-C, which may rely on the suppression of the Nrf2/HO-1 anti-oxidant signaling. These findings reveal the potential application of DHA as a promising new target to further enhance the efficacy of Ara-C chemotherapy.

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REFERENCES

1. Newell, L.F. and R.J. Cook, 2021. Advances in acute myeloid leukemia. BMJ, Vol. 375. 10.1136/bmj.n2026.
2. Ramos, N.R., C.C. Mo, J.E. Karp and C.S. Hourigan, 2015. Current approaches in the treatment of relapsed and refractory acute myeloid leukemia. J. Clin. Med., 4: 665-695.
3. Zehra, S., R. Najam, T. Farzana and T.S. Shamsi, 2016. Outcomes of 1st remission induction chemotherapy in acute myeloid leukemia cytogenetic risk groups. Asian Pac. J. Cancer Prev., 17: 5251-5256.
4. Schlenk, R.F., C. Müller-Tidow, A. Benner and M. Kieser, 2017. Relapsed/refractory acute myeloid leukemia: Any progress? Curr. Opin. Oncol., 29: 467-473.
5. Arwanih, E.Y., M. Louisa, I. Rinaldi and S.I. Wanandi, 2022. Resistance mechanism of acute myeloid leukemia cells against daunorubicin and cytarabine: A literature review. Cureus, Vol. 14. 10.7759/cureus.33165.
6. Abdul Mannan, Z.P. Germon, J. Chamberlain, J.R. Sillar, B. Nixon and M.D. Dun, 2021. Reactive oxygen species in acute lymphoblastic leukaemia: Reducing radicals to refine responses. Antioxidants, Vol. 10. 10.3390/antiox10101616.
7. Farge, T., E. Saland, F. de Toni, N. Aroua and M. Hosseini *et al*., 2017. Chemotherapy-resistant human acute myeloid leukemia cells are not enriched for leukemic stem cells but require oxidative metabolism. Cancer Discovery, 7: 716-735.
8. Glasauer, A. and N.S. Chandel, 2014. Targeting antioxidants for cancer therapy. Biochem. Pharmacol., 92: 90-101.
9. Ashton, T.M., W.G. McKenna, L.A. Kunz-Schughart and G.S. Higgins, 2018. Oxidative phosphorylation as an emerging target in cancer therapy. Clin. Cancer Res., 24: 2482-2490.
10. Harris, I.S. and G.M. DeNicola, 2020. The complex interplay between antioxidants and ROS in cancer. Trends Cell Biol., 30: 440-451.
11. Ma, Q., 2013. Role of Nrf2 in oxidative stress and toxicity. Annu. Rev. Pharmacol. Toxicol., 53: 401-426.
12. Mirzaei, S., A. Zarrabi, F. Hashemi, A. Zabolian and H. Saleki *et al*., 2021. Nrf2 signaling pathway in chemoprotection and doxorubicin resistance: Potential application in drug discovery. Antioxidants, Vol. 10. 10.3390/antiox10030349.
13. Chu, X., L. Zhong, W. Dan, X. Wang and Z. Zhang *et al*., 2022. DNMT3A R882H mutation drives daunorubicin resistance in acute myeloid leukemia via regulating NRF2/NQO1 pathway. Cell Commun. Signaling, Vol. 20. 10.1186/s12964-022-00978-1.
14. Cheng, C., F. Yuan, X.P. Chen, W. Zhang and X.L. Zhao *et al*., 2021. Inhibition of Nrf2-mediated glucose metabolism by brusatol synergistically sensitizes acute myeloid leukemia to Ara-C. Biomed. Pharmacother., Vol. 142. 10.1016/j.biopha.2021.111652.
15. Shang, Q., C. Pan, X. Zhang, T. Yang, J. Wang and Q. Fang, 2022. Overexpression of Nrf2 promotes OGG1 expression by activating AKT signaling pathway to mediate cytarabine resistance in acute myeloid leukemia cells. Blood, 140: 11480-11480.
16. Yu, R., G. Jin and M. Fujimoto, 2021. Dihydroartemisinin: A potential drug for the treatment of malignancies and inflammatory diseases. Front. Oncol., Vol. 11. 10.3389/fonc.2021.722331.
17. Dai, X., X. Zhang, W. Chen, Y. Chen, Q. Zhang, S. Mo and J. Lu, 2021. Dihydroartemisinin: A potential natural anticancer drug. Int. J. Biol. Sci., 17: 603-622.

18. Zhao, X., H. Zhong, R. Wang, D. Liu, S. Waxman, L. Zhao and Y. Jing, 2015. Dihydroartemisinin and its derivative induce apoptosis in acute myeloid leukemia through Noxa-mediated pathway requiring iron and endoperoxide moiety. Oncotarget, 6: 5582-5596.
19. Cao, J.T., H.M. Mo, Y. Wang, K. Zhao and T.T. Zhang *et al*., 2018. Dihydroartemisinin-induced apoptosis in human acute monocytic leukemia cells. Oncol. Lett., 15: 3178-3184.
20. Yao, Z., A. Bhandari, Y. Wang, Y. Pan and F. Yang *et al*., 2018. Dihydroartemisinin potentiates antitumor activity of 5-fluorouracil against a resistant colorectal cancer cell line. Biochem. Biophys. Res. Commun., 501: 636-642.
21. Feng, X., L. Li, H. Jiang, K. Jiang, Y. Jin and J. Zheng, 2014. Dihydroartemisinin potentiates the anticancer effect of cisplatin via mTOR inhibition in cisplatin-resistant ovarian cancer cells: Involvement of apoptosis and autophagy. Biochem. Biophys. Res. Commun., 444: 376-381.
22. Ianevski, A., A.K. Giri and T. Aittokallio, 2022. SynergyFinder 3.0: An interactive analysis and consensus interpretation of multi-drug synergies across multiple samples. Nucleic Acids Res., 50: W739-W743.
23. Matthews, J.H., X. Liang, V.J. Paul and H. Luesch, 2018. A Complementary chemical and genomic screening approach for druggable targets in the Nrf2 pathway and small molecule inhibitors to overcome cancer cell drug resistance. ACS Chem. Biol., 13: 1189-1199.
24. Kantarjian, H., T. Kadia, C. DiNardo, N. Daver and G. Borthakur *et al*., 2021. Acute myeloid leukemia: Current progress and future directions. Blood Cancer J., Vol. 11. 10.1038/s41408-021-00425-3.
25. Demain, A.L., 2006. From natural products discovery to commercialization: A success story. J. Ind. Microbiol. Biotechnol., 33: 486-495.
26. Yang, Y.H., J.W. Mao and X.L. Tan, 2020. Research progress on the source, production, and anti-cancer mechanisms of paclitaxel. Chin. J. Nat. Med., 18: 890-897.
27. Wu, J., Y. Li, Q. He and X. Yang, 2023. Exploration of the use of natural compounds in combination with chemotherapy drugs for tumor treatment. Molecules, Vol. 28. 10.3390/molecules28031022.
28. Lou, J.S., L.P. Zhao, Z.H. Huang, X.Y. Chen and J.T. Xu *et al*., 2021. Ginkgetin derived from *Ginkgo biloba* leaves enhances the therapeutic effect of cisplatin via ferroptosis-mediated disruption of the Nrf2/HO-1 axis in EGFR wild-type non-small-cell lung cancer. Phytomedicine, Vol. 80. 10.1016/j.phymed.2020.153370.
29. Lu, J.J., L.H. Meng, Y.J. Cai, Q. Chen, L.J. Tong, L.P. Lin and J. Ding, 2008. Dihydroartemisinin induces apoptosis in HL-60 leukemia cells dependent of iron and p38 mitogen-activated protein kinase activation but independent of reactive oxygen species. Cancer Biol. Ther., 7: 1017-1023.
30. Wang, Z., W. Hu, J.L. Zhang, X.H. Wu and H.J. Zhou, 2012. Dihydroartemisinin induces autophagy and inhibits the growth of iron-loaded human myeloid leukemia K562 cells via ROS toxicity. FEBS Open Bio, 2: 103-112.
31. Kim, S.J., M.S. Kim, J.W. Lee, C.H. Lee and H. Yoo *et al*., 2006. Dihydroartemisinin enhances radiosensitivity of human glioma cells *in vitro*. J. Cancer Res. Clin. Oncol., 132: 129-135.
32. Yang, S., D. Zhang, N. Shen, G. Wang, Z. Tang and X. Chen, 2019. Dihydroartemisinin increases gemcitabine therapeutic efficacy in ovarian cancer by inducing reactive oxygen species. J. Cell. Biochem., 120: 634-644.
33. Ianevski, A., A.K. Giri and T. Aittokallio, 2020. SynergyFinder 2.0: Visual analytics of multi-drug combination synergies. Nucleic Acids Res., 48: W488-W493.
34. Szakács, G., J.K. Paterson, J.A. Ludwig, C. Booth-Genthe and M.M. Gottesman, 2006. Targeting multidrug resistance in cancer. Nat. Rev. Drug Discovery, 5: 219-234.
35. Rosell, R., M. Taron, A. Ariza, A. Barnadas and J.L. Mate *et al*., 2004. Molecular predictors of response to chemotherapy in lung cancer. Semin. Oncol., 31: 20-27.
36. No, J.H., Y.B. Kim and Y.S. Song, 2014. Targeting Nrf2 signaling to combat chemoresistance. J. Cancer Prev., 19: 111-117.
37. Ren, D., N.F. Villeneuve, T. Jiang, T. Wu, A. Lau, H.A. Toppin and D.D. Zhang, 2011. Brusatol enhances the efficacy of chemotherapy by inhibiting the Nrf2-mediated defense mechanism. Proc. Natl. Acad. Sci. U.S.A., 108: 1433-1438.
38. Tang, X., H. Wang, L. Fan, X. Wu, A. Xin, H. Ren and X.J. Wang, 2011. Luteolin inhibits Nrf2 leading to negative regulation of the Nrf2/ARE pathway and sensitization of human lung carcinoma A549 cells to therapeutic drugs. Free Radical Biol. Med., 50: 1599-1609.