Effects of anthocyanidin on the inhibition of proliferation and induction of apoptosis in human gastric adenocarcinoma cells

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Abstract

Anthocyanins are naturally occurring reddish pigments that abundant in fruits and vegetables. To investigate the mechanistic basis for the anti-tumor properties of anthocyanins, five aglycone (cyanidin, delphinidin, malvidin, pelargonidin, and peonidin) and four glycosylated (cyaniding-3-glucoside, malvidin-3-glucoside, pelargonidin-3-glucoside and peonidin-3-glucoside) anthocyanins were used to examine their effects on cell cycle progression and induction of apoptosis in human gastric adenocarcinoma AGS cells. The data from cell viability assay showed that malvidin exhibited the most potent anti-proliferation effect on AGS cells in a time- and dose-dependent manner (P < 0.05). This event is accompanied the arrest of AGS cells at the G0/G1 phase by malvidin at the tested concentrations of 0–200 μM. Cellular uptake of anthocyanin and anthocyanidin was confirmed by HPLC analysis and the intracellular accumulation of malvidin (24.9 ± 1.1 μM/mg protein) was observed when treatment of AGS cells with malvidin for 12 h. In addition, an accumulation of AGS cells in sub-G1 phase (20% and 30% increase for 100 and 200 μM of malvidin, respectively) was observed as well as by the appearance of a fraction of cells with an aneuploid DNA content. The occurrence of apoptosis induced by malvidin was confirmed by morphological and biochemical features, including apoptotic bodies formation, caspase-3 activation and poly(ADP-ribose) polymerase proteolysis. Furthermore, the mitochondrial membrane potential of apoptotic cells after treatment with malvidin was significantly lost and resulted in the elevation of Bax/Bcl-2 ratio for 1.6-fold against control for 100 μM treatment. In addition, the malvidin treatment significantly increased the p38 kinase expression and inhibited the ERK activity, and the effects of malvidin on caspase-3 activation were blocked, respectively, by the ERK and p38 inhibitors. These findings suggest that growth inhibition and cytotoxicity of AGS cells by malvidin is involved in the induction of apoptosis rather than necrosis.

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Keywords: Anthocyanidin; Apoptosis; AGS cells; MAPK pathway; Bcl-2 family

1. Introduction

Following the change of dietary habits and the excessive process of food, the tendency of gastrointestinal disease is on the rise. Gastric cancer is a kind of gastrointestinal (GI) tract cancer that is the leading cause of cancer-related mortality in the world and approximately 90% of stomach cancers are adenocarci- nomas (Kelley and Duggan, 2003). Studies have shown that a high intake of smoked, salted, nitrated foods and carbohydrates, but a low intake of vegetables, fruits, and milks, are linked to cancer incidence. These diets have been shown to significantly increase the risk for stomach cancer (Serafini et al., 2002). Epidemiological studies have provided convincing evidence that dietary factors can modify the process of carcinogenesis, including initiation, promotion and progression of several types of human cancer (Ray, 2005). The occurrence of gastrointestinal (GI) cancers has increased strikingly during the past decade. Despite advances in early
diagnosis and treatment modalities, the side effects of chemical drugs and recurrence are still the problems. Therefore, the development of chemotherapeutic/chemopreventive agents for gastric cancer is important for reducing the mortality caused by this disease.

Anthocyanins are glycosides of anthocyanidins universally associated with attractive, colorful, and flavorful fruits. Recently, there has been a resurgence of interest in anthocyanins due to their potential biological and pharmacology benefit, such as: antioxidant (Moyer et al., 2002), anti-inflammatory (Subarnas and Wagner, 2000), reducing the risk of cardiovascular diseases (Wang and Mazza, 2002), and anti-tumor properties (Katsube et al., 2003). Animal studies have also demonstrated that feeding with anthocyanin-rich extract protected against tert-butyl hydroperoxide-induced hepatic toxicity (Wang et al., 2000) and decrease lipid peroxidation and DNA damage in vitamin E-depleted rats (Ramirez-Tortosa et al., 2001). More recently, anthocyanins have been shown to be an effective chemopreventive agent against 1,2-dimethyhydrazine- and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine-induced mammary carcinogenesis in rats (Hagiwara et al., 2002). In addition, anthocyanins can be directly absorbed and distributed to the blood (Tsuda et al., 1999), and to be incorporated in cell cultures, both in the plasma membrane and in the cytosol (Youdim et al., 2000). Extensive studies indicate that anthocyanins have strong free radical scavenging and antioxidant activities (Wang et al., 1997), suggesting that they play an important role in preventing against mutagenesis and carcinogenesis (Ommen, 1995). Anthocyanins also showed inhibitory effects on the growth of some cancer cells (Kamei et al., 1995). Our recent study has reported (Yeh and Yen, 2005) that the induction of apoptosis by the anthocyanidins through regulation of Bcl-2 gene and activation of c-jun n-terminal kinase cascade in hepatoma cells. However, the research concerning the protective effect of anthocyanins against gastric adenocarcinoma is limited; thus, the objective of the present study was to determine whether anthocyanins would be useful in the prevention of human gastric adenocarcinoma carcinogenesis. In the present study, human cultured gastric adenocarcinoma (AGS) cells were chosen for study.

AGS cells line has been shown to grow in athymic mice and to have the same histochemical and cytological characteristics as the specimen taken from the patient. It is important to characterize human tumor cells in vitro in this detailed manner, since they serve as excellent model systems for other studies involving the heterogeneous responses to anticancer drugs (Barranco et al., 1983), and recently this cell line has been widely used as a model system for evaluating cancer cell apoptosis (Liu et al., 2003).

Apoptosis is a defined type of cell death and differs from traditional cell death, necrosis. Many recent studies have indicated that anticancer drugs or cancer chemopreventive agents act through the induction of apoptosis to prevent tumor promotion, progression, and the occurrence of cellular inflammatory responses other than necrosis. Apoptosis is also a gene-directed form of cell death with well-characterized morphological and biochemical features (Brown and Attardi, 2005). Initiation of apoptosis appears to be a common mechanism of many cytotoxic agents used in chemotherapy. Therefore, apoptosis inducing agents are expected to be ideal anticancer drugs. In addition, some flavonoids, such as quercetin, apigenin, and phloretin, inhibit cancer cell growth through the induction of apoptosis (Wang et al., 1999). Therefore, understanding the mechanism of apoptosis has important implications in the prevention and treatment of many diseases.

In this study, we investigated the induction of apoptosis by five typical anthocyanins and their relative anthocyanidins in human gastric adenocarcinoma cells. In addition, the molecular mechanisms of the apoptotic effects induced by malvidin were also determined.

2. Materials and methods

2.1. Materials

Anthocyanidins [cyanidin chloride, delphinidin chloride, malvidin chloride, pelargonidin chloride, peonidin chloride], anthocyanins [cyanidin-3-O-glucose chloride, malvidin-3-O-glucose chloride (oenin), pelargonidin-3-O-glucose chloride, and peonidine-3-O-glucose chloride] were obtained from Extrasynthese (Genay, France). Human adenocarcinoma AGS cells were purchased from the Bioresource Collection and Research Center (BCRC, Food Industry Research and Development Institute, Hsin Chu, Taiwan). Goat anti-rabbit IgG polyclonal antibody conjugated to peroxidase were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Anti-ERK, anti-phospho-ERK, anti-JNK, anti-phospho-JNK, anti-p38, anti-phospho-p38, anti-capase-3, anti-poly (ADP-ribose) polymerase, and anti-β-actin antibodies were obtained from Cell Signaling Technology (Beverly, MA). Anti-Bcl-2, and anti-Bax antibodies were obtained from Pharmingen (San Diego, CA). Molecular mass markers for proteins were obtained from Pharmacia Biotech (Saclay, France). Polyvinylidene fluoride (PVDF) membrane for Western blotting was obtained from Millipore (Bedford, MA, USA).

2.2. Cell culture

The AGS cells were cultured in Ham’s F-12K medium supplemented with 10% heat-inactivated fetal bovine serum (FBS) and antibiotics (100 U/ml penicillin, 100 µg/ml streptomycin) at 37°C in a humidified atmosphere of 5% CO2. During the experiments the concentration of FBS were adjusted to 5%.
2.3. Cell morphological observation

Morphology of control cells and samples-treated cells were detected by phase contrast microscope (Nicon).

2.4. Cell viability assay

Cell viability assay was performed with MTT photometric analysis and trypan blue dye exclusion method. In the MTT assay, 5 × 10^4 cells were seeded in 96-well microtiter plate. Cells were treated without or with various concentrations of different samples. At the end of incubation, the supernatants were exchanged with 90 µl fresh medium and 10 µl of MTT (1 mg/ml) solution. After 3 h incubated at 37 °C, aspirated the medium and dissolved the violet crystals with 100 µl of DMSO and the extent of the reduction of MTT was measured by ELISA reader.

The cytotoxicity of anthocyanin and anthocyanidin was achieved through incubation of 2 × 10^5 cells in the 24-well culture plate untreated or treated with different samples. Cells were harvested and added 10 µl of trypan blue (0.4%). The dye exclusion method was performed by calculating the number of died cells over the control.

2.5. Cell collection and HPLC analysis of intracellular anthocyanin and anthocyanidin content

Following incubation of anthocyanin and anthocyanidin (500 µM) with AGS cells for 4, 8, 12 and 16 h, cells were washed twice with PBS and then the method for the determination of intracellular anthocyanin and anthocyanidin was followed with Kader et al. (1996) and Youdim et al. (2000). Cell pellets were resuspended with 10 µl of 1% Triton X-100 and 10 µl of 1 N HCl, vortexed vigorously and allowed to stand at 4 °C for 20 min. The HPLC analysis (Hitachi L-6200 intelligent pump equipped with a photodiode array detector Hitachi L-7455) with a LiChroCART RP-18 reversed phase column (200 × 4 mm, 5 µm) and using the mobile phase consisting of water/formic acid (90:10, v/v) (solvent A) and water/acetonitrile/methanol/formic acid (40:22.5:10, v/v) (solvent B). Initial starting conditions were 20% B, between 0 and 15 min % B increased from 20% to 25%, 15–60 min % B increased from 25% to 40%, 60–80 min % B increased from 40% to 80%, at 80 min mobile phases were switched to original starting conditions (20% B) and held at this condition for 10 min prior to the next injection.

2.6. Flow cytometric analysis

To investigate the effects of malvidin on the cell cycle distribution of AGS cells, cells (2 × 10^6 cells/ml) treated with various concentrations of malvidin and cultured for 0–72 h were harvested, washed with phosphate-buffered saline and fixed in 75% of ethanol at 4 °C overnight. After washing twice with cold PBS, cells were resuspended in PBS containing 40 µg/ml propidium iodide (PI) and 0.1 mg/ml RNase followed with shaking at 37 °C for 15 min. Cells were analyzed with flow cytometer (Becton-Dickinson Immunocytometry Systems, San Jose, CA) and the data were consequently evaluated by Cell Quest and Mod-Fit.

2.7. Mitochondrial membrane potential (ΔΨm)

Mitochondrial membrane potential was analyzed with the J-aggregate forming lipophilic compound 5, 5′, 6, 6 tetrachloro-1, 1, 3, 3 tetraethylbenzamidazolocarbocyanin iodide (JC-1), which has been incorporated into the MitoPT™ kit (Serotec Inc., UK) that is reproducible detection of the mitochondrial permeability transition (PT) events in apoptotic cells. AGS cells (1 × 10^5 cells/ml) were seeded onto 96-well plate and treated with various concentrations of malvidin for indicated time followed by incubated the cells that stained with the MitoPT™ dye reagent at 37 °C for 15 min in the CO2 incubator. The aggregate red form was detected by FLUO star galaxy spectrophotometer (BMG Lab-technologies Ltd., Offenburg, Germany) after excitation at 485 nm and emission at 520 nm. The apoptotic cells were showed up the accumulation of green fluorescence compared with control.

2.8. Measurement of caspase-3 activity

For the determination of caspase activity, the downstream executor enzyme caspase-3 was evaluated. Cells were pretreated with various doses of ERK and p38 inhibitors, respectively, for 1 h, and following the treatment of malvidin (100 µM) for 48 h. At the end, the caspase-3 activity was measured by proteolytic cleavage of the fluorogenic substrate using the CaspaTag™ Caspase-3 (DEVD) Activity kit (BioTech Inc., USA). Cells (1 × 10^6 cells/ml) from different treatments were collected and incubated with the Working Dilution reagent at 37 °C for 1 h, and after washing with the wash buffer, the cells were resuspended with PBS and the fluorescent intensity was detected by the fluorescence spectrophotometer with the excitation wavelength of 485 nm and the emission wavelength of 520 nm.

2.9. Western blot

The cytosolic proteins were isolated from AGS cells (2 × 10^6 cells/ml) after treatment with 100 µM malvidin for various times. The total proteins were extracted by adding 800 µL of cold lysis buffer (50 mM Tris–HCl, pH 7.4; 1 mM NaF; 150 mM NaCl; 1 mM EGTA; 1 mM phenylmethylsulfonyl fluoride; 1% NP-40; and 10 µg/ml leupeptin) to the cell pellets on ice for...
30 min, followed by centrifugation at 12,000 × rpm for 10 min at 4°C. The supernatant protein concentration was measured by Bio-Rad DC kit with bovine serum albumin (BSA) as the standard. The cell lysate were mixed with 4 × sample buffer (8% SDS; 0.04% coomassie blue R-250; 40% glycerol; 200 mM Tris, pH 6.8 and 10% 2-mercaptoethanol) and boiled for 10 min. Samples were electrophoresed in a 10% SDS-PAGE minigel and then transferred onto polyvinylidenedifluoride membranes (PVDF; Millipore Corp., Bedford, MA).

Table 1
Effect of anthocyanins and anthocyanidins on human gastric adenocarcinoma cell AGS viability

<table>
<thead>
<tr>
<th>Samples (200 µM)</th>
<th>Cell viability (% of control)a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyanidin</td>
<td>78 ± 1.3</td>
</tr>
<tr>
<td>Cyanidin-3-glucose</td>
<td>83 ± 2.4</td>
</tr>
<tr>
<td>Peonidin</td>
<td>89 ± 1.1</td>
</tr>
<tr>
<td>Peonidin-3-glucose</td>
<td>84 ± 0.9</td>
</tr>
<tr>
<td>Pelargonidin</td>
<td>86 ± 3.1</td>
</tr>
<tr>
<td>Pelargonidin-3-glucose</td>
<td>81 ± 0.8</td>
</tr>
<tr>
<td>Malvidin</td>
<td>63 ± 1.1</td>
</tr>
<tr>
<td>Malvidin-3-glucose (oenin)</td>
<td>69 ± 2.3</td>
</tr>
<tr>
<td>Delphinidin</td>
<td>67 ± 0.9</td>
</tr>
</tbody>
</table>

a Cells were treated with 200 µM of nine samples for 48 h. The cell viability assay was determined by MTT assay. Data are as the mean ± SD of three independent experiments.

Fig. 1. Inhibition of AGS cells viability by anthocyanin and anthocyanidin. AGS cells were treated with various concentrations of three samples for 48 h. After treatment, cell viability was estimated by trypan blue dye exclusion method. Data are the mean ± SD of three independent experiments.

Fig. 2. HPLC determination of anthocyanin and anthocyanidin incorporated into AGS cells. Data are presented from three independent experiments.
with transfer buffer (48 mM Tris; 39 mM glycine; 0.0037% SDS and 20% methanol) at 350 mA for 60 min. The membranes were blocked with 5% nonfat milk in PBS solution containing 0.1% Tween-20 (PBST) for 1 h. The membrane was immunoblotted with primary antibodies of rabbit anti-human caspase-3, PARP, Bcl-2 family and MAPK family in PBS solution that contained 5% BSA in overnight at 4 °C. After consecutively washed with PBST for 30 min, the membrane was incubated with horseradish peroxidase-labeled secondary antibody for 60 min at room temperature and washed with PBST for 30 min. Final detection was performed with enhance chemiluminescence (ECL™ kit) western blotting reagents (Amersham Pharmacia Biotech, New Jersey, USA).

2.10. Statistical analysis

All data are presented as means ± SD. The statistical significant differences compared with untreated group were calculated by Duncan’s multiple range test.

3. Results

3.1. Effect of anthocyanins and anthocyanidins on the cell viability of human gastric adenocarcinoma AGS cells

In preliminary experiments, the effects of four anthocyanins and five anthocyanidins on the proliferation of
AGS gastric adenocarcinoma cells were evaluated. The proliferation assay was performed by MTT assay, and found that malvidin, delphinidin, and malvidin-3-glucoside (oenin) showed much efficacy of anti-cell proliferation abilities (Table 1). In view of the potential anti-tumor abilities of these compounds, the cell viability was further evaluated by trypan blue dye exclusion assay, the result is shown in Fig. 1. The IC50 value of delphinidin, oenin and malvidin was 198, 149 and 128 μM, respectively, for 48 h incubation.

To evaluate the bioavailability of anthocyanin and anthocyanidin, the cytosolic concentrations of delphinidin, oenin and malvidin were detected by HPLC method. As shown in Fig. 2, the incorporation of those three samples into AGS was very quickly and reached the limitation within 12 h. The cytoplasmic concentrations of those three samples, expressed in μM/mg protein, ranged from 25.1 for oenin to 16.4 for malvidin.

3.2. Apoptosis induction of AGS cells by malvidin

As the results shown in Fig. 3A, the classical apoptotic cells were observed in malvidin-mediated cell death from cell shrinkage and membrane blabbing to the formation of apoptotic bodies. In comparison with the control, malvidin treatment significantly accumulated the percentage of cells at G0/G1 phase (Fig. 3B) for 24 h of incubation (74% and 79% under 100 and 200 μM treatment, respectively). Interestingly, the sub-G1 phase of cells were significantly increased from 3% to 20% and 33% under 100 and 200 μM malvidin treatment for 48 h, respectively, and in a time and dose dependent manner (as shown in Fig. 4A and B). Further more, the enzymatic activation of caspase-3 was evident within 50–200 μM malvidin treatment, and similar characteristics of proteolytic PARP protein were also detected by western blotting in AGS cells treated for 48 h with 100–200 μM of malvidin (Fig. 4C).

3.3. Effect of malvidin on the mitochondrial stability of AGS cells

To further define the feature of malvidin-induced apoptosis in AGS cells, the mitochondrial membrane potential of AGS cells was detected. This was performed by using the MitoPT™ kit, which specific detects the apoptotic feature in the early phase. Fig. 5A shows that 100 μM of malvidin induced serious mitochondrial disruption after 24 h incubation (69.5% of total cells). The Bcl-2 family of pro- and anti-apoptotic proteins expression of AGS cells was further analyzed and found that the elevation of Bax/Bcl-2 ratio for 1.6- and 2.1-fold against control for 100 and 200 μM treatments (Fig. 5B and C). These data demonstrated an apoptotic effect of malvidin in AGS cells.

Fig. 4. Malvidin-induced apoptosis in AGS cells. (A) Cells were treated with 100 μM of malvidin for indicated time. (B) Cells were treated with 50, 100 and 200 μM malvidin for 48 h. The data was calculated by using FACScan software. All data represent the mean ± SD of three independent experiments. Asterisks indicate significant difference by comparison with control as determined by Duncan’s multiple range test, *p < 0.05; **p < 0.001. (C) Representative western blotting of caspase-3 activation and PARP proteolytic fragment in AGS cells by treated with different concentrations of malvidin for 48 h.
3.4. Involvement of the MAP-kinases p38 and ERK in malvidin-induced apoptosis

Since MAPK pathways involved the growth and death of the cell, the changes of phospho-JNK, ERK and p38 protein levels in the AGS cells treated with malvidin were examined by western blotting technique. As shown in Fig. 6, cells were exposed to 100 μM of malvidin and the level of phospho-ERK protein was reduced to 55% for 48 h incubation (Fig. 6A), and the expression of phospho-p38 protein showed a significant 2.3-fold increase for 36 h incubation as compared to the control (Fig. 6B). However, there was no significant difference in the expression of phospho-JNK levels (data not shown). Furthermore, the specific inhibitors were used to determine of the exact molecular mechanisms of malvidin-induced apoptosis through MAPK pathway. The data showed that treatment of AGS cells with malvidin (100 μM) induced a significant increase of the activity of caspase-3, and pre-incubation of cells with the ERK inhibitor PD98059 (40 μM) synergistically promoted the effect of malvidin. While pretreatment with p38 inhibitor SB203580 (50 μM) abrogated the effect of malvidin on caspase-3 activation.

4. Discussion

Among the nine kinds of anthocyanins and anthocyanidins studied in the present study, malvidin had more efficiency in anti-proliferation and cytotoxicity of AGS cells with a time- and dose-dependent manner. Thus, the molecular mechanisms of the apoptotic effects induced by malvidin were further evaluated. Some studies of the induction of apoptosis by malvidin in different tumor cell lines have been reported, including the mediation of programmed cell death in human promyelocytic leukemia cells (Katsube et al., 2003) and the arrest of cell cycle progression in human monocytic leukemia cells (Hyun and Chung, 2004). Although there are some studies of the bioavailability of anthocyanins in human (Matsumoto et al., 2001; McGhie et al., 2003) and animal models (Tsuda et al., 1999), this is the first study reported that the uptake of anthocyanins in human gastric cells. The stomach is a good organ for the stabilization of anthocyanins because of its acidic environment (Passamonti et al., 2003). The data showed that the maximum incorporation of anthocyanin and anthocyanidin into AGS cells was reached a maximum after 12 h of incubation (Fig. 2). Recently, Passamonti et al.
had compared the bioavailability of some samples including delphinidin, malvidin and malvidin-3-O-glucoside (oenin). The incorporation of anthocyanin into AGS cells was detected by HPLC followed the method of Kader et al. (1996) with a slight modification. In addition, there are still other methods for analysis of anthocyanins and anthocyanidins in lots of different vegetables and fruits (Merken and Beecher, 2000). Nyman and Kumpulainen (2001) and Zhang et al. (2004) showed that the retention time of malvidin was latter than delphinidin, cyanidin and other anthocyanidins. These data showed that oenin possesses the best bioavailability than others due to its best efficacy of binding to organic anion membrane carrier, bilitranslocase.

Gastric cancer is the second most common cause of cancer-related mortality in the world and the therapies such as surgical operation or radiotherapy at later stages may incur a poor prognosis with overall 5-year survival rates of less than 25% (Houghton et al., 2002). Lately the treatment for gastric prefer to the chemotherapy that intensive treatment of anti-cancer curatives (Cascini et al., 2004) or the genetic engineering that inhibition the proliferation and differentiation of cancer cells (Steele and Lane, 2000; Heideman et al., 2004). Our strategy is to evaluate the biological functions of naturally occurring flavonoids, especially for anthocyanins, whether they have the potent ability to induce a programmed cell death in gastric carcinoma cells. In the present study, the morphological damages of classical apoptotic cells were

Fig. 6. Effect of malvidin on the expression of MAPK family and caspase-3 activity in AGS cells. Cells were treated with 100 μM of malvidin for indicated time and the western blotted to reveal the expression of (A) phospho-ERK, (B) phospho-p38, and (C) caspase-3 activity treated with or without MAPK inhibitors for 48 h. Similar results were obtained in three independent experiments. *p < 0.05 versus control; #p < 0.01 versus malvidin.
observed in malvidin-mediated cell death from cell shrinkage and membrane blabbing to the formation of apoptotic bodies (Fig. 3A). In comparison of the control, malvidin treatment significantly accumulated the percentage of cells at G0/G1 phase (Fig. 3B) for 24 h of incubation; and further increased the number of sub-G1 cells, that was accompanied with activating the caspase-3 expression which resulted in the proteolysis of PARP (Fig. 4C). The results show that malvidin further affects cells proliferation by arrest cell cycle progression and sequentially induces apoptosis. Liu et al. (2004) also reported the similar results on the induction of apoptosis by lycorine on HL-cells.

The mitochondrial dysfunction is associated with the intrinsic pathway of apoptosis (Desagher and Martinou, 2000; Ly et al., 2003). Bel-2 family members were classified into two major roles, one is the pro-apoptotic and the other is anti-apoptotic function (Kelekar and Thompson, 1998). When the death signal has been taken over, the C-terminal signal anchor sequences will target them to the outer membrane of organelles included mitochondria and then shift the permeability of mitochondria. Our data showed that malvidin mediated the continued reduction of mitochondrial membrane potential following with the incubation time (Fig. 5A), and elevated the ratio of Bax/Bcl-2 expression in a dose-dependent manner (Fig. 5B and C), which represented the pro/anti-apoptotic functions, respectively.

Mitogen-activated protein kinases (MAPKs) family are well known of involving in the regulation of survival, proliferation and death of the cell (Johnson and Lapadat, 2002; Dent et al., 2003). In the present study, malvidin treatment had a profound effect in the expression of the subfamilies of MAPKs, consist of ERK and p38 kinase. The data showed that malvidin could persistently decrease the activities of ERK (Fig. 6A), which responsible for the regulation of meiosis, mitosis and post-mitotic functions in cells (Johnson and Lapadat, 2002), and then leaded to the anti-proliferation affect. It has been reported that malvidin mediated the activation of p38 enzyme expression, which is involved in apoptosis (Iwama et al., 2001). As shown in Fig. 6B, malvidin treatment revealed that the expression of p38 kinase seemed more activated than vehicle control. There are several specific inhibitors that are used for evaluating the exactly molecular mechanism. PD98059 specifically binds to MKK1/2, the ERK upstream kinases, and then inhibits ERK phosphorylation and activation (Chuang et al., 2000). Previous studies have shown an inhibitory effect of the p38 inhibitor SB203580 on the DNA level (Diep et al., 2000; Yilmaz et al., 2003). In the present study, the data showed that p38 inhibitor SB203580 abrogates malvidin-induced expression of caspase-3, while ERK inhibitor PD98059 inhibits the stimulatory effect of malvidin on caspase-3 activity. These data indicate the malvidin mediates the apoptosis of AGS cells is through the effect of pro- and anti-apoptotic molecules of the MAPK family.

Based on the results of this study, malvidin has a much efficacy than other samples tested on the induction of the programmed cell death in human gastric adenocarcinoma AGS cells. The molecular mechanisms are due to the loss of mitochondrial membrane permeability and through the MAPK pathway. Therefore, anthocy-anidin should be good naturally occurring neutrauteicals for cancer preventions.

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References


