Embelin-induced MCF-7 breast cancer cell apoptosis and blockade of MCF-7 cells in the G2/M phase via the mitochondrial pathway

YANG LI1*, DALEI LI2*, SHENGGUANG YUAN3, ZHENRAN WANG4, FANG TANG4, RONGRONG NIE4, JUN WENG3, LINA MA2 and BO TANG3

1Department of Medical Oncology, Affiliated Hospital of Guilin Medical University, Guilin 541001; 2Gamma Knife Center, The Second Hospital of Changchun, Changchun 130062; Departments of 3Hepatobiliary Surgery and 4General Surgery, Affiliated Hospital of Guilin Medical University, Guilin 541001, P.R. China

Received September 23, 2012; Accepted December 11, 2012

DOI: 10.3892/ol.2012.1084

Abstract. Embelin is a small molecular inhibitor extracted from Myrsinaceae plants that specifically inhibits XIAP, affecting the proliferation and apoptosis of various types of tumor cells. In our previous studies, we have demonstrated that embelin is able to induce the apoptosis of MCF-7 breast cancer cells in a dose-dependent manner. However, its mechanism of action is not yet clear. The purpose of this study was to investigate the involvement of the mitochondrial pathway in embelin-induced apoptosis and the effect of embelin on the cell cycle. Different doses of embelin were added to MCF-7 breast cancer cells and it was found that embelin was able to induce apoptosis of MCF-7 breast cancer cells in a dose- and time-dependent manner. Flow cytometry analysis revealed that embelin caused changes in the MCF-7 cell mitochondrial membrane potential and blocked the cell cycle of MCF-7 cells in the G2/M phase. Moreover, embelin was demonstrated to promote mitochondrial release of cytochrome C via regulation of Bax and Bcl-2, resulting in the activation of caspase-3 and -9, while no significant changes in the level of caspase-8 were observed. The results have demonstrated that embelin-induced apoptosis of MCF-7 breast cancer cells involves the mitochondrial pathway.

Introduction

Breast cancer is a serious disease that threatens the health of individuals and is the most common malignant tumor in females. It is a major disease affecting females in particular, and its incidence is increasing annually (1-3). The key therapeutic approach for most breast cancer patients is to find an effective antitumor drug to assist surgical treatment. Embelin is a small molecular inhibitor extracted from Myrsinaceae plants. It is a polyphenolic compound that inhibits XIAP by binding to the Smac binding site in the BIR3 domain of XIAP protein molecules (4-6). Previous studies have demonstrated that embelin has anti-inflammatory and anti-oxidative biological effects (7-10). It has been demonstrated that embelin has an extensive antitumor role and is able to restrain the growth of various tumor cells, including those of breast, colon, prostate and pancreatic cancer (11-14). However, the detailed mechanism of the antitumor activity of embelin remains unknown.

This study was designed to investigate the effect of embelin on cell apoptosis and the cell cycle of MCF-7 breast cancer cells in vitro, and to explore the embelin-induced cell apoptosis signaling pathway in MCF-7 cells. We have demonstrated that by regulating the Bax and Bcl-2 proteins, embelin induces the release of cytochrome C and activates the caspase family to induce the apoptosis of breast cancer cells.

Materials and methods

Cell culture. MCF-7 breast cancer cells were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). Following cell passage, cells were inoculated in RPMI-1640 culture medium (Gibco-BRL, Grand Island, NY, USA) containing 10% fetal calf serum (HyClone Laboratories, Inc., Logan, UT, USA), 100 U/ml penicillin and 100 U/ml streptomycin. The cells were then cultured in an incubator containing 5% CO2 and 95% O2, at 37°C.

Cell viability. MCF-7 cells in the logarithmic growth phase were collected and cultured in 96-well culture plates inoculated at a density of 1x104 cells/ml for 24 h. When the cells had grown to adherence, different doses of embelin were administered to
Embelin induces apoptosis of MCF-7 breast cancer cells via the mitochondrial pathway. JC-1 coloration was used to examine the changes in the mitochondrial membrane potential. The results demonstrated a gradual decrease in the mitochondrial membrane potential along with an increase in the concentration of embelin. Additionally, the change in the mitochondrial membrane potential was observed prior to apoptosis (Fig. 3A). Western blot analysis was employed to examine the levels of Bax, Bcl-2 and cytochrome C inside the cytoplasm. It was revealed that when the concentration of embelin was increased, the level of Bax protein gradually decreased, while the level of cytochrome C increased (Fig. 3B). Our data demonstrated that embelin is able to change the mitochondrial membrane potential to promote a change in the levels of Bax and Bcl-2 as well as the release of cytochrome C, which results in the apoptosis of MCF-7 breast cancer cells.
Effect of embelin on the expression levels of apoptosis-related proteins in MCF-7 breast cancer cells. In order to study the effect of embelin on the expression levels of MCF-7 breast cancer cell apoptosis-related proteins, different concentrations of embelin were administered to MCF-7 cells for 48 h, and western blot analysis was conducted to investigate the expression levels of procaspase-3, -8 and -9 proteins (Fig. 4). It was found that following treatment with different concentrations of embelin, the expression levels of MCF-7 breast cancer cell procaspase-3 and -9 proteins significantly decreased, while no significant differences were observed in the expression level of procaspase-8 protein. Our data demonstrated that embelin-induced MCF-7 apoptosis may occur through the mitochondria-mediated caspase-3 and -9 pathways.
Embelin-induces MCF-7 breast cancer cell cycle blockade in G2/M phase. Flow cytometry was conducted to investigate whether embelin affected the cell cycle of MCF-7 breast cells. The results revealed that 48 h following the addition of different concentrations of embelin to MCF-7 cells, a cell cycle blockade was observed in the G2/M phase compared with the control group (Fig. 5). This suggests that embelin is able to increase the percentage of MCF-7 cells in the G2/M phase to decrease the proliferation of breast cancer cells.

Discussion

Kerr et al (15) first proposed the concept of apoptosis. Apoptosis is ubiquitous in the majority of tumor cells, and is important in the genesis and progression of tumors (16). Previous studies have demonstrated that antitumor drugs typically inhibit tumors by inducing apoptosis of sensitive tumor cells. Therefore, the intervention in apoptosis to treat tumors has become a new target in the search for antitumor drugs and a new development direction in present tumor pharmacology.

Breast cancer is a potentially life-threatening malignant tumor; it is important to study the disease to find effective antitumor drugs. Antitumor drugs that originate from plants have benefits including an extensive variety, low toxicity and few side-effects and adverse reactions. Therefore, highly effective antitumor drugs from plants are being explored. Embelin is a small molecular inhibitor with specific inhibition of XIAP that affects the proliferation and apoptosis of various tumor cells. Certain studies have demonstrated that embelin inhibits the proliferation of various tumor cells; significant effects have been observed in breast cancer and other solid tumor cells (17). The results of the present study are concordant with these findings. We demonstrated that when breast cancer cells had been treated with different concentrations of embelin for 48 h, the rate of cell apoptosis increased in a dose-dependent manner, indicating that embelin is able to induce breast cancer cell apoptosis as opposed to directly causing cell death. Moreover, embelin has the potent effect of restraining the cell cycle transition of breast cancer cells to blockade the cell cycle in G2/M phase, therefore altering the progression of the cell cycle to induce apoptosis.
There are various signaling pathways of apoptosis within an organism, of which the mitochondrial pathway is one of the most important. Bcl-2 family proteins are key regulatory factors of the mitochondrial pathway (18-20). We demonstrated that when breast cancer cells had been treated with different doses of embelin for 48 h, Bax and Bcl-2 migrated, the mitochondrial membrane potential increase expression of Bax, while Bcl-2 expression decreased and cytochrome C was released. These results indicated that the induction of breast cancer cell apoptosis by embelin was closely associated with the mitochondrial pathway. As demonstrated in previous studies, with the stimulation of pro-apoptosis factors, the Bax protein migrated from the cytoplasm to the outer mitochondrial membrane, changing the permeability of the outer mitochondrial membrane to promote the mitochondrial release of cytochrome C (21). Moreover, Bcl-2 protein is able to stabilize the mitochondrial permeability transition pore (mPTP) and maintain the normal functioning of the pore. In the present study, we demonstrated that with embelin treatment, the Bcl-2 protein level inside the cytoplasm gradually decreased while the release of cytochrome C increased in a stepwise manner. This indicates that embelin induces the activity of Bcl-2, causing the mPTP to open irreversibly, which further changes the permeability of the mitochondrial membrane and promotes the release of cytochrome C. The combination of these factors results in apoptosis.

The caspase family activates apoptosis-related protease when apoptosis occurs (22,23), and a series of subsequent biological effects occur in turn. Therefore, activation of the caspase family is important in the process of apoptosis. We analyzed the changes in procaspase-3, -8 and -9 proteins following treatment of breast cancer cells with embelin. A significant decrease in procaspase-3 and -9 expression was observed when breast cancer apoptosis occurred, but no decrease in procaspase-3 and -9 expression was observed when treatment of breast cancer cells with embelin was realized via the endogenous mitochondrial pathway as results suggest that embelin is an important step of the apoptotic pathway (24,25). These effects occur in turn. Therefore, activation of the caspase family promotes the release of cytochrome C. The combination of these factors results in apoptosis.

In summary, our study has demonstrated that embelin releases cytochrome C and activates the caspase family to result in the induction of breast cancer apoptosis through regulation of the action of the Bcl-2/Bax family in the mitochondrial pathway. Embelin may offer important contributions for the development of a novel drug to prevent and cure breast cancer in the future.

References

1. McPherson K, Steel CM and Dixon JM: ABC of breast diseases. Breast cancer-epidemiology, risk factors, and genetics. BMJ 321: 624-628, 2000.
2. Benson JR and Jatoi I: The global breast cancer burden. Future Oncol 8: 697-702, 2012.
3. Anderson WF, Katki HA and Rosenberg PS: Incidence of breast cancer in the United States: current and future trends. J Natl Cancer Inst 103: 1397-1402, 2011.
4. Reuter S, Prasad S, Phromnoi K, Kannappan R, Yadav VR and Aggarwal BB: Embelin suppresses osteoclastogenesis induced by receptor activator of NF-kB ligand and tumor cells in vitro through inhibition of the NF-kB cell signaling pathway. Mol Cancer 8: 1425-1436, 2010.
5. Hu R, Zhu K, Li Y, Yao K, Zhang R, Wang H, Yang W and Liu Z: Embelin induces apoptosis through down-regulation of XIAP in human leukemia cells. Med Oncol 28: 1584-1588, 2011.
6. Nikolovska-Coleska Z, Xu L, Hu Z, Tomita Y, Li P, Roller PP, Wang R, Fang X, Guo R, Zhang M, Lippman ME, Yang D and Wang S: Discovery of embelin as a cell-permeable, small-molecular weight inhibitor of XIAP through structure-based computational screening of a traditional medicinal herb-like compound database. J Med Chem 47: 2340-2440, 2004.
7. Joshi R, Kamat JP and Mukherjee T: Free radical scavenging reactions and antioxidant activity of embelin: biochemical and pulse radiolysis studies. Chem Biol Interact 167: 125-134, 2007.
8. Sreeprya M and Bali C: Effects of administration of Embelin and Curcumin on lipid peroxidation, hepatic glutathione antioxidant defense and hematopoietic system during N-nitrosodiethylamine/Phenobarbital-induced hepatocarcinogenesis in Wistar rats. Mol Cell Biochem 284: 49-55, 2006.
9. Mahendran S, Badami S, Ravi S, Thippeswamy BS and Veerapur VP: Synthesis and evaluation of analogs and anti-inflammatory activity with wide-ranging active free radical scavenging derivatives of embelin-A structure-activity relationship. Chem Pharm Bull 59: 913-919, 2011.
10. Thippeswamy BS, Mahendran S, Biradar MI, Raj P, Srivastava K, Badami S and Veerapur VP: Protective effect of embelin against acetic acid induced ulcerative colitis in rats. Eur J Pharmacol 654: 100-105, 2011.
11. Danquah M, Li F, Duke CB III, Miller DD and Mahato RI: Micellar delivery of bicalutamide and embelin for treating prostate cancer. Pharm Res 26: 2081-2092, 2009.
12. Dai Y, Qiao L, Chan KW, Yang M, Ye J, Ma J, Zou B, Gu Q, Wang J, Frangione B, Lan HY and Wang BC: Peroxisome proliferator-activated receptor-gamma contributes to the inhibitory effects of Embelin on colon carcinogenesis. Cancer Res 69: 4776-4783, 2009.
13. Aird KM, Ding X, Baras A, Wei J, Morse MA, Clay T, Lyerly HK and Devi GR: Trastuzumab signaling in ErbB2-overexpressing inflammatory breast cancer correlates with X-linked inhibitor of caspase protein expression. Mol Cancer Ther 7: 38-47, 2008.
14. Mori T, Doi R, Kida A, Nagai K, Kami K, Ito D, Toyoda E, Kawauchi Y and Uemoto S: Effect of the XIAP inhibitor Embelin on TRAIL-induced apoptosis of pancreatic cancer cells. J Surg Res 142: 281-286, 2007.
15. Kerr JF, Wyllie AH and Currie AR: Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer 22: 239-257, 1972.
16. Chiarugi P and Giannoni E: Anokis: a necessary death program for anchorage-dependent cells. Biochim Pharmacol 76: 1352-1364, 2008.
17. Altenworth JL, Aird KM, Aldrich AJ, Batinic-Haberle I and Devi GR: XIAP inhibition and generation of reactive oxygen species enhances TRAIL sensitivity in inflammatory breast cancer cells. Mol Cancer Ther 11: 1518-1527, 2012.
18. Yang B, Zhang Y, Liang R, Yuan P, Du J, Wang H and Wang L: Activation of the δ-opoid receptor inhibits serum deprivation-induced apoptosis of human liver cells via the activation of PKC and the mitochondrial pathway. Int J Mol Med 28: 1077-1085, 2011.
19. Mattson MP and Kroemer G: Mitochondria in cell death: novel targets for neuroprotection and cardioprotection. Trends Mol Med 9: 196-205, 2003.
20. Buralacu A: Regulation of apoptosis by Bcl-2 family proteins. iCell Mol Med 7: 249-257, 2003.
21. Saito M, Korsmeyer SJ and Schlesinger PH: BAX-dependent transport of cytochrome c reconstituted in pure liposomes. Nat Cell Biol 2: 553-555, 2000.
22. Nicholson DW, Ali A, Thornberry NA, Vaillancourt JP, Ding CK, Gallant M, Gareau Y, Griffin PR, Labelle M and Laeznevik YA: Identification and inhibition of the ICE/CED-3 protease necessary for mammalian apoptosis. Nature 376: 37-43, 1995.
23. Hyman BT and Yuan J: Apoptotic and non-apoptotic roles of caspases in neuronal physiology and pathophysiology. Nat Rev Neurosci 13: 395-406, 2012.
24. Riedl SJ and Shi Y: Molecular mechanisms of caspase regulation. Annu Rev Biochem 75: 73-105, 2006.
25. Shi LG, Zhang GP and Jin HM: Inhibition of microvascular endothelial cell apoptosis by angiopoietin-1 and the involvement of cytochrome C. Chin Med J (Engl) 119: 725-730, 2006.