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Inhibitory effects of maslinic acid upon human esophagus, stomach and pancreatic cancer cells

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ABSTRACT

Apoptotic, anti-invasive and anti-migratory effects of maslinic acid (MA) at 4, 8, or 16 μM in human esophageal squamous cancer cell line, OE33; gastric cancer cell line, SGC-7901; and pancreatic cancer cell line, Panc-1, were examined. MA treatments inhibited OE33 and SGC-7901 cells growth at 21–66% and 32–75%, respectively; but lowered Panc-1 viability at 13–27% only. MA treatments increased cleaved caspase-3 and Bax expression, and raised caspase-3 and caspase-8 activities in OE33 and SGC-7901 cells. MA treatments also increased DNA fragmentation and decreased reactive oxygen species production in these two cell lines. MA treatments declined invasion and migration in OE33 and SGC-7901 cells, and lowered vascular endothelial growth factor and transforming growth factor-beta1 levels in these cells. MA suppressed hypoxia-inducible factor-1alpha, matrix metalloproteinase (MMP)-2 and MMP-9 expression in OE33 and SGC-7901 cells. These findings indicated that this triterpene was a potent agent against esophagus and stomach cancers.

1. Introduction

Esophagus, stomach, pancreatic, liver and colon cancers are common gastrointestinal (GI) tract cancers (Khamly, Jefford, Michael, & Zalcberg, 2006). The development and application of agent with inhibitory effects upon these GI tract cancer cells benefits the prevention and/or therapy for these cancers.

Maslinic acid (MA) is a pentacyclic triterpenic acid naturally occurring in many herbs and plant foods such as glossy privet fruit (*Ligustrum lucidum* Ait.), hawthorn fruit (*Crataegi Pinnatifidae* Fructus) and olive (Cui et al., 2006; Yin, Lin, Mong, & Lin, 2012). It is reported that this triterpene could inhibit the proliferation of human colon cancer cell lines, HT-29 and Caco-2 (Juan, Planas, Ruiz-Gutierrez, Daniel, & Wenzel, 2008; Reyes, Centelles, Lupiáñez, & Cascante, 2006). Our previous study found that MA at 2–16 μM declined invasion and migration in three hepatic cancer cell lines via suppressing mRNA expression of angiogenic factors including hypoxia-inducible factor (HIF)-1alpha, vascular endothelial growth factor (VEGF) and urokinase-type plasminogen activator (Lin, Huang, Mong, Chan, & Yin, 2011). These previous studies implied that this triterpene was a potent cytotoxic agent for GI tract cancers. However, it is unknown that MA could retard the growth of other GI tract cancer cells such as esophagus, stomach or pancreatic cancer cells. Human esophageal squamous cancer cell line, OE33;

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gastric cancer cell line, SGC-7901; and pancreatic cancer cell line, Panc-1, have been widely used for anti-cancer studies to investigate the apoptotic effects and possible mechanisms of certain compounds (Li et al., 2012; Milano et al., 2013; Zhang et al., 2013). In our present study, these cancer cell lines were used as representative cell lines for esophageal cancer, gastric cancer and pancreatic cancer, respectively, to evaluate the potential of MA as an anti-GI tract cancer agent. The apoptotic effects, anti-invasive and anti-migratory activities of MA at various doses in these cancer cell lines were examined.

Cancer cells apoptosis could be induced by increasing the expression of apoptotic factors such as Bax and cleaved caspase-3, and/or decreasing the expression of anti-apoptotic factors such as Bcl-2 (Manikandan, Murugan, Priyadarsini, Vinothini, & Nagini, 2010; Prasad, Vaid, & Katiyar, 2012). In addition, activated caspase cascade is another important pathway to promote cell apoptosis (Frejlich et al., 2013). So far, less information is available regarding the influence of MA upon caspase activity, expression of apoptotic or anti-apoptotic molecules in these cancer cell lines. On the other hand, VEGF, transforming growth factor (TGF)-beta1, matrix metalloproteinase (MMP)-2 and MMP-9 are involved in GI tract cancer cell adhesion, invasion and migration, which contribute to cancer metastasis (Augoff et al., 2009; Xue et al., 2013). HIF-1alpha regulates the essential adaptive responses of cancer cells to hypoxia; and reactive oxygen species (ROS) could be generated by mitochondria under hypoxic condition (Pugh & Ratcliffe, 2003). Increased HIF-1alpha expression and ROS overproduction favor the metastasis of gastric and pancreatic cancers (Park et al., 2003; Shimojo et al., 2013). Therefore, if MA could mediate these factors such as MMPs, HIF-1alpha or TGF-beta1 in OE33, SGC-7901 or Panc-1 cells, its anti-invasive or anti-migratory actions upon these cancer cells could be explained.

2. Materials and methods

2.1. Chemicals

MA (98%) was purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). Medium, plates, antibiotics and chemicals used for cell culture were purchased from Difco Laboratory (Detroit, MI, USA). All chemicals used in these measurements were of the highest purity commercially available.

2.2. Cell culture

OE33, SGC-7901 and Panc-1 cell lines were obtained from American Type Culture Collection (ATCC, Rockville, MD, USA). Cells were cultured in RPMI 1640 medium, containing 10% fatal bovine serum (FBS), 100 units/mL of penicillin and 100 units/mL of streptomycin (pH 7.4) at 37 °C in 5% CO_2. The culture medium was changed every 3 days, and cells were subcultured once a week. A phosphate buffer saline (PBS, pH 7.2) was added to adjust the cell number to 10^5/mL for various experiments and analyses.

2.3. Experimental design

Stock solution of MA was prepared in dimethyl sulfoxide (DMSO) and diluted with medium. An equal volume of DMSO (final concentration <0.5%) was added to the controls. Test cells (10^5/mL) were treated with MA at 4, 8 or 16 μM for 48 h at 37 °C. Control group contained no MA.

2.4. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

MTT assay was performed to examine cell viability. After 48 h treatment with MA at various doses, cells were further incubated with 0.25 mg MTT/mL for 3 h at 37 °C. The amount of MTT formazan product was determined by measuring absorbance at 570 nm (630 nm as a reference) using a microplate reader (Bio-Rad, Hercules, CA, USA). Cell viability was expressed as a percent of control group.

2.5. Measurement of DNA fragmentation

Cell death detection ELISA plus kit (Roche Molecular Biochemicals, Mannheim, Germany) was used to quantify DNA fragmentation. After incubation with MA at various concentrations, cells were lysed for 30 min at room temperature and followed by centrifugation at 200 × g for 10 min. Then, 20 μL of supernatant were transferred onto the streptavidin-coated plate, and 80 μL freshly prepared immunoreagent were added to each well and incubated for 2 h at room temperature. After washing with PBS, the substrate solution was added and incubated for 15 min. The absorbance at 405 nm (reference wavelength 490 nm) was measured using a microplate reader. DNA fragmentation was expressed as the enrichment factor using the following equation:

enrichment factor
= (absorbance of the sample)/(absorbance of the control)

sample: cells treated with MA; control: cells without MA treatment.

2.6. Measurement of caspases activity

Activity of caspase-3 and -8 was detected by using fluorometric assay kits (Upstate, Lake Placid, NY, USA) according to the manufacturer's protocol. In brief, control or treated cells were lysed in 50 mL cold lysis buffer and incubated in ice for 10 min. Fifty microlitres of cell lysates were mixed with 50 mL reaction buffer and 5 mL fluorogenic substrates specific for caspase-3 or -8 in a 96-well microplate. After incubation at 37 °C for 1 h, fluorescent activity was measured using a fluorophotometer with excitation at 400 nm and emission at 505 nm. Data were expressed as percentage of the control, and the control group was designated as 100%.

2.7. Analysis of VEGF, TGF-beta1 and ROS levels

Cells were pre-incubated with MA at various concentrations for 48 h. After washing by PBS, samples were incubated with 10 ng/mL TNF-alpha for 18 h. VEGF and TGF-beta1 levels in cell culture supernatant were determined using ELISA kits (R&D Systems, Minneapolis, MN, USA). The dye $DCFH_2$-DA was used to measure ROS level. Briefly, cells were washed and suspended in RPMI 1640 medium. After incubating with 50 μM dye