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Delphinidin suppresses ultraviolet B-induced cyclooxygenases-2 expression through inhibition of MAPKK4 and PI-3 kinase

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Cyclooxygenase-2 (COX-2), a key mediator of inflammation, and its product, prostaglandin E2 (PGE2), enhance carcinogenesis, particularly in skin. Ultraviolet (UV) B is the most carcinogenic component of solar irradiation, and a crucial role of COX-2 in UVB-mediated skin carcinogenesis has been reported. Here, we investigated the effects of delphinidin, an abundant dietary anthocyanin, on UVB-induced COX-2 upregulation and the underlying molecular mechanism. We found that delphinidin suppressed UVB-induced COX-2 expression in JB6 P+ mouse epidermal cells. COX-2 promoter activity and PGE2 production were also suppressed by delphinidin treatment within non-cytotoxic concentrations. Activator protein-1 and nuclear factor-xB, crucial transcription factors involved in COX-2 expression, were activated by UVB and delphinidin abolished this activation. UVB-induced phosphorylation of c-Jun N-terminal kinase, p38 kinase and Akt was inhibited by delphinidin. The activities of mitogen-activated protein kinase kinase kinase (MAPKKK) 4 and phosphatidylinositol-3 kinase-3 (PI-3K) were inhibited markedly by delphinidin.

A pull-down assay using delphinidin-Sepharose beads revealed that delphinidin binds directly with MAPKK4 or PI-3K in a manner that was competitive with adenosine triphosphate. Moreover, in vivo investigations using mouse skin revealed that the upregulation of COX-2 expression, MAPKK4 activity and PI-3K activity induced by UVB was abolished with delphinidin treatment. Collectively, our results demonstrated that delphinidin targets MAPKK4 and PI-3K directly to suppress COX-2 overexpression, suggesting a potential protective role for delphinidin against UVB-mediated skin carcinogenesis.

Introduction

A strong link between inflammation and carcinogenesis has been reported (1,2). Cyclooxygenase-2 (COX-2), a major mediator of inflammation, and its product, prostaglandin E2 (PGE2), enhance carcinogenesis, particularly skin cancer (3,4). The pivotal role of COX-2 in UVB-mediated skin carcinogenesis was reported (5). Cyclooxygenase-2 (COX-2), a major component of solar UV radiation, is suggested as a major cause of skin cancer (5). UVB radiation activates a variety of cellular signaling pathways and molecular targets linked to photocarcinogenesis. Chronic exposure to UVB irradiation leads to induction of COX-2 expression and inflammatory responses, resulting in the development of skin cancer (6). UVB irradiation stimulates activator protein-1 (AP-1) and nuclear factor-xB (NF-xB), which are crucial transcription factors involved in COX-2 expression and carcinogenesis (7,8), especially skin cancer development (9).

Major signaling pathways that are known to mediate UVB-induced biological responses involve mitogen-activated protein kinase kinases (MAPKKs) (10). MAPKKs mediate a wide range of intracellular signaling molecules involved in biological processes, including cell proliferation, differentiation and apoptosis. Three types of MAPKKs have been characterized, including extracellular signal-regulated kinases (ERKs), c-Jun N-terminal kinases (JNKs) and p38. They are activated by specific mitogen-activated protein kinase kinases (MAPKKs), including mitogen-activated protein kinase/extracellular signal-regulated kinase (MEK) 1/2, MAPKKK4/7 and MAPKKK3/6, respectively (11). Specific inhibitors of these kinase families cause a downregulation of MAPK activity and could be used to treat MAPKs-mediated diseases, including cancer. Another key mediator of the UVB-induced cellular response is the phosphatidylinositol-3 kinase (PI-3K) pathway. PI-3K is a major upstream kinase of Akt. This pathway regulates various cellular processes, such as apoptosis, proliferation and growth (12), and requires UVB-induced COX-2 expression (13,14). Therefore, MAPKKs and PI-3K are possible molecular target candidates for suppressing UVB-induced COX-2 expression.

Anthocyanins are abundant natural polyphenolic compounds that contribute intense color to fruits and vegetables, including berries, red grapes, purple sweet potato and red cabbage (15). Anthocyanins have been noted for their health-promoting effects and biological function associated with a lower risk of cancer (16,17). Among the anthocyanidins, the aglycon form of anthocyanin, delphinidin, has the most potent anticarcinogenic properties. Delphinidin was reported to exert a stronger inhibitory potency against cancer cell migration (18) and cell transformation (19) than other anthocyanidin compounds tested. However, direct protein targets of delphinidin and antitumor-promoting mechanisms remain largely unknown. In the present study, we investigated the chemopreventive effects of delphinidin on UVB-induced tumor promotion in vitro and in vivo and examined the underlying molecular mechanism. Here, we report that delphinidin suppresses UVB-induced COX-2 expression by acting as a potent inhibitor of MAPKK4 and PI-3K.

Materials and methods

Delphinidin was purchased from Indofine Chemical (Hillsborough, NJ) and the purity of the chemical was >99% according to the manufacturer’s information. Dimethylsulfoxide was obtained from Sigma–Aldrich (St Louis, MO). Delphinidin was prepared as a 40 mM stock in dimethylsulfoxide, 40 μl aliquots were stored at −80°C and fresh aliquots were used for each experiment. Eagle’s minimum essential medium (EMEM), basal medium Eagle, gentamicin, fetal bovine serum (FBS) and 1-glutamine were from Gibco BRL (Carlsbad, CA). The antibodies against phosphorylated MEK1/2 (Ser217/221), phosphorylated MAPKK3/6 (Ser189/207), total MAPKK3/6, phosphorylated MAPK4 (Ser257/Thr261) and total p90RSK were purchased from Cell Signaling Technology (Beverly, MA). The antibodies against total MEK1/2, total MAPK4 phosphorylated ERKs (Thr202/Tyr204) and total ERKs were from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-β-actin was purchased from Sigma–Aldrich. The MAPKK4 and PI-3K assay kits were obtained from Upstate Biotechnology (Lake Placid, NY). Cytokine bromide–Sepharose 4B, glutathione–Sepharose 4B, [γ-32P] adenosine triphosphate (ATP) and the

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chemiluminescence detection kit was purchased from Amersham Pharmacia Biotech (Piscataway, NJ), and the protein assay kit was acquired from Bio-Rad Laboratories (Hercules, CA). A PGE$_2$ enzyme immunoassay kit was obtained from Cayman Chemical (Ann Arbor, MI). G$_{418}$ and the luciferase assay substrate were purchased from Promega (Madison, WI).

**Cell viability assay**

To estimate cell viability, JB6 P+ cells were seeded (10$^3$ cells per well) in 96-well plates with 5% FBS and EMEM and incubated at 37°C in a 5% CO$_2$ incubator. The cells were treated with delphinidin at the concentrations indicated (0, 5, 10 or 20 µM) for 4 h. After incubation, 20 µl of CellTiter 96 Aqueous One Solution (Promega) were added to each well, and the cells were then incubated for 1 h at 37°C in a 5% CO$_2$ incubator. Absorbance was measured at 492 and 690 nm.

**UVB irradiation**

A UVB irradiation system was used to stimulate cells in serum-free media. The UVB irradiation system was used to stimulate cells in serum-free media. The UVB was set at 0.5 kJ/m$^2$. Cells were exposed to UVB at a dose of 0.5 kJ/m$^2$ at 312 nm. Cells were exposed to UVB at a dose of 0.5 kJ/m$^2$ and then harvested after 18 h later. The amount of PGE$_2$ released was measured as for the in vitro MAPK4 kinase assay described above. Data are presented as the mean of data points from three mice in each treatment group.

**In vivo MAPK4 kinase assay and kinase assay**

Mice were treated with delphinidin (0, 40 or 200 nmol) applied topically in 200 µl of acetone, and dorsal skin was prepared 2 h after UVB exposure (0.5 kJ/m$^2$). Proteins were extracted as described above and centrifuged at 16 000 g for 15 min. In advance, 700 µg of mouse skin extract was mixed with protein A/G beads (20 ml) for 1 h at 4°C. The mixture was processed and radiolabeled spots were visualized by autoradiography.

**In vivo PI-3K immunoprecipitation and kinase assay**

An active PI-3K protein (100 ng) was incubated with delphinidin (0, 10 and 20 µM) for 10 min at 30°C. The mixtures were then incubated with 20 µl of 0.5 µM phosphatidylinositol (Avanti Polar Lipids, Alabaster, AL) for 5 min at room temperature, followed by incubation with reaction buffer [100 mM N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (pH 7.6), 50 mM MgCl$_2$ and 250 µM ATP containing 10 µCi of [γ-32P]ATP] for an additional 10 min at 30°C. The reaction was stopped by adding 15 µl of 4 N HCl and 130 µl of 30% chloroform/methanol (1:1). After vortexing, 30 µl of the lower chloroform phase was spotted onto nitrocellulose or nitrocellulose-coated silica gel plate that was previously activated for 1 h at 110°C. The resulting [32P]-labeled phosphatidylinositol-3-phosphate was separated by thin layer chromatography and radiolabeled spots were visualized by autoradiography.
Direct, cell-based and in vivo pull-down assays
Active MAPKK4 protein (0.2 μg) or a JB6 P+ cellular supernatant fraction (500 μg) was incubated with the delphinidin–Sepharose 4B (or Sepharose 4B only as a control) (100 μl, 50% slurry) in a reaction buffer (50 mM Tris (pH 7.5), 5 mM EDTA, 150 mM NaCl, 1 mM DTT, 0.01% Nonidet P-40 and 0.02 mM PMSF), and proteins bound to the beads were analyzed by western blotting. For the in vivo pull-down assay, mice received a topical application of 200 μl acetone alone 1 h before UVB irradiation (0.5 kJ/m² UVB and harvested 6 h later). Relative activities were determined using a luciferase assay as described in Materials and Methods. Delphinidin suppresses UVB-induced COX-2 promoter activity. JB6 P+ cells, which were stably transfected with a COX-2 luciferase reporter plasmid, were treated with delphinidin at the concentrations indicated (0, 10 or 20 μM) for 1 h before being exposed to 0.5 kJ/m² UVB and harvested 6 h later. Relative activities were determined using a luciferase assay as described in Materials and Methods. Delphinidin suppresses UVB-induced COX-2 activity. JB6 P+ cells were treated with delphinidin at the concentrations indicated (0, 5, 10 or 20 μM) for 1 h before being exposed to 0.5 kJ/m² UVB and harvested 6 h later. Relative activities were determined using a luciferase assay as described in Materials and Methods. Delphinidin suppresses UVB-induced PGE2 production. JB6 P+ cells were treated with delphinidin at the concentrations indicated (0, 10 or 20 μM) for 1 h before being exposed to 0.5 kJ/m² UVB and harvested 6 h later. PGE2 production was measured using a PGE2 assay kit as described in Materials and Methods. For (B–D) and (E–F), data are shown as mean ± SD and asterisks indicate a significant inhibition by delphinidin compared with the group treated with UVB alone (*P < 0.05 and **P < 0.01).

ATP and delphinidin competition assay
Recombinant MAPKK4 or p110 for PI-3K (0.2 μg each) was incubated with 100 μl of delphinidin–Sepharose 4B or 100 μl of Sepharose 4B in a reaction buffer (see direct and cell-based pull-down assays) for 12 h at 4°C, and ATP (10 or 100 μM) was added to a final volume of 500 μl and incubated for 30 min. The samples were washed, and proteins were then detected by western blotting. Molecular modeling
The homology model structure of MAPKK4 was generated by Geno3D (http://geno3d-pbil.ibcp.fr) using the co-ordinates of MAPKK7 (Protein Data Bank)
code 2DYL) as a template. The co-ordinates of PI-3K in complex with ATP or myricetin (Protein Data Bank codes 1E8X or 1E90, respectively) were used for the docking of delphinidin to PI-3K. Insight II (Accelrys, San Diego, CA) was used for the modeling study and structure analysis.

Statistical analysis

When necessary, data are expressed as means ± SDs and the Student’s t-test was used for single statistical comparisons. A probability value of $P < 0.05$ was used as the criterion for significance.

Results

Delphinidin inhibits UVB-induced COX-2 protein expression and PGE$_2$ production in JB6 P$^+$ cells

Because abnormal upregulation of COX-2 and inflammation were reported to play an important role in skin cancer (6), we first investigated the effect of delphinidin on UVB-induced COX-2 upregulation. The optimal time for induction of COX-2 protein expression by UVB irradiation was determined by a time-course study (data not shown).

The results of cell viability assay data indicated that delphinidin (Figure 1A) between 5 and 20 μM concentration had no effect on cell viability (Figure 1B). However, delphinidin at 5–20 μM suppressed UVB-induced COX-2 protein expression in JB6 P$^+$ cells (Figure 1C and D). UVB-induced COX-2 promoter activity was dose-dependently suppressed by delphinidin treatment in JB6 P$^+$ cells, which were stably transfected with a COX-2 luciferase plasmid (Figure 1E). The production of PGE$_2$, which increased following exposure to UVB, was also suppressed by delphinidin treatment (Figure 1F). These results indicated that delphinidin could effectively repress UVB-induced COX-2 protein expression and PGE$_2$ production in JB6 P$^+$ cells.

Delphinidin suppresses UVB-induced transactivation of AP-1 and NF-κB and phosphorylation of JNKs, p38 and Akt in JB6 P$^+$ cells

Next, we measured the effect of delphinidin on transactivation of AP-1 and NF-κB using JB6 P$^+$ cells stably transfected with an AP-1 or NF-κB luciferase reporter plasmid, were treated with delphinidin at the concentrations indicated (0, 5, 10 or 20 μM) for 1 h before being exposed to 0.5 kJ/m$^2$ UVB and harvested 6 h later. Relative activities were determined by luciferase assay as described in Materials and Methods. (C–F) JB6 P$^+$ cells were treated with delphinidin at the concentrations indicated (0, 10 or 20 μM) for 1 h before being exposed to 0.5 kJ/m$^2$ UVB and harvested after 30 min. Western blot analysis was conducted as described in Materials and Methods using specific antibodies as indicated. Data are presented as means ± SDs and asterisks for (A) and (B) indicate significant inhibition of luciferase activity by delphinidin compared with the group treated with UVB alone (*$P < 0.05$ and **$P < 0.01$).

**Fig. 2.** Effect of delphinidin on UVB-induced signaling in JB6 P$^+$ cells. (A and B) Delphinidin suppresses UVB-induced AP-1 (A) or NF-κB (B) transactivation. JB6 P$^+$ cells, which were stably transfected with an AP-1 or NF-κB luciferase reporter plasmid, were treated with delphinidin at the concentrations indicated (0, 5, 10 or 20 μM) for 1 h before being exposed to 0.5 kJ/m$^2$ UVB and harvested 6 h later. Relative activities were determined by luciferase assay as described in Materials and Methods. (C–F) JB6 P$^+$ cells were treated with delphinidin at the concentrations indicated (0, 10 or 20 μM) for 1 h before being exposed to 0.5 kJ/m$^2$ UVB and harvested after 30 min. Western blot analysis was conducted as described in Materials and Methods using specific antibodies as indicated. Data are presented as means ± SDs and asterisks for (A) and (B) indicate significant inhibition of luciferase activity by delphinidin compared with the group treated with UVB alone (*$P < 0.05$ and **$P < 0.01$).
NF-κB luciferase reporter plasmid. Consistent with the results for COX-2 expression, delphinidin inhibited UVB-induced transactivation of AP-1 (Figure 2A) or NF-κB (Figure 2B) in a dose-dependent manner, which may contribute to the antitumor-promoting and anti-inflammatory activities of delphinidin. We found that delphinidin suppressed UVB-induced phosphorylation of JNKs, c-Jun, p38, activating transcription factor 2, ERK1/2 and p90RSK but not MAPKK4, MAPKK3/6 or MEK1/2 (Figure 2C–E). We could not detect UVB-induced phosphorylation of MAPKK7. Delphinidin also strongly suppressed the phosphorylation of the PI-3K downstream kinases, including Akt and p70S6K (Figure 2F). These results suggested that the inhibition of the JNKs, p38, ERKs and Akt pathways by delphinidin leads to the suppression of AP-1 and NF-κB transactivation, resulting in decreased COX-2 expression.

**Delphinidin inhibits MAPKK4 or PI-3K activity in vitro**

Because delphinidin strongly suppressed the JNKs and Akt signaling pathways, we investigated the effects of delphinidin on the kinase activity of MAPKK4 and PI-3K. Kinase assay data revealed that delphinidin strongly suppressed MAPKK4 and PI-3K activity in vitro (Figure 3A and C). However, delphinidin had no effect on MAPKK6 activity (data not shown). Further, ex vivo kinase assay data revealed that delphinidin inhibited MAPKK4 and PI-3K activity in UVB-treated cell lysates (Figure 3B and D).

**Delphinidin binds directly to MAPKK4 and PI-3K**

We next determined whether delphinidin interacts directly with MAPKK4 or PI-3K. Delphinidin binds directly to the MAPKK4 (A) or PI-3K (C) protein. MAPKK4– or PI-3K–delphinidin binding was confirmed by immunoblotting using an antibody against MAPKK4 (A) or p110 for PI-3K (C); lane 1 (input control), MAPKK4 (A) or PI-3K (C) protein standard; lane 2 (control), Sepharose 4B was used for a pull-down assay as described in Materials and Methods and lanes 3 and 4, MAPKK4 (A) or PI-3K (C) was pulled down using delphinidin–Sepharose 4B beads as described in Materials and Methods. Delphinidin binds to UVB-activated MAPKK4 (B) or PI-3K (D) as described in Materials and Methods. Delphinidin–Sepharose 4B as described in Materials and Methods.

**Delphinidin suppresses UVB-induced COX-2, MAPKK4 and PI-3K activity in vivo**

We examined the effect of delphinidin on UVB-treated mouse skin in vivo. UVB irradiation highly upregulated COX-2 expression in
mouse skin, and this was suppressed by delphinidin (Figure 5A). We also found that UVB-induced MAPKK4 or PI-3K activity in mouse skin was suppressed significantly by delphinidin treatment (Figure 5B and C). The pull-down assay data indicated direct binding of delphinidin with MAPKK4 (Figure 5D, upper panel) or PI-3K (Figure 5D, lower panel) using \textit{in vivo} skin lysates. Together, these findings provide evidence that delphinidin suppresses UVB-induced COX-2 expression mainly by targeting MAPKK4 and PI-3K.

**Delphinidin binds directly to MAPKK4 and PI-3K in an ATP-competitive manner**

We next determined the means by which delphinidin interacts directly with MAPKK4 or PI-3K and results indicated that ATP competed with delphinidin for binding with MAPKK4 (Figure 6A) or PI-3K (Figure 6B). These results suggested that delphinidin inhibits MAPKK4 and PI-3K activity competitively with ATP. Using a computer modeling study, we found that delphinidin easily docked to the ATP-binding site of MAPKK4 (Figure 6C) or PI-3K (Figure 6D).

**Discussion**

Dietary anthocyanins or anthocyanin-rich fruits and extracts exhibit protective effects against a variety of chronic diseases. Previous studies have shown that anthocyanins possess a strong antioxidant capacity, preventing oxidative stress-induced apoptosis (20) and doxorubicin-induced cardiotoxicity (21). Anthocyanins possess strong antioxidant capacity and antiproliferative activity against cancer cell growth (22) and suppress tumor cell invasion and migration (23). Delphinidin was shown to inhibit cell transformation and migration most strongly among several anthocyanidins tested (18,19). The beneficial effects of anthocyanidins have been explained by their antioxidant effects (24). However, due to their low effective dose and specific signaling inhibition, antioxidant effects cannot account for all the beneficial effects (25). Therefore, our previous studies and other studies suggested that flavonoids, including anthocyanidins, can inhibit certain cellular kinase activities. Myricetin suppresses MEK1, Fyn and PI-3K activities and quercetin inhibits MEK1, Raf1 and PI-3K, whereas kaempferol attenuates p90 RSK activity. However, the targets of delphinidin have not been fully elucidated (26–29).

Accumulating data suggest that inflammation is associated with cell transformation and the development of cancer. COX-2, a key player in the inflammatory response, is highly upregulated during carcinogenesis (30). Evidence suggests that high levels of COX-2 expression and PGE$_2$ production enhance tumorigenesis of various cell types, particularly in skin (3,4,31). UVB, the most carcinogenic component of solar irradiation, is a major cause of skin cancer (32).
and COX-2 plays a critical role in UVB-induced skin cancer. A recent study indicated that UVB-induced photocarcinogenesis could be abolished by COX-2 inhibition (33). A transgenic mouse study using COX-2 knockout or COX-2 overexpressing mice also revealed that COX-2 is required for UVB-induced skin carcinogenesis (34,35). AP-1 and NF-jB are the most important transcription factors involved in COX-2 expression. UVB activates AP-1 and NF-jB through the MAPKs and PI-3K/Akt pathways (10). Therefore, targeting these pathways could be effective to inhibit COX-2 expression and prevent skin carcinogenesis.

In this study, we found that delphinidin effectively suppressed the phosphorylation of JNKs but not p38. A new molecular target of delphinidin that regulates p38 must be elucidated. To investigate the molecular basis of MAPKK4 inhibition by delphinidin, we carried out a docking study (Figure 6C) using a homology model structure of the MAPKK4 kinase domain derived from the crystal structure of MAPKK7, which has 66% amino acid sequence homology with MAPKK4. The kinase domain of MAPKK4 consists of an N-lobe and a C-lobe. These N- and C-lobes are linked through a loop called the ‘hinge region’. The backbone of this loop interacts with the adenine moiety of ATP by hydrogen bonding. Considering the experimental result indicating that delphinidin is an ATP-competitive kinase inhibitor, we docked the compound to the ATP-binding site of MAPKK4 and delphinidin was easily docked to this site of MAPKK4. Delphinidin can form hydrogen bonds with the backbone of the hinge region of MAPKK4 as do other ATP-competitive kinase inhibitors. The hydroxyl groups at the 3# and 5# positions can form hydrogen bonds with the backbone carbonyl groups of Glu179 and Met181. The hydroxyl group at the 4# position forms a hydrogen bond with the enzyme. Therefore, inhibition of MAPKK4 activity by delphinidin causes a decrease in JNKs phosphorylation but not p38. A new molecular target of delphinidin that regulates p38 must be elucidated. To investigate the molecular basis of MAPKK4 inhibition by delphinidin, we carried out a docking study (Figure 6C) using a homology model structure of the MAPKK4 kinase domain derived from the crystal structure of MAPKK7, which has 66% amino acid sequence homology with MAPKK4. The kinase domain of MAPKK4 consists of an N-lobe and a C-lobe. These N- and C-lobes are linked through a loop called the ‘hinge region’. The backbone of this loop interacts with the adenine moiety of ATP by hydrogen bonding. Considering the experimental result indicating that delphinidin is an ATP-competitive kinase inhibitor, we docked the compound to the ATP-binding site of MAPKK4 and delphinidin was easily docked to this site of MAPKK4. Delphinidin can form hydrogen bonds with the backbone of the hinge region of MAPKK4 as do other ATP-competitive kinase inhibitors. The hydroxyl groups at the 3# and 5# positions can form hydrogen bonds with the backbone carbonyl groups of Glu179 and Met181. The hydroxyl group at the 4# position forms a hydrogen bond.
with the backbone amide group of Met181. The hydroxyl groups at positions 3 and 7 form hydrogen bonds with the side chains of Lys187 and Asp247, respectively. In addition, the inhibitor would be sandwiched by the side chains of the hydrophobic residues in the ATP-binding site, including Ala120, Met178, Ile108, Val116, Cys156, Leu236 and Met181.

We suggest that another molecular target of delphinidin is PI-3K. The PI-3K/Akt pathway has been pointed out as another potential target for suppressing UVB-mediated COX-2 expression (13,14). Inhibition of Akt phosphorylation by a PI-3K inhibitor or dominant-negative Akt mutant suppressed UVB-induced COX-2 transcription in human keratinocytes (14). UVB-induced phosphorylation of Akt/p70S6K was attenuated effectively by delphinidin because PI-3K activity, a well-known upstream kinase of Akt, was inhibited by delphinidin. We also created a model structure (Figure 6D) of PI-3K in complex with delphinidin using the crystal structure of PI-3K in complex with ATP or delphinidin (38). PI-3K comprises four domains: a Ras-binding domain, a C2 domain, a helical domain and a catalytic domain. Although the substrate of PI-3K is not a protein, the catalytic domain of the enzyme consists of an N-lobe, a C-lobe and a hinge loop with a fold similar to protein kinases, and this structural similarity is also conserved in the ATP-binding site that is flanked by these two lobes. Consequently, ATP binds between these lobes in a manner similar to the ATP binding in protein kinases. Because delphinidin was an ATP-competitive inhibitor of PI-3K in our experiment, we docked the compound to the ATP-binding site of PI-3K. The hydroxyl groups at the 3' and 4' positions of delphinidin could form hydrogen bonds with the backbone atoms of Val887 in the hinge loop of PI-3K. The hydroxyl groups at positions 3 and 5 could also make hydrogen bonds with the side chains of Lys833, Asp841 and Tyr867. Delphinidin could hydrophobically interact with the side chains of Met804, Trp812, Ile831 and Ile879 from the N-lobe and Ala885, Phe961, Met953 and Ile963 from the C-lobe. The high inhibitory activity of delphinidin for PI-3K could be due to the hydrogen bonding and hydrophobic interactions.

MEK1 is another molecular target of delphinidin. Flavonoids that possess a hydroxyl group at the 3' position (e.g. myricetin, quercetin but not kaempferol) inhibit MEK1 activity. The hydroxy group at the 3' position plays a key role in the formation of the hydrogen bond between flavonoids and the backbone amide group of Ser212 in MEK1. Because delphinidin has a hydroxyl group at the 3' position, it also suppresses MEK1 activity. Delphinidin attenuated 12-O-tetradecanoylphorbol 13-acetate-induced neoplastic transformation by inhibiting the MEK/ERK signaling pathway. In the present study, the UVB-induced phosphorylation of ERKs was decreased by delphinidin, which suggests that the broad reactivity of multi-kinase inhibitors allows the multiple applications for a number of conditions. A potential drawback with the use of a multi-target kinase inhibitor is the appearance of undesirable side effects. Safety is a pivotal concern in using multi-target kinase inhibitors. Food components such as delphinidin are generally regarded as safe due to their longtime use. In this regard, delphinidin should be considered as a potential chemopreventive agent.

To summarize (Figure 6E), delphinidin inhibits UVB-induced COX-2 expression in JB6 P+ cells by blocking the MAPK4 and PI-3K pathways and subsequently suppressing AP-1 and NF-kB activities. Our results suggest MAPKK4 and PI-3K as potent molecular targets of delphinidin in suppressing UVB-mediated skin carcinogenesis. Taken together, these results provide insight into the molecular action of delphinidin and indicate the potential of delphinidin as a novel chemopreventive agent. Further studies, including X-ray crystallography, to determine the inhibitor complex structure would elucidate the exact binding mode of delphinidin to targeted kinases.

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