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Effect of evodiamine on cell death pathways in human gastric cancer cells

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Abstract: Evodiamine (EVO) exerts anti-cancer effect in a majority of cancer cells. BGC-823 and SGC-7901 cells were used to study EVO-induced cytotoxicity in human gastric cancer cell. Our results demonstrated that EVO exposure elicited cell vialibility decrease and G2/M arrest caused by induction of cdc2/cyclin B1 complex activation. EVO also induced caspase-dependent apoptosis and necroptosis caused by induction of actication of RIP, RIP3 and MLKL. Moreover, increase of reactive oxygen species (ROS) levels and cytotoxicity induced by EVO were significantly attenuated by co-treatment with a ROS scavenger, EUK134. In conclusion, EVO induced ROS-dependent cytotoxicity, which may involve apoptosis and necroptosis, in human gastric cancer cells.

Keywords: Evodiamine, human gastric cancer, ROS, cytotoxicity, apoptosis, necroptosis.

INTRODUCTION

In 2020, 1,089,103 newly registered cases and 768,793 deaths make gastric cancer the 6th most common cancer worldwide(Sung *et al.*, 2021). Importantly, for the therapy of gastric cancer, traditional treatment have no satisfactory therapeutic effect (Digklia & Wagner, 2016).

Reactive oxygen species (ROS), a heterogeneous group of oxygen-containing radical species, is involved in a variety of biochemical processes in tumor cells (Srinivas, Tan, Vellayappan, & Jeyasekharan, 2019). The function of ROS is decided by its level. For instance in tumor cells, persistent high level of ROS could be beneficial to tumorigenesis and metastasis. And in gastric cancer cells, ROS participate in the initiation, progression and metastasis as an important factor (Butcher, den Hartog, Ernst, & Crowe, 2017). However, further exposure to higher ROS could induce serious oxidative stress that is able to cause cell death (Sies & Jones, 2020; Srinivas *et al.*, 2019). Thus, it is an effective method to treat gastric cancer by inducing oxidative damage in the cells.

Evodia rutaecarpa, a herbal medicine, is applied for therapeutic treatment for gastrointestinal diseases in traditional Chinese medicine (Lee *et al.*, 2008). And in the fruit of evodia rutaecarpa, the main effective component is evodiamine (EVO), a quinolone alkaloid that involves a variety of biological functions (D. Li, Li, Jiang, Liu, & Zhao, 2022). Some previous studies demonstrated that EVO is endowed with several antitumor effects which may include inducing inhibition of proliferation and metastasis, G2/M arrest and cell apoptosis (Hwang *et al.*, 2020; Panda & Biswal, 2022; J. Y. Yang, Woo, Lee, & Kim, 2022; R. Yang, Wen, Yang, Dai, & Zhao, 2021; Zhou & Hu, 2018). Although previous studies reported that EVO treatment could induce ROS generation (Wang *et al.*, 2018; J. Yang, Wu, Tashino, Onodera, & Ikejima, 2010), the role of ROS in EVO-induced cytotoxicity remains unclear. In our study, the EVO-induced cell death in human gastric cancer cells are investigated.

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MATERIALS AND METHODS

Reagents

EVO, Z-VAD-fmk (A pan-caspase inhibitor), AG14361 (A PARP1 inhibitor), Nec-1 (A RIP inhibitor) and EUK134 (A ROS quencher) were all supplied by Selleck.

Cell culture

Two human gastric adenocarcinoma cell lines, BGC-823 and SGC-7901, were supplied from the National Infrastructure of Cell Line Resource. The cells were cultured in DMEM/high glucose (10% FBS; Gibco) at 37˚C, 5% CO_2.

Cell viability assay

Cells seeding was performed in 96-well plates ($1x10^4$ cells per well). After attachment, cells were treated as follows: 1) for fig. 1, cells were treated with EVO (5-15μM) for three time points (24-72 h); 2) for fig. 5, cells were pre-treated with Z-VAD-fmk, AG14361, Nec-1 or EUK134 for 1h, followed by EVO plus the consequent inhibitor for another 24h. Then a staining with CCK-8 incubation solution (Yeasen) for 1h (37°C). At last, absorbance at 450 nm was recorded.

Cell cycle assay

Cells seeding was performed in 6-cm dishes ($6x10^5$ cells per dish). After attachment, cells were treated with EVO (10μM, 24h) and then harvested (trypsinization and consequent centrifugation). Next, a fixation with ethanol (70%; 24h, 4°C) was performed. After a wash with PBS, a staining with PI (40mg/mL; Yeasen) for 30 min (room

temperature) was performed and flow cytometric analysis (Beckman) were performed.

Cell apoptosis assay

Cells seeding was performed in 6-cm dishes ($6x10^5$ cells per dish). After attachment, cells were treated with 10μM EVO (10μM, 24h) and then harvested (trypsinization and consequent centrifugation). Then a staining with Annexin V-FITC+PI incubation solution (30 min, room temperature; Yeasen) and flow cytometric analysis (Beckman) were performed.

Mitochondrial membrane potential (Δψm) assay

The cells samples were collected as in "Cell apoptosis assay". Then a staining with JC-1 solutions (Yeasen) for 40 min (room temperature) and flow cytometric analysis (Beckman) were performed.

ROS assay

The cells samples were collected as in "Cell apoptosis assay". Then a staining with DCFH-DA solutions (Yeasen) for 30 min (37°C) and flow cytometric analysis (Beckman) were performed.

Western blot

This procedure was carried out as previously represented (Liu, Li, Chen, & Ge, 2020). The list of antibodies are as below: p-cdc2 (#9114), cdc2 (#77055), cyclin B1 (#12231), cdc25C (#4688), caspase3 (#14220), cleaved-caspase3 (#9664), PARP1 (#9532), p-RIP (#65746), RIP (#3493), p-RIP3 (#93654), RIP3 (#13526), p-MLKL (#91689), MLKL (#14993), β-actin (AC028) and anti-Rabbit IgG (#7074). The dilution ration of β-actin and anti-Rabbit IgG were 1:5000 and the others were 1:1000. HRP-conjugated β-actin was purchased from ABclonal and the others were purchased from CST. Representative blots from three independent experiments are represented.

STATISTICAL ANALYSIS

Data analysis was performed using Prism 7.0 software. Data are shown as means ± SD. Student's t-test and one-way ANOVA were applied for two-group comparison and multiple-group comparison, respectively.

RESULTS

Evodiamine induces cell viability decrease

We use CCK-8 kit to identify the effects of EVO on the cell viability. As shown in fig. 1, EVO elicited a decrease in cell viability of BGC-823 and SGC-7901 cells in both a dose- and time-dependent manner.

Evodiamine induces G2/M cell cycle arrest

We use PI staining to identify the effects of EVO on the cell cycle of human gastric cancer cells. As shown in fig. 2A, EVO exposure induced a significant increase in G2/M cell population. As shown in fig. 2B, in the cells exposed to EVO, the protein levels of p-cdc2 (Thr161) and cyclin B1 appeared to increase over time, indicating that cdc2/cyclin B1 complex may be activated after EVO treatment. Furthermore, EVO exposure elicited a decrease in the protein levels of interphase cdc25C (60 kDa) and an increase in mitotic cdc25C (75 kDa).

Evodiamine induces apoptosis

We use Annexin-V/PI staining to identify whether EVO induced apoptosis in human gastric cancer cells. As shown in fig. 3A, EVO exposure elicited a significant increase in the apoptotic cell population in BGC-823 and SGC-7901 cells, indicating that apoptosis occurred after EVO treatment. Furthermore, as shown in fig. 3B, EVO exposure elicited an increase in cleaved caspase-3, caspase-9 and PARP1, indicating that EVO exposure activated caspase-3, caspase-9 and PARP1. In addition, a significant decrease of BCL2/BAX ratio (fig. 3C) and a Δψm dissipation (fig. 3D) were found in cells exposed to EVO.

Evodiamine induces necroptosis

We also examined whether necroptosis was induced by EVO exposure in human gastric cancer cells. Necroptosis is a regulated cell death (RCD) type that involves activation of RIP, RIP3 and MLKL. As shown in fig. 4, EVO exposure elicited an increase in RIP phosphorylation (Ser166), RIP3 phosphorylation (Ser227) and MLKL phosphorylation (Ser358), indicating that EVO may induce necroptosis in human gastric cancer cells.

Evodiamine induces ROS-dependent cell death

We also used Z-VAD-fmk (pan-caspase inhibitor), AG14361 (PARP1 inhibitor) and Nec-1 (RIP1 inhibitor) to confirm that EVO induce apoptosis and necroptosis. As shown in fig. 5A, to our surprise, pretreatment of all the three inhibitors had no effects on EVO-induced cytotoxicity, indicating that EVO-induced cytotoxicity was a complicated process. We also used DCFH-DA staining to identify the effect of EVO on ROS levels. As shown in fig. 5B, significantly increased ROS levels were observed after EVO treatment. Furthermore, EUK143, a ROS quencher (Ajith, 2018) was used to identify the role of ROS in EVO-induced cytotoxicity. We found that EUK134 significantly alleviated the increase of ROS levels (fig. 5B) and cell death (fig. 5C) induced by EVO, indicating that ROS may be involved in EVO-induced cytotoxicity.

DISCUSSION

Evodiamine, a key active ingredient of the fruit of Evodia rutaecarpa, has been demeonstrated anti-tumor activity in a variety of tumor cells, including colon (Kim *et al.*, 2019; F. S. Li *et al.*, 2020), lung (Jiang *et al.*, 2020; Panda & Biswal, 2022), osteosarcoma (S. Yang *et al.*, 2020; Zhou & Hu, 2018), breast (Du *et al.*, 2017), hepatocellular (Guo *et al.*, 2018), pancreatic (Hong *et al.*, 2020) and prostate cancer cells (Cheng *et al.*, 2022; Lei *et al.*, 2022).

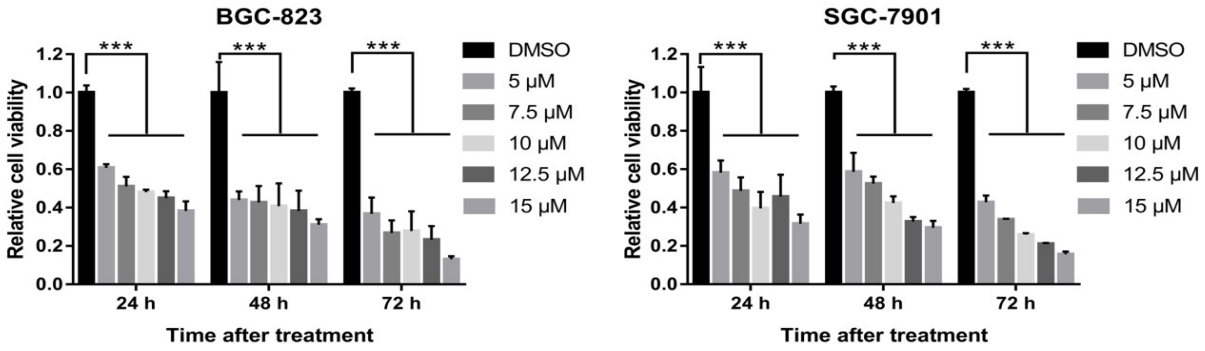

Fig. 1: Evodiamine induces cell death in human gastric cancer cells. BGC-823 and SGC-7901 cells were treated with DMSO and EVO (5, 7.5,10,12.5, 15μM) for 24, 48 and 72h and then the cell viability were measured using CCK-8 assay.

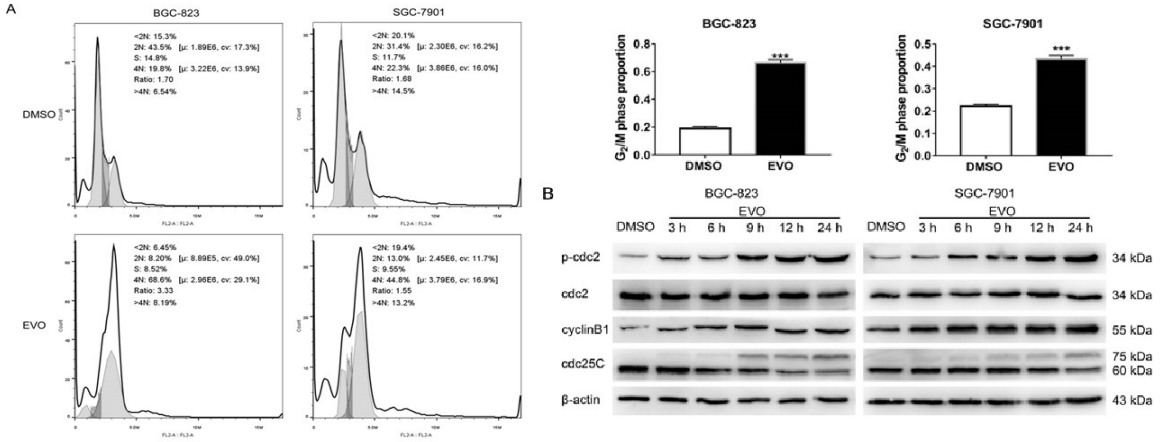

Fig. 2: Evodiamine induces G2/M cell cycle arrest in human gastric cancer cells. a. Cell cycle of BGC-823 and SGC-7901 cells treated with DMSO or 10μM EVO for 24 h were examined using flow cytometry. b. BGC-823 and SGC-7901 cells were treated with DMSO or 10μM EVO for 3, 6, 9, 12 or 24h and the levels of p-cdc2 (Thr161), cdc2, cyclin B1 and cdc25C were examined by western blotting. ***P<0.001 vs. DMSO.

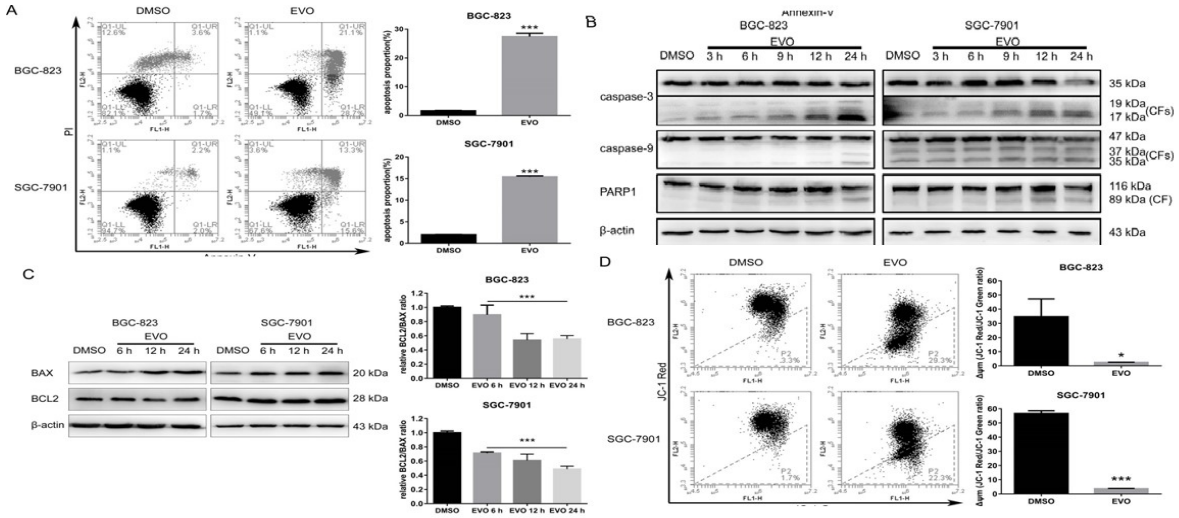

Fig. 3: Evodiamine induces apoptosis in human gastric cancer cells. BGC-823 and SGC-7901 cells were treated with 10μM EVO for 24 h. a. The apoptotic rates were examined using Annexin-V/PI staining assay. b. The protein levels of caspase-3, caspase-9 and PARP1 were examined using western blotting. c. The protein levels of BCL2 and BAX were examined by western blotting and the BCL2/BAX protein ratios were calculated. d. The values of Δψm were examined using JC-1 assay and expressed as the ratio of red to green fluorescence intensity. *P<0.05 and ***P<0.001 vs. DMSO.

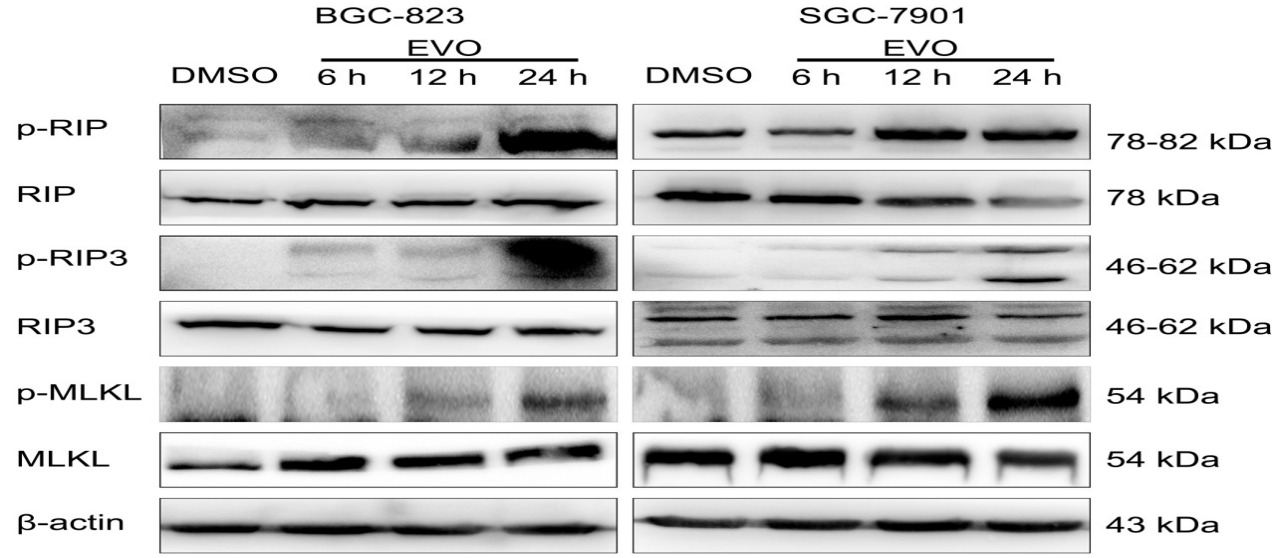

Fig. 4: Evodiamine induces necroptosis in human gastric cancer cells. BGC-823 and SGC-7901 cells were treated with 10μM EVO for 3, 6, 9, 12 or 24h and the protein levels of p-RIP, RIP, p-RIP3, RIP3, p-MLKL and MLKL were examined using western blotting.

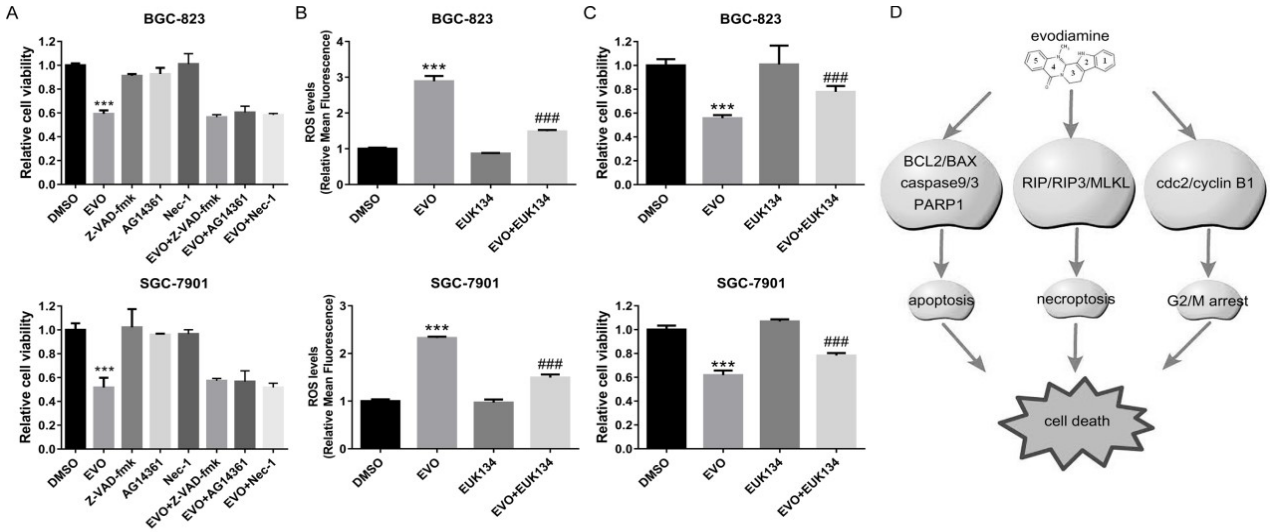

Fig. 5: Evodiamine induces ROS-dependent cell death in human gastric cancer cells. a. Co-treatment with 100μM Z-VAD-fmk or 50μM AG14361 or 50μM Nec-1 did not attenuate the 10μM EVO-induced cytotoxicity. b. EVO increased intracellular ROS levels, which were blocked by 75μM EUK134. c. Co-treatment with 75μM EUK134 significantly attenuated EVO-induced cytotoxicity. ***P<0.001 vs. DMSO; ###P<0.001 vs. EVO. d. Schematic illustration of effect of EVO on cell death pathways in human gastric cancer cells.

The anti-tumor mechanism of evodiamine in gastric cancer cells may include inhibiting PTEN-mediated EGF/PI3K (R. Yang *et al.*, 2021) and inhibiting FAK/AKT/mTOR (J. Y. Yang *et al.*, 2022). However, the underlying mechanism of EVO-induced cytotoxicity in gastric cancer cells has not been fully elucidated.

Firstly, the cytotoxic effect of EVO was examined and our results demonstrated that EVO exposure elicited a significant decrease of cell viability in human gastric cancer cells. Moreover, the effect of EVO on cell cycle of human gastric cancer cells was examined in the present study and the results show that EVO induces increase of cell population in G2/M phase that is in agreement with previous studies (J. Yang *et al.*, 2010; Zhou & Hu, 2018). There are a serious of biological effectors that control progression of cell cycle, such as cyclins and cyclin-dependent kinases (Malumbres & Barbacid, 2009). For

example, cdc2/cyclin B1 complex is the most critical regulatory factor of G2-M progression (Taylor & Stark, 2001). Our results show EVO exposure elicited an increase of the cdc2 phosphorylation at Thr161 and cyclin B1 protein level in BGC-823 and SGC-7901 cells, indicating that cdc2/cyclin B1 complex may be activated by EVO exposure. Moreover, we found cdc25C, a regulatory effector of G2-M progression that can trigger cdc2 activation (Booher, Holman, & Fattaey, 1997), was also affected after EVO treatment. Our results show that EVO exposure elicited an increase of the mitotic cdc25C (75 kDa) and a decrease of interphase cdc25C (60 kDa) in BGC-823 and SGC-7901 cells, confirming that EVO induces G2/M arrest.

Cytotoxicity of EVO involves several RCD types, among them, apoptosis plays an important role (F. Yang *et al.*, 2017; Zhou & Hu, 2018). Likewise, our results show that EVO exposure elicited a significant increase of the apoptotic cell population in BGC-823 and SGC-7901 cells. The crucial step of mitochondrial-dependent intrinsic apoptosis is irreversible mitochondrial outer membrane permeabilization (MOMP) which is mediated by BAX (Delbridge, Grabow, Strasser, & Vaux, 2016) and BCL2 (Czabotar, Lessene, Strasser, & Adams, 2014). Our results show that EVO exposure elicited $\Delta\psi m$ dissipation and a decrease of the BCL2/BAX ratio in BGC-823 and SGC-7901 cells, indicating that EVO may induce MOMP. Furthermore, MOMP can promote apoptotic factors usually located in the intermembrane space of mitochondria (Delbridge *et al.*, 2016), leading to caspase-9 activation (P. Li *et al.*, 1997) and subsequent caspase-3 and -7 activation which are precipitate apoptosis (Riedl & Salvesen, 2007). Our results show that in BGC-823 and SGC-7901 cells, EVO exposure elicited an increase in cleaved caspase-9 and cleaved caspase-3, that is, activation of caspase-9 and caspase-3, indicating that EVO may induce intrinsic apoptosis. Moreover, we also found that EVO exposure elicited an increase of cleaved PARP1, indicating that PARP1 was activated. PARP1 activation can occur in response to DNA damage, leading to $\Delta\psi m$ dissipation and consequent MOMP (Fatokun, Dawson, & Dawson, 2014).

Necroptosis is a type of RCD that manifests a necrotic morphotype, its main mechanism is the sequential activation of RIP, RIP3 and MLKL (Galluzzi *et al.*, 2018). Our results show that in BGC-823 and SGC-7901 cells, EVO exposure elicited increase of RIP, RIP3 and MLKL phosphorylation, indicating that RIP, RIP3 and MLKL may be activated by EVO exposure to precipitate necroptosis.

A lot of evidence suggests that for the processes that are referred to as necessary steps for execution of cell death, pharmacologic inhibition only changes its biochemical and morphologic manifestations, but could not effectively prevent cell death (Galluzzi *et al.*, 2015). For example, although the inhibitors of caspase, PARP1 and RIP can significantly block the activation of caspases, PARP1 and RIP induced by pharmacologic interventions, cell death still remain unchanged (Dunai *et al.*, 2012; Prabhakaran, Li, Borowitz, & Isom, 2004; Steinhart, Belz, & Fulda, 2013). Likewise, our results show that Z-VAD-fmk, AG14361 or Nec-1 failed to protect BGC-823 and SGC-7901 cells from cell death, but rather impel the cell death to switch towards other types.

In whole process of RCD, reverse of early phase biochemical changes such as decreasing ATP levels and oxidative stress could restore cellular homeostasis (Galluzzi *et al.*, 2015). And our results show that in BGC-823 and SGC-7901 cells, EVO exposure elicited a significant increase of ROS levels and pretreatment of EUK134 which is a ROS scavenger significantly attenuated this effect. Moreover, pretreatment of EUK134 effectively inhibited the decrease of cell viability induced by EVO exposure, indicating that EVO-induced cytotoxicity is ROS-dependent.

CONCLUSION

Our results demonstrate that EVO-induced cytotoxicity involves intrinsic apoptosis and necroptosis in human gastric cancer BGC-823 and SGC-7901 cells and ROS may play an important role in this effect.

CONFLICTS OF INTEREST

None of the authors has any conflict of interest to declare.

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REFERENCES

Ajith TA (2018). Role of mitochondria and mitochondria-targeted agents in non-alcoholic fatty liver disease. *Clin. Exp. Pharmacol. Physiol.,* **45**(5): 413-421.

Booher RN, Holman PS and Fattaey A (1997). Human Myt1 is a cell cycle-regulated kinase that inhibits Cdc2 but not Cdk2 activity. *J. Biol. Chem.,* **272**(35): 22300-22306.

Butcher LD, den Hartog G, Ernst PB and Crowe SE (2017). Oxidative stress resulting from helicobacter pylori infection contributes to gastric carcinogenesis. *Cell Mol. Gastroenterol Hepatol.,* **3**(3): 316-322.

Cheng P, Zhang X, Wang X, Liu C, Zhao X, Fan J and Xu C (2022). Identification of evodiamine as a suppressor of prostate cancer progression by reducing AR transcriptional activity via targeting Src. *Endocrine,* **75**(2): 635-645.

Czabotar PE, Lessene G, Strasser A and Adams JM (2014). Control of apoptosis by the BCL-2 protein family: Implications for physiology and therapy. *Nat Rev. Mol. Cell Biol.,* **15**(1): 49-63.

Delbridge AR, Grabow S, Strasser A and Vaux DL (2016). Thirty years of BCL-2: Translating cell death discoveries into novel cancer therapies. *Nat Rev Cancer,* **16**(2): 99-109.

Digklia A and Wagner AD (2016). Advanced gastric cancer: Current treatment landscape and future perspectives. *World J. Gastroenterol,* **22**(8): 2403-2414.

Du J, Sun Y, Lu YY, Lau E, Zhao M, Zhou QM and Su SB (2017). Berberine and evodiamine act synergistically against human breast cancer mcf-7 cells by inducing cell cycle arrest and apoptosis. *Anticancer Res.,* **37**(11): 6141-6151.

Dunai ZA, Imre G, Barna G, Korcsmaros T, Petak I, Bauer PI and Mihalik R (2012). Staurosporine induces necroptotic cell death under caspase-compromised conditions in U937 cells. *PLoS One,* **7**(7): e41945.

Fatokun AA, Dawson VL and Dawson TM (2014). Parthanatos: mitochondrial-linked mechanisms and therapeutic opportunities. *Br. J. Pharmacol.,* **171**(8): 2000-2016.

Galluzzi L, Bravo-San Pedro JM, Vitale I, Aaronson SA, Abrams JM, Adam D, Alnemri ES, Altucci L, Andrews D, Annicchiarico-Petruzzelli M, Baehrecke EH, Bazan NG, Bertrand MJ, Bianchi K, Blagosklonny MV, Blomgren K, Borner C, Bredesen DE, Brenner C, Campanella M, Candi E, Cecconi F, Chan FK, Chandel NS, Cheng EH, Chipuk JE, Cidlowski JA, Ciechanover A, Dawson TM, Dawson VL, De Laurenzi V, De Maria R, Debatin KM, Di Daniele N, Dixit VM, Dynlacht BD, El-Deiry WS, Fimia GM, Flavell RA, Fulda S, Garrido C, Gougeon ML, Green DR, Gronemeyer H, Hajnoczky G, Hardwick JM, Hengartner MO, Ichijo H, Joseph B, Jost PJ, Kaufmann T, Kepp O, Klionsky DJ, Knight RA, Kumar S, Lemasters JJ, Levine B, Linkermann A, Lipton SA, Lockshin RA, Lopez-Otin C, Lugli E, Madeo F, Malorni W, Marine JC, Martin SJ, Martinou JC, Medema JP, Meier P, Melino S, Mizushima N, Moll U, Munoz-Pinedo C, Nunez G, Oberst A, Panaretakis T, Penninger JM, Peter ME, Piacentini M, Pinton P, Prehn JH, Puthalakath H, Rabinovich GA, Ravichandran KS, Rizzuto R, Rodrigues CM, Rubinsztein DC, Rudel T, Shi Y, Simon HU, Stockwell BR, Szabadkai G, Tait SW, Tang HL, Tavernarakis N, Tsujimoto Y, Vanden Berghe T, Vandenabeele P, Villunger A, Wagner EF, Walczak H, White E, Wood WG, Yuan J, Zakeri Z, Zhivotovsky B, Melino G and Kroemer G (2015). Essential versus accessory aspects of cell death: Recommendations of the NCCD 2015. *Cell Death Differ,* **22**(1): 58-73.

Galluzzi L, Vitale I, Aaronson SA, Abrams JM, Adam D, Agostinis P, Alnemri ES, Altucci L, Amelio I, Andrews DW, Annicchiarico-Petruzzelli M, Antonov AV, Arama E, Baehrecke EH, Barlev NA, Bazan NG, Bernassola F, Bertrand MJM, Bianchi K, Blagosklonny MV, Blomgren K, Borner C, Boya P, Brenner C, Campanella M, Candi E, Carmona-Gutierrez D, Cecconi F, Chan FK, Chandel NS, Cheng EH, Chipuk JE, Cidlowski JA, Ciechanover A, Cohen GM, Conrad M, Cubillos-Ruiz JR, Czabotar PE, D'Angiolella V, Dawson TM, Dawson VL, De Laurenzi V, De Maria R, Debatin KM, DeBerardinis RJ, Deshmukh M, Di Daniele N, Di Virgilio F, Dixit VM, Dixon SJ, Duckett CS, Dynlacht BD, El-Deiry WS, Elrod JW, Fimia GM, Fulda S, Garcia-Saez AJ, Garg AD, Garrido C, Gavathiotis E, Golstein P, Gottlieb E, Green DR, Greene LA, Gronemeyer H, Gross A, Hajnoczky G, Hardwick JM, Harris IS, Hengartner MO, Hetz C, Ichijo H, Jaattela M, Joseph B, Jost PJ, Juin PP, Kaiser WJ, Karin M, Kaufmann T, Kepp O, Kimchi A, Kitsis RN, Klionsky DJ, Knight RA, Kumar S, Lee SW, Lemasters JJ, Levine B, Linkermann A, Lipton SA, Lockshin RA, Lopez-Otin C, Lowe SW, Luedde T, Lugli E, MacFarlane M, Madeo F, Malewicz M, Malorni W, Manic G, Marine JC, Martin SJ, Martinou JC, Medema JP, Mehlen P, Meier P, Melino S, Miao EA, Molkentin JD, Moll UM, Munoz-Pinedo C, Nagata S, Nunez G, Oberst A, Oren M, Overholtzer M, Pagano M, Panaretakis T, Pasparakis M, Penninger JM, Pereira DM, Pervaiz S, Peter ME, Piacentini M, Pinton P, Prehn JHM, Puthalakath H, Rabinovich GA, Rehm M, Rizzuto R, Rodrigues CMP, Rubinsztein DC, Rudel T, Ryan KM, Sayan E, Scorrano L, Shao F, Shi Y, Silke J, Simon HU, Sistigu A, Stockwell BR, Strasser A, Szabadkai G, Tait SWG, Tang D, Tavernarakis N, Thorburn A, Tsujimoto Y, Turk B, Vanden Berghe T, Vandenabeele P, Vander Heiden MG, Villunger A, Virgin HW, Vousden KH, Vucic D, Wagner EF, Walczak H, Wallach D, Wang Y, Wells JA, Wood W, Yuan J, Zakeri Z, Zhivotovsky B, Zitvogel L, Melino G and Kroemer G (2018). Molecular mechanisms of cell death: Recommendations of the nomenclature committee on cell death 2018. *Cell Death Differ,* **25**(3): 486-541.

Guo XX, Li XP, Zhou P, Li DY, Lyu XT, Chen Y, Lyu YW, Tian K, Yuan DZ, Ran JH, Chen DL, Jiang R and Li J (2018). Evodiamine induces apoptosis in smmc-7721 and hepg2 cells by suppressing nod1 signal pathway. *Int. J. Mol. Sci.,* **19**(11): 3419.

Hong Z, Wang Z, Zhou B, Wang J, Tong H, Liao Y, Zheng P, Jamshed MB, Zhang Q and Chen H (2020). Effects of evodiamine on PI3K/Akt and MAPK/ERK signaling pathways in pancreatic cancer cells. *Int. J. Oncol.,* **56**(3): 783-793.

Hwang ST, Um JY, Chinnathambi A, Alharbi SA, Narula AS, Namjoshi OA, Blough BE and Ahn KS (2020). Evodiamine mitigates cellular growth and promotes apoptosis by targeting the c-met pathway in prostate cancer cells. *Molecules,* **25**(6): 1320.

Jiang ZB, Huang JM, Xie YJ, Zhang YZ, Chang C, Lai HL, Wang W, Yao XJ, Fan XX, Wu QB, Xie C, Wang MF and Leung EL (2020). Evodiamine suppresses non-small cell lung cancer by elevating CD8(+) T cells and downregulating the MUC1-C/PD-L1 axis. *J. Exp. Clin. Cancer Res.,* **39**(1): 249.

Kim H, Yu Y, Choi S, Lee H, Yu J, Lee JH and Kim WY (2019). Evodiamine eliminates colon cancer stem cells via suppressing notch and Wnt signaling. *Molecules,* **24**(24): 4520.

Lee SH, Son JK, Jeong BS, Jeong TC, Chang HW, Lee ES and Jahng Y (2008). Progress in the studies on rutaecarpine. *Molecules,* **13**(2): 272-300.

Lei Y, Chan M, Liu H, Lyu W, Chen L, Zhong Y, Gan H, Wang M, Qi M, Guo Y, Liu J and Zhang E (2022). Evodiamine as the active compound of evodiae fructus to inhibit proliferation and migration of prostate cancer through PI3K/AKT/NF-kappaB signaling pathway. *Dis. Markers,* **2022**: 4399334.

Li D, Li Y, Jiang X, Liu W and Zhao Q (2022). Evodiamine: A privileged structure with broad-ranging biological activities. *Mini. Rev. Med. Chem.,* **22**(21): 2680-2701.

Li FS, Huang, J, Cui MZ, Zeng JR, Li PP, Li L, Deng Y, Hu Y, He, BC and Shu DZ (2020). BMP9 mediates the anticancer activity of evodiamine through HIF1alpha/p53 in human colon cancer cells. *Oncol Rep,* **43**(2): 415-426.

Li P, Nijhawan D, Budihardjo I, Srinivasula SM, Ahmad M, Alnemri ES and Wang X (1997). Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. *Cell,* **91**(4): 479-489.

Liu N, Li Y, Chen G and Ge K (2020). Evodiamine induces reactive oxygen species-dependent apoptosis and necroptosis in human melanoma A-375 cells. *Oncol Lett,* **20**(4): 121.

Malumbres M and Barbacid M (2009). Cell cycle, CDKs and cancer: A changing paradigm. *Nat. Rev. Cancer,* **9**(3): 153-166.

Panda M and Biswal BK (2022). Evodiamine inhibits stemness and metastasis by altering the SOX9-beta-catenin axis in non-small-cell lung cancer. *J. Cell Biochem.,* **123**(9): 1454-1466.

Prabhakaran K, Li L, Borowitz JL and Isom GE (2004). Caspase inhibition switches the mode of cell death induced by cyanide by enhancing reactive oxygen species generation and PARP-1 activation. *Toxicol. Appl. Pharmacol.,* **195**(2): 194-202.

Riedl SJ and Salvesen GS (2007). The apoptosome: Signalling platform of cell death. *Nat. Rev. Mol. Cell Biol.,* **8**(5): 405-413.

Sies H and Jones DP (2020). Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. *Nat. Rev. Mol. Cell Biol.,* **21**(7): 363-383.

Srinivas US, Tan BWQ, Vellayappan BA and Jeyasekharan AD (2019). ROS and the DNA damage response in cancer. *Redox Biol.,* **25**: 101084.

Steinhart L, Belz K and Fulda S (2013). Smac mimetic and demethylating agents synergistically trigger cell death in acute myeloid leukemia cells and overcome apoptosis resistance by inducing necroptosis. *Cell Death Dis.,* **4**(9): e802.

Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A and Bray F (2021). Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J. Clin.,* **71**(3): 209-249.

Taylor WR and Stark GR (2001). Regulation of the G2/M transition by p53. *Oncogene,* **20**(15): 1803-1815.

Wang R, Deng D, Shao N, Xu Y, Xue L, Peng Y, Liu Y and Zhi F (2018). Evodiamine activates cellular apoptosis through suppressing PI3K/AKT and activating MAPK in glioma. *Onco Targets Ther.,* **11**: 1183-1192.

Yang F, Shi L, Liang T, Ji L, Zhang G, Shen Y, Zhu F and Xu L (2017). Anti-tumor effect of evodiamine by inducing Akt-mediated apoptosis in hepatocellular carcinoma. *Biochem. Biophys. Res. Commun.,* **485**(1): 54-61.

Yang J, Wu LJ, Tashino S, Onodera S and Ikejima T (2010). Protein tyrosine kinase pathway-derived ROS/NO productions contribute to G2/M cell cycle arrest in evodiamine-treated human cervix carcinoma HeLa cells. *Free Radic Res.,* **44**(7): 792-802.

Yang JY, Woo HJ, Lee P and Kim SH (2022). Induction of apoptosis and effect on the fak/akt/mtor signal pathway by evodiamine in gastric cancer cells. *Curr. Issues Mol. Biol.,* **44**(9): 4339-4349.

Yang R, Wen J, Yang T, Dai C and Zhao Y (2021). Evodiamine inhibits gastric cancer cell proliferation via pten-mediated egf/pi3k signaling pathway. *Evid Based Complement Alternat Med.,* **2021**: 5570831.

Yang S, Chen J, Tan T, Wang N, Huang Y, Wang Y, Yuan X, Zhang P, Luo J and Luo X (2020). Evodiamine exerts anticancer effects against 143b and mg63 cells through the wnt/beta-catenin signaling pathway. *Cancer Manag Res,* **12**: 2875-2888.

Zhou Y and Hu J (2018). Evodiamine induces apoptosis, g2/m cell cycle arrest and inhibition of cell migration and invasion in human osteosarcoma cells via raf/mek/erk signalling pathway. *Med. Sci. Monit,* **24**: 5874-5880.