Tangeretin induces cell-cycle G₁ arrest through inhibiting cyclin-dependent kinases 2 and 4 activities as well as elevating Cdk inhibitors p21 and p27 in human colorectal carcinoma cells

Min-Hsiung Pan¹,², Wei-Jen Chen¹, Shoei-Yn Lin-Shiau³, Chi-Tang Ho³ and Jen-Kun Lin¹*  

Institutes of ¹Biochemistry and ²Toxicology, College of Medicine, National Taiwan University, Taipei, Taiwan, ³Department of Food Science, Cook College, Rutgers University, New Brunswick, NJ 08901, USA and ⁴Department of Marine Food Science, National Kaohsiung Institute of Marine Technology, Kaohsiung, Taiwan  

*To whom correspondence should be sent at Institute of Biochemistry, College of Medicine, National Taiwan University, No 1, Section 1, Jen-Ai Road, Taipei, Taiwan  
Email: jklin@ha.mc.ntu.edu.tw

Tangeretin (5,6,7,8,4’-pentamethoxyflavone) is concentrated in the peel of citrus fruits. DNA flow cytometric analysis indicated that tangeretin blocked cell cycle progression at G₁ phase in colorectal carcinoma COLO 205 cells. Over a 24 h exposure to tangeretin, the degree of phosphorylation of Rb was decreased after 12 h and G₁ arrest developed. The protein expression of cyclins A, D1, and E reduced slightly under the same conditions. Immunocomplex kinase experiments showed that tangeretin inhibited the activities of cyclin-dependent kinases 2 (Cdk2) and 4 (Cdk4) in a dose-dependent manner in the cell-free system. As the cells were exposed to tangeretin (50 µM) over 48 h a gradual loss of both Cdk2 and 4 kinase activities occurred. Tangeretin also increased the content of the Cdk inhibitor p21 protein and this effect correlated with the elevation in p53 levels. In addition, tangeretin also increased the level of the Cdk inhibitor p27 protein within 18 h. These results suggest that tangeretin either exerts its growth-inhibitory effects through modulation of the activities of several key G₁ regulatory proteins, such as Cdk2 and Cdk4, or mediates the increase of Cdk inhibitors p21 and p27.

Introduction

Flavonoids are widespread in fruit and vegetables. They are intensively studied for their role in human health, including cancer prevention. Citrus flavonoids have a broad spectrum of biological activity, including anticarcinogenic and antitumor activities (1). It is suggested that cancer induction can be prevented by ingestion of certain food ingredients (2), and flavonoids, in citrus fruits and juices are among the most prominent cancer-preventing agents (3–5). The biological effects of flavonoids seem to occur mainly through their interaction with protein tyrosine kinases (6) and cyclooxygenase (7). Polymethoxylated flavonoids, such as tangeretin and nobiletin, are more potent inhibitors of tumor cell growth than free hydroxylated flavonoids. They also possess potent anti-inflammatory and anti-metastatic activities (8,9).

Tangeretin is a polymethoxylated flavone, 5,6,7,8,4’-pentamethoxyflavone, which is concentrated in the peel of citrus fruits (10) (Figure 1), and probably acts as a natural resistance factor against pathogenic fungi (11). Grapefruit and orange juice have been shown to inhibit human breast cancer cell proliferation (12) and to interact with drug administration (13). Several biological activities have been shown for tangeretin itself, including the ability to enhance gap junctional intercellular communication (14), to counteract tumor promoter-induced inhibition of intercellular communication (15) and to inhibit cancer cell proliferation (16).

Previous studies have shown that many flavonoids exhibit potent antitumor activity against several rodent and human cancer cell lines (17,18). The antitumor properties of some flavonoids have been studied with respect to apoptosis and cell cycle arrest. Quercetin has been shown to impair the G₁ to the S phase transition in a human gastric cancer cell line and also to cause apoptosis in several cell lines (19). The molecular mechanisms of cell cycle arrest by flavonoids remain largely unclear, but appear to involve modulation of multiple cell cycle regulatory proteins. The eukaryotic cell cycle is regulated through the sequential activation and inactivation of cyclin-dependent kinases (Cdks) that drive cell cycle progression through the phosphorylation (20) and dephosphorylation of several regulatory proteins.

In normal cells, Cdks exist predominantly in quaternary complexes consisting of a Cdk, a cyclin, a proliferating cell nuclear antigen (PCNA) and a 21 kDa protein (p21) (21). Cdk activation requires cyclin binding and phosphorylation of conserved threonine residue by Cdk-activating kinase (CAK). The activated Cdk–cyclin complexes can be changed to an inactive state by phosphorylation of a conserved threonine–tyrosine pair or binding to Cdk inhibitory subunits (CKIs). Progression from G₁ to S phase in mammalian cells is regulated by the accumulation of cyclins D, E and A, which bind to and activate different Cdk catalytic subunits. The activation of Cdk4–cyclin D and/or Cdk6–cyclin D complex is necessary for transition from early to mid G₁ phase. Transition through mid G₁ to S phase is regulated by activation of the Cdk2–cyclin E complex. Progression through late G₁ to S phase also requires the presence of Cdk2–cyclin A complex (22). The retinoblastoma tumor suppressor protein (Rb) is a critical target protein that is phosphorylated via these Cdk–cyclin complexes (23). Rb controlled gene expression is mediated by a family of heterodimeric transcriptional regulators, collectively termed the E2F, which can transactivate genes whose products are important for transition from G₁ to S phase (24). Phosphorylation of Rb frees these regulators, enabling them to transactivate the target genes. Therefore, the hypophosphorylated forms of Rb are predominantly found in G₀–G₁ phase, but the hyperphosphorylated forms of Rb are required during S and G₂–M phase (23).

Recent studies show that Cdk regulation involves a diverse family of proteins, termed the CKIs (Cdk inhibitors), that bind and inactivate Cdk–cyclin complexes. In mammalian cells CKIs fall into two classes: (i) p21 (Cip1/Waf1/Cap20/Sdi1/
The effect of p21 is largely exerted during the G1 phase of the cell in vivo (25). The majority of related CKIs, specifically p16INK4A, p15INK4B, p18INK4C, and p19INK4D, are closely related. These proteins are expressed in 205 cells, and their expression is regulated by the status of the cell cycle, with preferential binding to Cdk4- and Cdk2-containing complexes (26). In addition, the expression of p21 in a variety of tissues has been shown to exert its anti-tumorigenic activity through a p53-independent mechanism (29). These effects probably also occur in p53 null mice, suggesting that it is also regulated by a mechanism independent of p53 (28). However, the expression of p21 in a variety of tissues has been shown to exert its anti-tumorigenic activity through a p53-independent mechanism (29).

In this study, we examined the effects of tangeretin and related flavonoids on the growth of colorectal carcinoma COLO 205 cells, expression of G1 cyclins, phosphorylation state of Rb, and kinase activities of Cdk2 and Cdk4 in a cell-free system and in cultured cells. The effects of these flavonoids on the p53 protein levels were also investigated.

Materials and methods

Materials

Apigenin, kaempferol, myricetin, quercetin, rutin, luteolin, and all protease inhibitors were purchased from Sigma (St. Louis, MO). Tangeretin and nobiletin were isolated from orange peel extract rich in polymethoxyflavones which was provided by Florida Flavors Inc. (Lakeland, FL). The extract (5 g) was subjected to silica gel column chromatography. The column was eluted with chloroform containing increasing amounts of ethyl acetate followed by ethyl acetate and methanol. Tangeretin and nobiletin were isolated as two major products at 72 mg and 52 mg, respectively. The purity of these two compounds was determined by HPLC. Tangeretin and nobiletin were identified by comparison of 1H and 13C NMR spectra with literature values (34). The antibodies to p27, cyclins A, D1, E, and Cdk2 were obtained from the Santa Cruz Biotechnology (Santa Cruz, CA); anti-p21 monoclonal antibody was obtained from Transduction Laboratory (Lexington, KY); anti-3T3 tubulin monoclonal antibody from Oncogene Science (Cambridge, UK); anti-Rb monoclonal antibody and Cdk4 from Upstate Biotechnology (Lake Placid, NY); p53 from Oncogene Research Products (Cambridge, MA); anti-pRb antibody from Cell Signaling (Beverly, MA). It is important to be precise about which antibodies are used, especially with respect to anti-phospho-Rb.

Cell culture

The cell line COLO 205 (CCL-222, American Type Culture Collection) was developed from a poorly differentiated human colon adenocarcinoma. Cell lines were maintained in RPMI-1640 supplemented with 10% FCS (Gibco BRL, Grand Island, NY). The cell line COLO 205 (CCL-222; American Type Culture Collection) was used, especially with respect to anti-phospho-Rb.

Determination of cell growth curve

Human colon cancer (5 × 10^5) cells were plated in 35 mm petri dishes. The next day, the medium was changed and various flavonoids were added. Control cells were treated with DMSO to a final concentration of 0.05% (v/v). At the end of incubation, cells were harvested for cell count using a hemocytometer (17).

Flow cytometric cell analysis

Cell cycle distribution was analyzed by flow cytometry as described previously (35). Briefly, cells were trypsinized, washed once with PBS, and fixed in 100% ethanol for 1 h at −20°C. Fixed cells were washed with PBS, incubated with 0.5 ml PBS containing 0.05% RNase and 0.5% Triton X-100 for 30 min at 37°C and stained with propidium iodide. The stained cells were analyzed using a FACSscan laser flow cytometer (Becton Dickinson, San Jose, CA).

Western blot analysis

Equal amounts of total cellular proteins (50 µg) were resolved by SDS-polyacrylamide gel electrophoresis (PAGE), transferred onto polyvinylidene difluoride (PVDF) membranes (Amersham, Arlington, IL), and then probed with primary antibody followed by secondary antibody conjugated with horseradish peroxidase or alkaline phosphatase. The immunocomplexes were visualized using enhanced chemiluminescence kits (α-tubulin, Rb, cyclin A, cyclin E, Cdk2, Cdk4, p21, p27, p53 and cyclin D1).

In vitro and cell culture Cdkks kinase assay

For in vitro Cdkks kinase assay, commercially growing COLO 205 cells were washed with cold PBS and lysed with Gold lysis buffer (10% glycerol, 1% Triton X-100, 1 mM sodium orthovanadate, 1 mM EGTA, 5 mM EDTA, 10 mM NaF, 1 mM sodium pyrophosphate, 20 mM Tris–HCl pH 7.9, 100 µM β-glycerophosphate, 137 mM NaCl, 1 mM PMSF, 10 µg/ml aprotinin and 10 µg/ml leupeptin) for 30 min at 4°C. The cell lysate was clarified by centrifugation at 12000 g for 10 min at 4°C. 4 mg of protein were incubated with anti-Cdk2 or anti-Cdk4 antibody and protein A/G plus agarose (Santa Cruz Biotechnology, Santa Cruz, CA) for 18 h at 4°C. The immunoprecipitate was washed three times with immunoprecipitate buffer (1% Triton X-100, 150 mM NaCl, 10 mM Tris pH 7.4, 1 mM EDTA, 1 mM EGTA, 0.2 mM sodium orthovanadate, 0.2 mM PMSF, 0.5% NP-40) and three times with kinase buffer (50 mM HEPES, pH 7.4, 10 mM MgCl2, 2.5 mM EDTA, 10 mM β-glycerophosphate, 1 mM NaF, 1 mM DTT for Cdk4; 50 mM HEPES, pH 7.4, 10 mM MgCl2, 2.5 mM EDTA, 1 mM DTT for Cdk2) another three times and separated into 6-8 tubes. The kinase reactions were carried out in a final volume of 40 µl containing 2 µCi of [γ-32P]ATP (5000 Ci/mmol, Amersham) and incubated for 20 min at 25°C. Each sample was mixed with 10 µl of 5% Laemmli’s loading buffer to stop the reaction, heated for 10 min at 100°C, and subjected to SDS–PAGE. The gels were dried, visualized by...
**Table I.** Effect of different flavonoids on the growth of COLO 205 cells

<table>
<thead>
<tr>
<th>Compounds</th>
<th>IC₅₀ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apigenin</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Kaemperol</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Myricetin</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Quercetin</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Rutin</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Luteolin</td>
<td>47.6 ± 0.15</td>
</tr>
<tr>
<td>Tangeretin</td>
<td>37.5 ± 0.12</td>
</tr>
<tr>
<td>Nobiletin</td>
<td>66.2 ± 1.25</td>
</tr>
</tbody>
</table>

Cells were treated with various concentrations of flavonoids for 24 h. The numbers of viable cells were determined by counting the trypan blue-excluding cells in a hemocytometer. Each experiment was independently performed three times and expressed as mean ± SE.

**Results**

**Several flavonoids inhibit cell proliferation**

Previous studies have shown that flavonoids are potent anti-proliferation (17–18) and anticancer agents (36). Here we investigated the anti-proliferation of eight structurally related flavonoids: apigenin, luteolin, quercetin, rutin, tangeretin, nobiletin, myricetin and kaempferol. The structures of these flavonoids are illustrated in Figure 1. To assess the inhibitory effects of selected flavonoids on the growth of colon cancer cells, we first determined the growth rates of COLO 205 colon cancer cells. Exponentially growing cultures of COLO 205 cells were continuously cultured in the absence or presence of different concentrations of flavonoids. After 48 h of treatment, the cell growth rates were determined by trypan blue exclusion. As shown in Table I, luteolin and nobiletin induced a dose-dependent inhibited cell proliferation, assuming an IC₅₀ value of ~47.6 µM and 66.2 µM, but the potencies of their inhibition were lower than that by tangeretin (IC₅₀, 37.5 µM). Tangeretin strongly inhibited COLO 205 cell growth (Table I). Exponentially growing COLO 205 cultures rapidly underwent growth inhibition with the addition of various concentrations of tangeretin, as evidenced by a decrease of cell proliferation over the experimental period (Figure 2).

**Tangeretin induces G₀/G₁ cell cycle arrest in COLO 205 cells**

In this study, we demonstrated that tangeretin induced significant growth inhibition of human colon cancer cells (Figure 2). In order to determine whether tangeretin has a cell cycle arrest effect in human COLO 205 colon cancer cells, the cells treated with DMSO or tangeretin for 12 and 24 h were subjected to flow cytometric analysis after staining their DNA. Histograms of flow cytometric data are shown in Figure 3. Control cells (DMSO) progressed through the cell cycle well. In contrast, tangeretin-treated COLO 205 cells were blocked in the G₀/G₁ phase (over 90%) after 24 h treatment.

**Effects of tangeretin on pRb phosphorylation, cyclins and Cdk2 and 4 protein expression**

The phosphorylation mediated by both cyclin D/Cdk4 and cyclin E/Cdk2 of the Rb protein is required for the cells to progress from G₁ into S phase in those cells possessing a functional pRb. To elucidate the arrest point of tangeretin-treated COLO 205 cells in the G₁ phase we analyzed the phosphorylation state of pRb and the expression of G₁ cyclin protein and Cdk2 and 4. As shown in Figure 4A, the degree of phosphorylation of Rb was decreased after 12 h of 50 µM
tangeretin treatment compared with total Rb protein. Exposure to 50 µM tangeretin, the levels of cyclins A, D1 and E were analyzed by immunoblotting over a 24 h period. As shown in Figure 4B and C, cyclin A, D1 and E levels did not change with increasing time of 0.05% DMSO exposure, while tangeretin appeared to have a suppressing effect on the levels of cyclin A, cyclin D1 and E. By contrast, there was no change in the protein expression of Cdk2 and 4, which are associated with Rb phosphorylation. However, small changes in the levels of cyclins were not sufficient to explain the reduction of the phosphorylation of Rb. Therefore, we subsequently investigated the effects of tangeretin on the activities of Cdk2 and 4 in a cell-free system and in cultured cells, as well as its effects on the expression of Cdk inhibitory subunits (CKIs).

Effects of tangeretin on the activities of Cdk2 and 4 in a cell-free system and in cultured cells

To examine whether tangeretin would directly inhibit Cdkks in COLO 205 cells, we first evaluated the effect of tangeretin on the kinase activities of Cdkks 2 and 4 in cell-free systems. The kinase activities of Cdkks 2 and 4 were measured after immunoprecipitation from exponentially growing COLO 205 cells using anti-Cdk2 or anti-Cdk4 antibodies, then tangeretin was added and histone H1 (for Cdk2) or Gst–Rb fusion protein (for Cdk4) as substrates. As shown in Figures 5A and 6A, the in vitro inhibition of Cdkks 2 and 4 by tangeretin was concentration-dependent, assuming an IC50 of ~26.5 µM and...
Tangeretin induces G₁ arrest in human colorectal carcinoma cells

Fig. 6. Effects of tangeretin on the activities of Cdk4 kinase in cell-free system and cultured cell. (A) Cdk4 immuno complex was prepared from growing COLO 205 cells and reacted with [γ-\(^{32}\)P]ATP, various concentrations of tangeretin and substrates (GST-Rb) for 20 min at room temperature as described under Materials and methods. Quantification of the phosphorylated-GST-Rb was performed by densitometric analysis (IS-1000 Digital System). Data represent the means ± SE of three samples. (B) COLO 205 cells were treated with 50 µM of tangeretin for increasing periods. (C) Cells were treated with different concentrations of tangeretin for 24 h. Total cell lysates were used for immunoprecipitation, the kinase activities were assayed with GST-Rb as substrates. The experiments were performed as described under Materials and methods. Data shown are representative of at least three independent experiments.

19.2 µM for Cdk2 and Cdk4 respectively. We next examined the activities of Cdk2 and Cdk4 from tangeretin-treated COLO 205 cells. The cells were treated with 50 µM tangeretin for increasing periods, and the kinase activity was then determined by the immunoprecipitation method described above. As shown in Figure 5B and 6B, the kinase activities of both Cdk2 and Cdk4 were inhibited in a time-dependent manner, and more than 90% of the activities were inhibited compared with untreated control cells (100%) after 12 h treatment. On the other hand, the inhibition of Cdk2 and Cdk4 activities by tangeretin were also concentration-dependent in cultured COLO 205 cells (Figure 5C and 6C). These findings were consistent with the lack of Cdk2 and Cdk4 activities in tangeretin-treated cells, the underphosphorylated form of Rb, and the failure of these cells to progress from G₁ to S phase.

Fig. 7. Effects of tangeretin on protein expression of p27, p21 and p53 in COLO 205 cells. (A) COLO 205 cells were treated with 50 µM of tangeretin for the time indicated. (B) Cells were treated with various concentrations of tangeretin for 24 h. Cell lysis and western blotting were performed as described under Materials and methods. Data shown are representative of at least three independent experiments.

Effects of tangeretin on protein expression of Cdk inhibitors p27Kip1, p21Cip1 and p53 in colon cancer cells

To examine further whether tangeretin could induce other members of the Cdk inhibitor protein family, we investigated the effect of tangeretin on the expression of p27 and p21 proteins in COLO 205 cells. The expression of p27 protein was significantly increased within 18 h after exposure to 50 µM tangeretin (Figure 7A). As with p27 protein, tangeretin treatment induced a significant increase of p21 protein. We studied further the effect of tangeretin on the levels of p27 and p21 proteins. As shown in Figure 7B, tangeretin markedly up-regulated the level of p27 and p21 in a concentration-dependent manner. The increase of p21 is reported to be regulated by either a p53-dependent or p53-independent mechanism. To determine whether the growth-inhibitory response to tangeretin was dependent on the p53 status, additional experiments were performed as described below, and it was found that p53 protein was also induced by tangeretin in COLO 205 cells.

Effects of tangeretin and related compounds on the expression of p53 in COLO 205 colon cancer cell line

The p53 tumor suppressor has been shown to be a key regulator in interpreting the extrinsic signals that induce cell cycle arrest (37). Flavonoids such as apigenin have been reported to induce the expression of p53 protein in mouse fibroblasts (38). To
Flavonoids are naturally occurring plant polyphenols found in abundance in diets rich in fruit, vegetable, and plant-derived beverages such as tea. Epidemiological studies have shown that the intake of certain vegetables, fruits and tea in the daily diet provides effective cancer prevention (39). Polymethoxylated flavonoids (1), tangeretin, and nobiletin, are more potent inhibitors of tumor antitumor activities. Polymethoxylated flavonoids have a broad spectrum of biological activity including anticarcinogenic and anti-inflammatory activity relations the effects of selected flavonoids on the expression of p53 were examined by western blotting. Among these compounds, tangeretin and nobiletin exhibited rather strong induced activities in the COLO 205 cells (Figure 8A). However, tangeretin was the most potent inducer of p53 and induced p53 protein expression in a dose-dependent manner (Figure 8B). These results suggest that p53 protein plays a critical role in the tangeretin-induced G0/G1 cell cycle arrest.

Discussion
Flavonoids are a critical role in the negative regulation of cell division. Cdk2 and Cdk4, which are regulators for Cdks. Additional studies are needed to determine whether the inhibition of Cdk(s) activation or inhibition of Cdk-inactivating kinases contributes to the inhibition of Cdk(s) 2 and 4 inhibition due to these direct and indirect actions inhibited phosphorylation of Rb by tangeretin (Figure 4A). However, Cdk activation and inhibition also requires phosphorylation or dephosphorylation at some conserved amino acid residues (20). It is therefore possible that tangeretin inhibits the Cdk-activating kinases (CAKs) or activates the Cdk-inactivating phosphatases, which are regulators for Cdks. Additional studies are needed to determine whether the inhibition of CAs or activation of Cdk-inactivating phosphatases contributes to the inhibition of Cdk(s) 2 and 4. In addition, Cdk6 might phosphorylate pRb in cells. Issues of whether or not tangeretin directly inhibits Cdk6 activity remain to be addressed.

There was little change in the levels of cyclin D1 and E after 24 h of tangeretin exposure (Figure 4B). But, it seems that the protein amount of cyclins was not the main causative effect on the activities of Cdks in tangeretin-treated COLO 205 cells. However, we cannot rule out the possibility that tangeretin might block cyclin binding to Cdk, thereby inhibiting the kinase complex activities of Cdks.

The tumor suppressor, p53, has been implicated in a variety of cellular processes (48). However, the undisputed roles of p53 are the induction of cell growth arrest and apoptosis (27). Among the transcriptional targets of p53, the Cdk inhibitor p21Cip1 plays a key role in mediating G1 arrest (49). Another CKI is p27Kip1, which mediates growth arrest and is thought to play a critical role in negative regulation of cell division in vivo (22,50). The outcome of CKIs induction in most cells is cessation of cell proliferation, differentiation, or even cell death. Since an inhibition of CKIs activity is one of the factors causing uncontrolled proliferation of tumor cells, one possible strategy to control cancer cell proliferation is to induce CKIs expression, which would lead to G0/G1 arrest and stop tumor cell growth. In this study, we observed that tangeretin can cease cell proliferation in COLO 205 cells which possess functional p53 and induce the increase of p53 protein in a time-dependent manner. G1 arrest produced by tangeretin occurred with an accompanying p21Cip1 and p27Kip1 accumulation (Figure 7). The p21Cip1 continued to show a markedly up-regulation from 18 h to 24 h after exposure to tangeretin. Inhibition of Cdk activity might also have occurred through up-regulation of p21Cip1 by tangeretin. These effects might be triggered by an increased expression of p53 by tangeretin. In this study, tangeretin also increased the p27 protein level in...
Tangeretin induces \( G_1 \) arrest in human colorectal carcinoma cells


Wang,J.K., Lin-Shiau,S.Y. and Lin,J.K. (1999) Induction of apoptosis by apigenin and related flavonoids through cytochrome c release and activation of colon cancer cells, suggesting that p27 is also important for G1 arrest by tangeretin. These results suggest that tangeretin inhibited not only the increase of CKIs levels but also the decrease of Cdk2 and Cdk4 kinase activities in both the cell-free system and in cultured cells.

Additional studies are needed to clarify whether transcriptional, translational or post-translational levels control the activation of the p21 gene by tangeretin. In summary, we have shown that the growth-inhibitory response to tangeretin, including the inhibition of Cdk2 and Cdk4 kinase activities, and the increase in the level of p21 and p27, is dependent on p53 activity. The p21 and/or p27 up-regulation by tangeretin might play an important role in its anti-proliferative activity in cultured cells. A predominance of polymethoxyflavonoids (tangeretin and nobiletin) over polyhydroxyflavonoids was demonstrated by the induced p53 levels (Figure 8). In previous reports polymethoxyflavonoids were more potent than polyhydroxyflavonoids in antitumor activities using a cell culture system (42). Differences in their activities may be derived from the relatively greater membrane uptake efficiencies of polymethoxyflavonoids than those of polyhydroxyflavonoids because methylation of the phenolic hydroxyl groups, in general, increases the molecular hydrophobicity that promotes the transportation rates (5). To our knowledge this is the first report to demonstrate that tangeretin does, indeed, play an important role in the function of anti-proliferation and anti-tumor capacity through inhibiting Cdns kinase activities and elevating Cdk inhibitors, and may provide a pivotal mechanism for its cancer chemopreventive action.

Acknowledgments:

This study was supported by the National Science Council NSC 90-2320-B-002-163, NSC 90-2320-B-002-164, NSC 90-2313-B-022-004; by the National Health Research Institute, NHRRI-EX98193BL, by the National Research Institute for Chinese Medicine, NRICM 90102; and by the Ministry of Education, ME 99-B-A01-1.

References


Received March 14, 2002; revised May 27, 2002; accepted July 1, 2002