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ORIGINAL PAPER

A novel alkylating deacetylase inhibitor molecule EDO-S101 in combination with cytarabine synergistically enhances apoptosis of acute myeloid leukemia cells

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Abstract

Acute myeloid leukemia (AML) is a devastating disease. Hybrid agents with dual activity, which have been shown to possess anti-cancer effect, are expected to potentially improve the prognosis of AML patients. EDO-S101 is a novel alkylating deacetylase inhibitor molecule synthesized by the addition of the hydroxamic acid of histone deacetylases inhibitor vorinostat into bendamustine, a DNA-damaging agent. However, the effect of EDO-S101 in combination with traditional chemotherapy drugs has not been studied in AML. In this study, we investigated the effect of EDO-S101 in combination with cytarabine in treating AML cells. The synergic activity against AML was identified by remarkable reduction of cell viability, significant apoptosis enhancement and the upregulation of the cleaved PARP, Casepase-3 and -7 proteins compared with monotherapy. To explain the drivers, we detected the DNA damage pathway including DNA double-strand breaks marker γ-H2AX and DNA damage checkpoint proteins, which was supposed to be responsible for the enhanced apoptosis activity. In summary, our data demonstrated that EDO-S101 in combination with cytarabine could synergistically induce the apoptosis of AML cells and it might be a potential regimen for treating leukemia.

Keywords Acute myeloid leukemia · EDO-S101 · Cytarabine · Apoptosis · DNA damage

Introduction

Acute myeloid leukemia (AML) is a heterogeneous disease characterized by myeloid differentiation suppression and abnormal proliferation of hematopoietic blast cells [1, 2]. Currently, anthracycline plus cytarabine (Ara-C) is the traditional induction chemotherapeutic regimen for AML patients [3, 4]. A number of new agents have been added to the AML management [5, 6]. However, it is regrettable that relatively few new agents have been approved in the past 20 years [7] and AML remains incurable when adverse risk features exist [8]. While evidence showed that there was modest improvement in prognosis for younger patients with AML, the overall outcome remains dissatisfied [9]. Therefore, to overcome the dilemma, new treatments are required.

Cooperative or at least additive effects has been observed in the treatment of cancer with Histone deacetylase inhibitors (HDACis) in combination with DNA damage agents [10]. Bendamustine is an alkylating agent consisting of purine-like structural group and an alkylating nitrogen mustard moiety [11, 12], which has been shown to have valid clinical activity and manageable toxicity in treating

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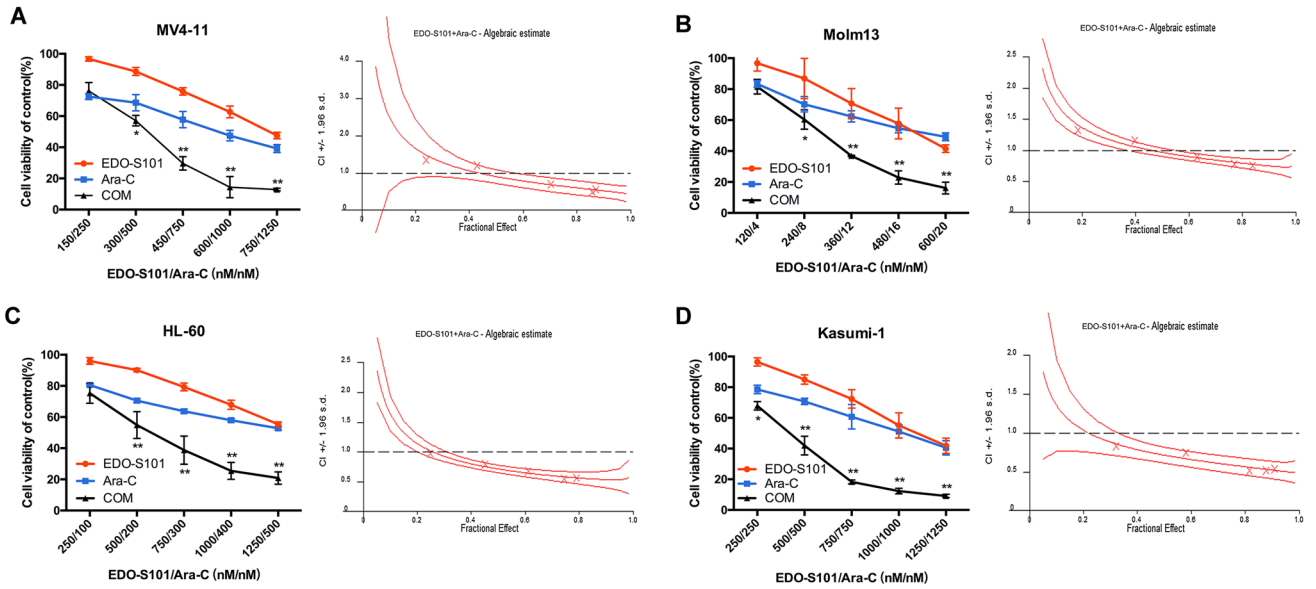


Fig. 1 Proliferation inhibition of AML cell lines treated by EDO-S101 and Ara-C alone or simultaneously. MV4-11 cells (**a**), Molm13 cells (**b**), HL-60 cells (**c**) and Kasumi-1 cells (**d**) were treated with EDO-S101 and Ara-C alone or simultaneously for 48 h. The rate of viability was measured by a MTT assay and the synergistic effect curves were determined by Calcusyn software. In all of the above, error bars represent the SD. The *p* values were determined using two-tailed unpaired *t* test, $*p < 0.05$, and $**p < 0.001$

hematologic malignancies [12, 13]. SAHA is one of the HDACis that plays an important role in the epigenetic regulation of gene expression to anticancer [14]. EDO-S101 is a novel first-in-class hybrid structurally fused of bendamustine and vorinostat (SAHA) [15, 16]. Several trials have demonstrated that the EDO-S101 provides superior efficacy to single bendamustine or SAHA [15, 17, 18]. It is being investigated in the phase I clinical study in hematologic malignancies currently [19, 20]. Nevertheless, the synergistic effect of this novel hybrid in combination with other anticancer drugs remains unknown in experimental and clinical settings. Our hypothesis is that this hybrid in combination with traditional drug might exert more efficient therapeutic activity in AML.

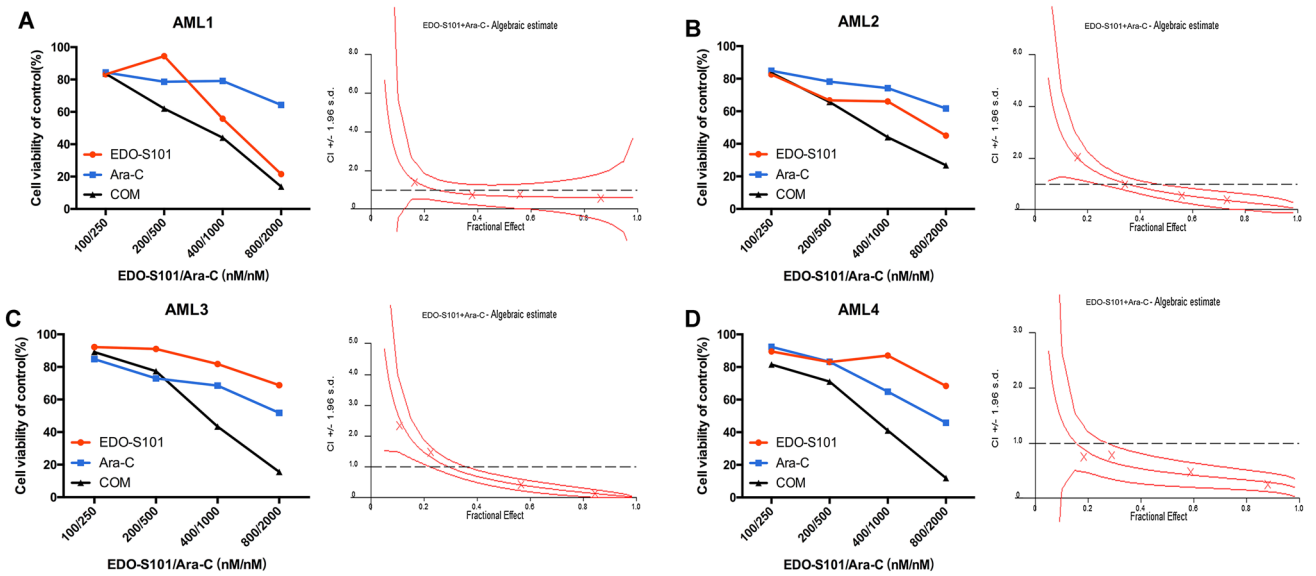


Fig. 2 Proliferation inhibition of AML primary cells by EDO-S101 and Ara-C alone or simultaneously. AML patients' primary cells were treated with EDO-S101 EDO-S101 and Ara-C alone or simultaneously for 48 h. The detection and analysis methods used were same as those shown in Fig. 1

Table 1 The synergistic effect of EDO-S101 and Ara-C on MV4-11, Molm13, HL-60 and Kasumi-1 cells was determined by CalcuSyn software. CIs calculated at the ED50, ED75 and ED90 are as follows:

	Combination indexes		
	ED-50	ED-75	ED-90
MV4-11	0.90593	0.64786	0.52541
Molm13	0.96831	0.81864	0.75074
HL-60	0.71631	0.58307	0.53891
Kasumi-1	0.71644	0.58923	0.52521

Table 2 The synergistic effect of EDO-S101 and Ara-C on primary AML cells was determined by CalcuSyn software. CIs calculated at the ED50, ED75 and ED90 are as follows:

	Combination Indexes		
	ED-50	ED-75	ED-90
AML1	0.67488	0.62086	0.61532
AML2	0.67361	0.34521	0.183
AML3	0.51316	0.22388	0.09838
AML4	0.47198	0.35619	0.28393

As an antimetabolite, Ara-C plays the pharmacological role through DNA damage [21], and is one of the most effective and common cytotoxic agents for treatment of AML [22, 23]. In this study, we investigated the effect of EDO-S101 in combination with Ara-C on AML and further explained the potential synergic mechanisms.

Materials and methods

Materials

Antibodies PARP, Caspase-3, Caspase-7, γ-H2AX, P-ATM, P-ATR, P-CHK1, P-CHK2 and GAPDH were purchased from Cell Signaling Technology (Beverly, MA, USA). ATR, ATM, CHK1 and CHK2 were purchase from ProteinTech (Rosemont, USA). EDO-S101 was presented from Minsheng Institute of Pharmaceutical Research (Hangzhou, China). Cytarabine was bought from Selleck Chemicals (Houston, USA). EDO-S101 and Ara-C would be diluted to 100–1000 μM with Dimethyl sulfoxide (DMSO) and phosphate-buffered saline (PBS), respectively.

Methods

AML cell lines and primary cells

MV4-11 and Molm13 cell lines were kindly gifted by Professor Ravi Bhatia (City of Hope National Medical Center, Duarte, CA). Kasumi-1 and HL-60 cell lines were purchased from Cell Bank of Type Culture Collection of Chinese Academy of Science (Shanghai, China). MV4-11 and Molm13 cells were cultured in Iscove's Modified Dulbecco's Medium (IMDM) (Gibco, Billings, MT, USA), while HL-60 and Kasumi-1 cells were cultured in RPMI 1640 medium (Gibco, Billings, MT, USA), supplemented with 10% fetal bovine serum (Gibco) at 37 °C in a humid incubator containing 5% CO_2. Peripheral blood mononuclear cells (PBMCs) were collected through venipuncture and isolated by Ficoll-Hypaque (Sigma-Aldrich) density gradient centrifugation. Informed consents were acquired from patients according to institutional guidelines.

Cell viability assay

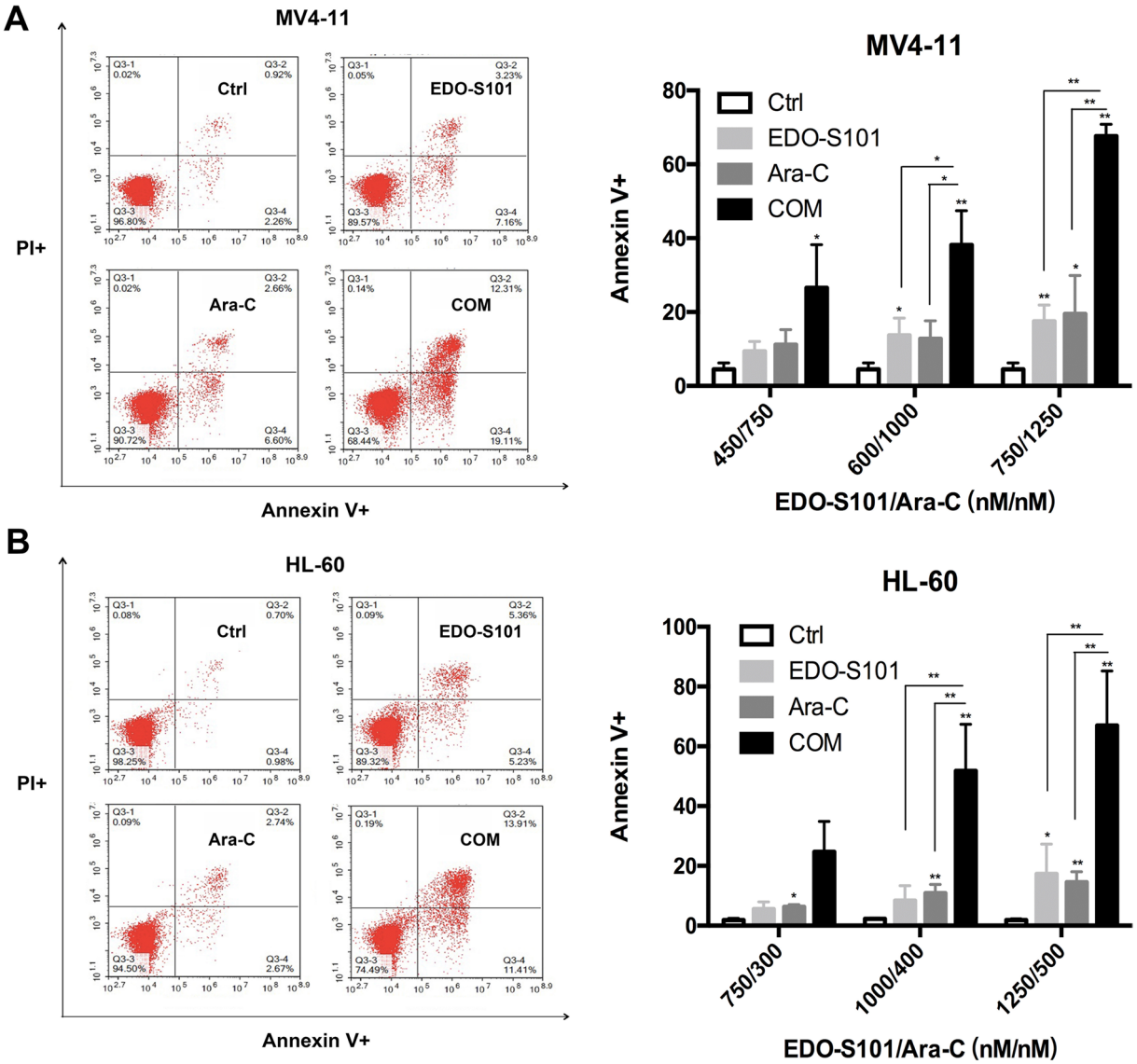
Cells were seeded into 96-well plates at 2 × 105 (cell-line cells) or 1 × 106 (primary AML cells) and treated with EDO-S101, Ara-C and the combination for 48 h at 37 °C incubator, 20 μl MTS solution (Promega) was added and the mixtures were incubated for 4 h in the incubator. Then, plates were read at an absorbance of 490 nm.

Apoptosis assay

Cells were treated with EDO-S101, Ara-C and the combination for 48 h and harvested. After washed three times with PBS, cells were suspended in 1 × binding buffer and co-stained with 5 μl AnnexinV-Fluorescein Isothiocyanate (FITC) plus 10 μl Propidium Iodide (PI) in an apoptosis detection kit (BD Pharmingen, San Diego, C. Apoptotic cells were analyzed by FACS flow cytometer (Becton–Dickinson, San Diego, CA, USA).

Western blot analysis

Treated cells were harvested and lysed in 1 × RIPA buffer (Cell Signaling Technology, Beverly, MA, USA), then centrifuged at 12000×*g* for 15 min at 4 °C. The supernatant that contained proteins was collected. BCA reagent was used to detect the protein concentration. Proteins were separated via different concentrations SDS-PAGE gel (Life Technology, USA), then transferred to a PVDF membrane (Millipore, Billerica, MA, USA) for 1.5–2 h and blocked in Tris-buffered solution (TBS) containing 5% non-fat milk for 1–2 h. The membrane was divided according to protein size and incubated in corresponding primary antibodies overnight at 4 °C. Then the membranes were washed with TBS-T buffer for three times and incubated with secondary antibodies (CST, Beverly, MA, USA) for 1–1.5 h. The protein bands were visualized using an ECL kit (Thermo scientific, USA)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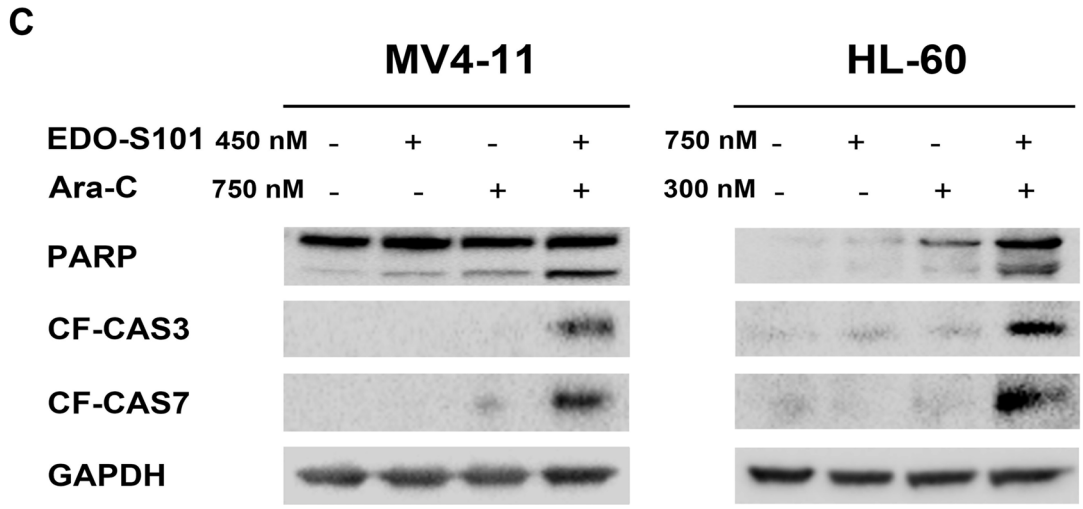
C
MV4-11
HL-60
EDO-S101 450 nM - + - +
750 nM - + - +
Ara-C 750 nM - - + +
300 nM - - + +
PARP
CF-CAS3
CF-CAS7
GAPDH

◂**Fig. 3** Apoptosis of AML cells induced by EDO-S101 and Ara-C alone or simultaneously. MV4-11 (**a**) and HL-60 cells (**b**) were treated with EDO-S101 and Ara-C alone or simultaneously for 48 h and the apoptosis was measured by flow cytometry. Expression of PARP, caspase-3 and -7 were analyzed by Western blot (**c**). In all of the above, error bars represent the SD. The *p* values were determined using two-tailed unpaired *t* test, $*p < 0.05$, and $**p < 0.001$

and analysis by the image lab software (Bio-Rad, California, USA).

Statistical analysis

CalcuSyn software (Biosoft, Cambridge, UK) was used to calculate Combination Index (CI). GraphPad Prism software (San Diego, CA) was used to calculate IC_{50}. Statistical analyses were performed using two-tailed unpaired *t* test.

Results

EDO-S101 plus Ara-C synergistically inhibited proliferation of AML cell lines and primary AML cells

Cells were plated into 96-well plates and exposed to increasing concentrations of corresponding drugs. The results showed that EDO-S101 in combination with Ara-C synergistically inhibited growth of AML cell lines (Fig. 1) ($p < 0.05$) and primary AML cells (Fig. 2). The synergistic effect curves were determined by Calcusyn analyses. Combination indexes (CIs) at the ED50, ED75 and ED90 are presented (Tables 1, 2). Our finding indicated that EDO-S101 in combination with Ara-C had a synergistic therapeutic effect on AML cells.

EDO-S101 in combination with Ara-C synergistically induced apoptosis of AML cells

AML cells were treated with EDO-S101, Ara-C and the combination for 48 h. Compared with untreated and single agents treated, EDO-S101 plus Ara-C significantly ($p < 0.05$) increases apoptosis of MV4-11 and HL-60 cells (Fig. 3a, b). Then, we analyzed the apoptosis-related pathway proteins by western blot analysis. As showed in Fig. 3c, we observed that the combined treatment obviously upregulated the expression of cleaved PARP, Caspase-3 and Caspase-7 at 48 h. Therefore, we investigated ESO-S101 and Ara-C could synergistically induce apoptosis of AML cells.

EDO-S101 in combination with Ara-C synergistically enhanced DNA damage response

To explain the cause of apoptosis, we identified the level of DNA damage-related proteins P-ATM, P-ATR, P-CHK1 and P-CHK2 were obviously increased while ATM, ATR, CHK1 and CHK2 had no significant change (Fig. 4). We speculated that DNA damage might be the main reason of AML cells apoptosis.

Discussion

In this study, we found the synergistic anti-AML effect of EDO-S101 and Ara-C on AML cells, which probably attributed to apoptosis enhancement, and to potentially improve AML prognosis. Further, as we hypothesized, the mechanism behind the apoptosis enhancement might be the synergistic increase of DNA damage.

Our study comprehensively profiled the synergistic effect of EDO-S101 and Ara-C in treating AML in vitro. EDO-S101 was demonstrated as an agent with anti-hematological disease activity as monotherapy [17, 19, 24]. Combination chemotherapies are main options for acute leukemia [25]. However, the effect of EDO-S101 in combination with other agents in treating AML is still unknown. In this study, AML cell lines and patients' primary cells were treated by EDO-S101 in combination with Ara-C. Consequently, CIs < 1 at ED50, ED75 and ED90 were detected, which was consistently indicating the presence of synergistic effect.

Subsequent investigation found that EDO-S101 had the ability to augment the apoptosis induced by Ara-C, which was detected by flow cytometer, resulting in improved effect on AML cells in comparison to EDO-S101 or Ara-C alone. It is well known that the feature of the apoptotic process constantly involves of caspases [26], and Poly (ADP-ribose) Polymerase (PARP) [27]; therefore, we detected the level of cleaved PARP, Caspase-3 and Caspase-7 and found consistently upregulation of these proteins. Given that the resistance to Ara-C commonly leads to the failure of AML treatment [28, 29], our results that EDO-S101 enhanced the apoptosis of AML cells induced by Ara-C suggested that potentially have implications to improve the therapeutic effect of traditional chemotherapy regimens and the tolerability for the patients who are unable to tolerate intensive chemotherapy.

Finally, we explored the mechanism of the combination chemotherapy and found that the enhanced apoptosis was possibly caused by strengthened DNA damage. It has been shown that HDACi has the ability to induce the growth arrest and apoptosis of cancer cells, and ultimately leading to cell death [30, 31], and it also improves the

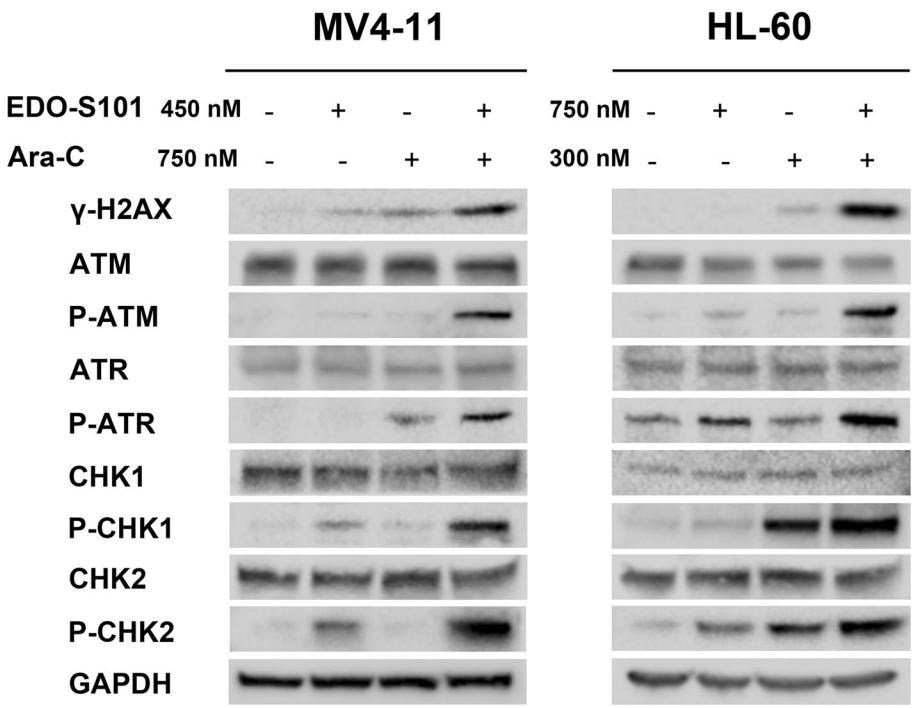

Fig. 4 EDO-S101 and Ara-C synergistically induced DNA damage signaling. MV4-11 and HL-60 cells were treated with EDO-S101 and Ara-C for 48 h. For the level of γ-H2AX, ATM, P-ATM, ATR, P-ATR, CHK1, P-CHK1, CHK2 and P-CHK2 proteins were analyzed by Western blot

sensitivity of tumor cells to chemotherapy or other therapeutic method [32, 33]. Ara-C as a first-choice chemotherapy drug in the AML treatment [28] is able to block DNA synthesis by incorporating into DNA [23, 34]. Unrepaired DNA damage promotes the pathways of cell apoptotic and necrotic death [35]. Therefore, we assumed that the combination of EDO-S101 and Ara-C could mutually promote DNA damage in AML cells. H2AX was demonstrated to be extensively phosphorylated (γ-H2AX) in the presence of DNA double-stranded breaks (DSBs) [36, 37]. ATM and ATR proteins are central to the entire DNA damage response [38]. Downstream of these proteins are CHK1 and CHK2 kinases and their homologues [39], which could be phosphorylated by ATM and ATR in response to replication stress or DNA damage [40–42]. Therefore, we detected the expression of γ-H2AX and some DNA damage checkpoint proteins. Our result showed that EDO-S101 in combination with Ara-C significantly upregulated the expression of γ-H2AX, P-ATM, P-ATR, P-CHK1 and P-CHK2, suggesting that, as we hypothesized, combination therapy of EDO-S101 plus Ara-C enhanced AML apoptosis possibly through promoting DNA damage.

There are some limitations in this study. We had studied the synergistic effects and explored its mechanism of the EDO-S101 in combination with Ara-C in vitro; however, for assessing the biological safety, in vivo study remains to be necessary. Moreover, whether the synergy results can be extended to other anti-AML drugs are waiting to be explored, and the optimal combination regimen is still an open issue to be studied.

In conclusion, the hybrid EDO-S101 in combination with conventional chemotherapeutic drug Ara-C had synergic effect on AML cells and patients' primary cells. This combination therapy synergistically increased the apoptosis of AML cells probably through the enhancement of DNA damage. Our data suggested that EDO-S101 in combination with Ara-C might be an effective and promising treatment option for AML.

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Authors' contributions JJ designed the experiments. JRJ performed the experiments and was a major contributor in writing the manuscript. SHM, FLL and XL analyzed data. XH and MXY collected the patients' information. WJG contributed to revise the manuscript. All authors read and approved the final manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Informed consent Informed consents were acquired from patients according to institutional guidelines.

References

1. Assi SA, Imperato MR, Coleman DJL, Pickin A, Potluri S, Ptasinska A, et al. Subtype-specific regulatory network rewiring in acute myeloid leukemia. Nat Genet. 2018;51(1):151–62. https://doi.org/10.1038/s41588-018-0270-1.
2. Miller KD, Siegel RL, Lin CC, Mariotto AB, Kramer JL, Rowland JH, et al. Cancer treatment and survivorship statistics, 2016. CA: a cancer journal for clinicians. 2016;66(4):271–89. https://doi.org/10.3322/caac.21349.
3. Fernandez HF, Sun Z, Yao X, Litzow MR, Luger SM, Paietta EM, et al. Anthracycline dose intensification in acute myeloid leukemia. N Engl J Med. 2009;361(13):1249–59. https://doi.org/10.1056/NEJMoa0904544.
4. Ferrara F, Schiffer CA. Acute myeloid leukaemia in adults. Lancet. 2013;381(9865):484–95. https://doi.org/10.1016/S0140-6736(12)61727-9.
5. Dohner H, Estey EH, Amadori S, Appelbaum FR, Buchner T, Burnett AK, et al. Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European LeukemiaNet. Blood. 2010;115(3):453–74. https://doi.org/10.1182/blood-2009-07-235358.
6. Wei AH, Tiong IS. Midostaurin, enasidenib, CPX-351, gemtuzumab ozogamicin, and venetoclax bring new hope to AML. Blood. 2017;130(23):2469–74. https://doi.org/10.1182/blood-2017-08-784066.
7. Burnett A, Wetzler M, Lowenberg B. Therapeutic advances in acute myeloid leukemia. J Clin Oncol. 2011;29(5):487–94. https://doi.org/10.1200/JCO.2010.30.1820.
8. Patel JP, Gonen M, Figueroa ME, Fernandez H, Sun Z, Racevskis J, et al. Prognostic relevance of integrated genetic profiling in acute myeloid leukemia. N Engl J Med. 2012;366(12):1079–89. https://doi.org/10.1056/NEJMoa1112304.
9. Jurcic JG. Highlights in hematologic malignancy treatments: leukemia, myelodysplastic syndromes, and allotransplant-new drugs on the horizon for acute myeloid leukemia. JAMA Oncol. 2017;3(3):299–300. https://doi.org/10.1001/jamaoncol.2016.3928.
10. Stiborova M, Eckschlager T, Poljakova J, Hrabeta J, Adam V, Kizek R, et al. The synergistic effects of DNA-targeted chemotherapeutics and histone deacetylase inhibitors as therapeutic strategies for cancer treatment. Curr Med Chem. 2012;19(25):4218–38.
11. Balfour JA, Goa KL. Bendamustine. Drugs. 2001;61(5):631–8. https://doi.org/10.2165/00003495-200161050-00009 **(Discussion 9–40)**.
12. Blumel S, Goodrich A, Martin C, Dang NH. Bendamustine: a novel cytotoxic agent for hematologic malignancies. Clin J Oncol Nurs. 2008;12(5):799–806. https://doi.org/10.1188/08.CJON.799-806.
13. Hoy SM. Bendamustine: a review of its use in the management of chronic lymphocytic leukaemia, rituximab-refractory indolent non-Hodgkin's lymphoma and multiple myeloma. Drugs. 2012;72(14):1929–50. https://doi.org/10.2165/11209510-000000000-00000.
14. Grant S, Easley C, Kirkpatrick P. Vorinostat. Nat Rev Drug Discov. 2007;6(1):21–2. https://doi.org/10.1038/nrd2227.
15. Qiu Y, Li Z, Copland JA, Mehrling T, Tun HW. Combined alkylation and histone deacetylase inhibition with EDO-S101 has significant therapeutic activity against brain tumors in preclinical models. Oncotarget. 2018;9(46):28155–64. https://doi.org/10.18632/oncotarget.25588.
16. Festuccia C, Mancini A, Colapietro A, Gravina GL, Vitale F, Marampon F, et al. The first-in-class alkylating deacetylase inhibitor molecule tinostamustine shows antitumor effects and is synergistic with radiotherapy in preclinical models of glioblastoma. J Hematol Oncol. 2018;11(1):32. https://doi.org/10.1186/s13045-018-0576-6.
17. Yu J, Qiu S, Ge Q, Wang Y, Wei H, Guo D, et al. A novel SAHA-bendamustine hybrid induces apoptosis of leukemia cells. Oncotarget. 2015;6(24):20121–31. https://doi.org/10.18632/oncotarget.4041.
18. Lopez-Iglesias AA, Herrero AB, Chesi M, San-Segundo L, Gonzalez-Mendez L, Hernandez-Garcia S, et al. Preclinical antimyeloma activity of EDO-S101, a new bendamustine-derived molecule with added HDACi activity, through potent DNA damage induction and impairment of DNA repair. J Hematol Oncol. 2017;10(1):127. https://doi.org/10.1186/s13045-017-0495-y.
19. Besse L, Kraus M, Besse A, Bader J, Silzle T, Mehrling T, et al. The first-in-class alkylating HDAC inhibitor EDO-S101 is highly synergistic with proteasome inhibition against multiple myeloma through activation of multiple pathways. Blood Cancer J. 2017;7(7):e589. https://doi.org/10.1038/bcj.2017.69.
20. Mehrling T, Chen Y. The alkylating-HDAC inhibition fusion principle: taking chemotherapy to the next level with the first in class molecule EDO-S101. Anticancer Agents Med Chem. 2016;16(1):20–8.
21. Grant S. Ara-C: cellular and molecular pharmacology. Adv Cancer Res. 1998;72:197–233.
22. Di Tullio A, Rouault-Pierre K, Abarrategi A, Mian S, Grey W, Gribben J, et al. The combination of CHK1 inhibitor with G-CSF overrides cytarabine resistance in human acute myeloid leukemia. Nat Commun. 2017;8(1):1679. https://doi.org/10.1038/s41467-017-01834-4.
23. Veuger MJ, Heemskerk MH, Honders MW, Willemze R, Barge RM. Functional role of alternatively spliced deoxycytidine kinase in sensitivity to cytarabine of acute myeloid leukemic cells. Blood. 2002;99(4):1373–80.
24. M'Kacher R, Frenzel M, Al Jawhari M, Junker S, Cuceu C, Morat L, et al. Establishment and characterization of a reliable xenograft model of hodgkin lymphoma suitable for the study of tumor origin and the design of new therapies. Cancers. 2018;10(11):E414. https://doi.org/10.3390/cancers10110414.
25. Koczian F, Naglo O, Vomacka J, Vick B, Servatius P, Zisis T, et al. Targeting the ER-mitochondria interface sensitizes leukemia cells towards cytostatics. Haematologica. 2018;104(3):546–55. https://doi.org/10.3324/haematol.2018.197368.
26. Testa U, Riccioni R. Deregulation of apoptosis in acute myeloid leukemia. Haematologica. 2007;92(1):81–94.
27. Kaufmann SH, Desnoyers S, Ottaviano Y, Davidson NE, Poirier GG. Specific proteolytic cleavage of poly(ADP-ribose) polymerase: an early marker of chemotherapy-induced apoptosis. Cancer Res. 1993;53(17):3976–85.
28. Yang C, Boyson CA, Di Liberto M, Huang X, Hannah J, Dorn DC, et al. CDK4/6 Inhibitor PD 0332991 sensitizes acute myeloid leukemia to cytarabine-mediated cytotoxicity. Cancer Res. 2015;75(9):1838–45. https://doi.org/10.1158/0008-5472.CAN-14-2486.
29. Wolach O, Itchaki G, Bar-Natan M, Yeshurun M, Ram R, Herscovici C, et al. High-dose cytarabine as salvage therapy for relapsed or refractory acute myeloid leukemia—is more better or more of the same? Hematol Oncol. 2016;34(1):28–35. https://doi.org/10.1002/hon.2191.
30. Marks PA, Rifkind RA, Richon VM, Breslow R. Inhibitors of histone deacetylase are potentially effective anticancer agents. Clin Cancer Res. 2001;7(4):759–60.
31. Feng R, Oton A, Mapara MY, Anderson G, Belani C, Lentzsch S. The histone deacetylase inhibitor, PXD101, potentiates bortezomib-induced anti-multiple myeloma effect by induction of oxidative stress and DNA damage. Br J Haematol. 2007;139(3):385–97. https://doi.org/10.1111/j.1365-2141.2007.06772.x.

32. Camphausen K, Burgan W, Cerra M, Oswald KA, Trepel JB, Lee MJ, et al. Enhanced radiation-induced cell killing and prolongation of gammaH2AX foci expression by the histone deacetylase inhibitor MS-275. Cancer Res. 2004;64(1):316–21.
33. Munshi A, Kurland JF, Nishikawa T, Tanaka T, Hobbs ML, Tucker SL, et al. Histone deacetylase inhibitors radiosensitize human melanoma cells by suppressing DNA repair activity. Clin Cancer Res. 2005;11(13):4912–22. https://doi.org/10.1158/1078-0432.CCR-04-2088.
34. Major PP, Egan EM, Beardsley GP, Minden MD, Kufe DW. Lethality of human myeloblasts correlates with the incorporation of arabinofuranosylcytosine into DNA. Proc Natl Acad Sci USA. 1981;78(5):3235–9.
35. Roos WP, Thomas AD, Kaina B. DNA damage and the balance between survival and death in cancer biology. Nat Rev Cancer. 2016;16(1):20–33. https://doi.org/10.1038/nrc.2015.2.
36. Paull TT, Rogakou EP, Yamazaki V, Kirchgessner CU, Gellert M, Bonner WM. A critical role for histone H2AX in recruitment of repair factors to nuclear foci after DNA damage. Curr Biol. 2000;10(15):886–95.
37. Fernandez-Capetillo O, Chen HT, Celeste A, Ward I, Romanienko PJ, Morales JC, et al. DNA damage-induced G2-M checkpoint activation by histone H2AX and 53BP1. Nat Cell Biol. 2002;4(12):993–7. https://doi.org/10.1038/ncb884.
38. Zou L, Elledge SJ. Sensing DNA damage through ATRIP recognition of RPA-ssDNA complexes. Science. 2003;300(5625):1542–8. https://doi.org/10.1126/science.1083430.
39. Zhou BB, Elledge SJ. The DNA damage response: putting checkpoints in perspective. Nature. 2000;408(6811):433–9. https://doi.org/10.1038/35044005.
40. Chen Y, Poon RY. The multiple checkpoint functions of CHK1 and CHK2 in maintenance of genome stability. Front Biosci. 2008;13:5016–29.
41. Lopez-Girona A, Tanaka K, Chen XB, Baber BA, McGowan CH, Russell P. Serine-345 is required for Rad3-dependent phosphorylation and function of checkpoint kinase Chk1 in fission yeast. Proc Natl Acad Sci USA. 2001;98(20):11289–94. https://doi.org/10.1073/pnas.191557598.
42. O'Neill T, Giarratani L, Chen P, Iyer L, Lee CH, Bobiak M, et al. Determination of substrate motifs for human Chk1 and hCds1/Chk2 by the oriented peptide library approach. J Biol Chem. 2002;277(18):16102–15. https://doi.org/10.1074/jbc.M111705200.