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Apigenin causes G_2/M arrest associated with the modulation of $p21^{Cip1}$ and Cdc2 and activates p53-dependent apoptosis pathway in human breast cancer SK-BR-3 cells

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Abstract

We studied the effects of apigenin on the cell cycle distribution and apoptosis of human breast cancer cells and explored the mechanisms underlying these effects. We first investigated the antiproliferative effects in SK-BR-3 cells exposed to between 1 and 100 μM apigenin for 24, 48 and 72 h. Apigenin significantly inhibited cell proliferation at concentrations over 50 μM, regardless of exposure time ($P<.05$), and resulted in significant cell cycle arrest in the G_2/M phase after 48 h of treatment at high concentrations (50 and 100 μM; $P<.05$). To investigate the regulatory proteins of cell cycle arrest affected by apigenin, we treated cells with 50 and 100 μM apigenin for 72 h. Apigenin caused a slight decrease in cyclin D and cyclin E expression, with no change in CDK2 and CDK4. In addition, the apigenin-induced accumulation of the cell population in the G_2/M phase resulted in a decrease in CDK1 together with cyclin A and cyclin B. In an additional study, apigenin also increased the accumulation of p53 and further enhanced the level of $p21^{Cip1}$, with no change in $p27^{Kip1}$. The expression of Bax and cytochrome *c* of p53 downstream target was increased markedly at high concentration treatment over 50 μM apigenin. Based on our findings, the mechanism by which apigenin causes cell cycle arrest via the regulation of CDK1 and $p21^{Cip1}$ and induction of apoptosis seems to be involved in the p53-dependent pathway.

Keywords: Apigenin; Apoptosis; CDK1; G_2/M phase; p53; SK-BR-3 cells

1. Introduction

Apoptosis is an important series of events that leads to programmed cell death and is essential for development and tissue homeostasis. The potential mechanisms underlying the apoptotic process involve factors regulating the balance between the induction and inhibition of apoptosis. Recently, the regulation of apoptosis has been proposed as a promising target for cancer chemotherapy [1–3]. Several studies have indicated that anticancer drugs or cancer chemopreventive agents act through the induction of apoptosis in various cancer cells [4]. In addition, the initiation of apoptosis appears to be a common mechanism of many new anticancer agents for chemotherapy.

Apigenin (4′,5,7-trihydroxyflavone) is a member of the flavone subclass of flavonoids present in fruits and vegetables [5] and is considered to have various biological activities such as anti-inflammatory, anticancer and free-radical scavenging properties [6–9]. Studies of human malignant cancer cell lines have shown that apigenin inhibits cancer cell growth via the promotion of cell cycle arrest and apoptosis [10,11]. As a candidate anticancer agent, apigenin is of particular interest because it exhibits selective induction of cell cycle arrest and apoptosis in human prostate carcinoma cells without affecting normal cells [12,13]. Apigenin is also reported to be nonmutagenic and of low toxicity compared to related flavonoids [14].

Although a recent study has shown that apigenin inhibits the growth of human breast cancer cells [15], the cellular mechanisms underlying the action of apigenin in the induction of cell cycle arrest and apoptosis remain unknown. Therefore,

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our objective was to investigate the cellular mechanisms underlying cell cycle arrest and apoptosis induced by apigenin using human breast cancer SK-BR-3 cells.

2. Materials and methods

2.1. The cell culture and apigenin treatment

Human breast cancer SK-BR-3 cells were purchased from the Korean Cell Line Bank. Cells were routinely maintained in RPMI 1640 (Gibco), supplemented with 10% FBS and antibiotics (50 U/ml of penicillin and 50 μg/ml streptomycin, Gibco) at 37°C in a humidified atmosphere containing 5% CO_2. Cells were treated with apigenin ranging from 1 to 100 μM and incubated for 24, 48 and 72 h. Apigenin was purchased from Sigma and dissolved in DMSO (final concentration 0.1% in medium).

2.2. Cell proliferation and cell death assay

Cell proliferation was determined using the methyl thiazolyl tetrazolium (MTT) assay. At the 24-, 48- and 72-h point, the cells exposed to apigenin ranging from 1 to 100 μM were added to MTT. Four hours later, DMSO was added to each well to dissolve the resulting formazan crystals and then absorbance was recorded at 490 nm in a microplate reader (SpectraMax Plus; Molecular Devices).

2.3. Cell cycle distribution

Cells were then harvested, washed with cold PBS and processed for cell cycle analysis. Briefly, the cells were fixed in absolute ethanol and stored at −20°C for later analysis. The fixed cells were centrifuged at 1000 rpm and washed with cold PBS twice. RNase A (20 μg/ml final concentration) and propidium iodide staining solution (50 μg/ml final concentration) was added to the cells and incubated for 30 min at 37°C in the dark. The cells were analyzed using a FACSCalibur instrument (BD Biosciences, San Jose, CA) equipped with CellQuest 3.3 software. ModFit LT 3.1 trial cell cycle analysis software was used to determine the percentage of cells in the different phases of the cell cycle.

2.4. Immunoblotting assay

Cells were lysed in RIPA buffer (1% NP-40, 150 mM NaCl, 0.05% DOC, 1% SDS, 50 mM Tris, pH 7.5) containing protease inhibitor for 1 h at 4°C. The supernatant was separated by centrifugation, and protein concentration was determined by Bradford protein assay kit II (Bio-Rad Laboratories, California). Proteins (25 μg/well) denatured with sample buffer were separated by 10% SDS-polyacrylamide gel. Proteins were transferred onto nitrocellulose membranes (0.45 μm). The membranes were blocked with a 1% BSA solution for 3 h and washed twice with PBS containing 0.2% Tween-20 and incubated with the primary antibody overnight at 4°C. Antibodies against CDK1, CDK2, CDK4, cyclin A, cyclin B, cyclin D, cyclin E, $p21^{Cip1}$, $p27^{Kip1}$ and β-actin were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA) and used to probe the separate membranes. The next day, the immunoreaction was continued with the secondary goat anti-rabbit horseradish-peroxidase-conjugated antibody after washing for 2 h at room temperature. The specific protein bands were detected by Opti-4CN Substrate Kit (Bio-Rad Laboratories).

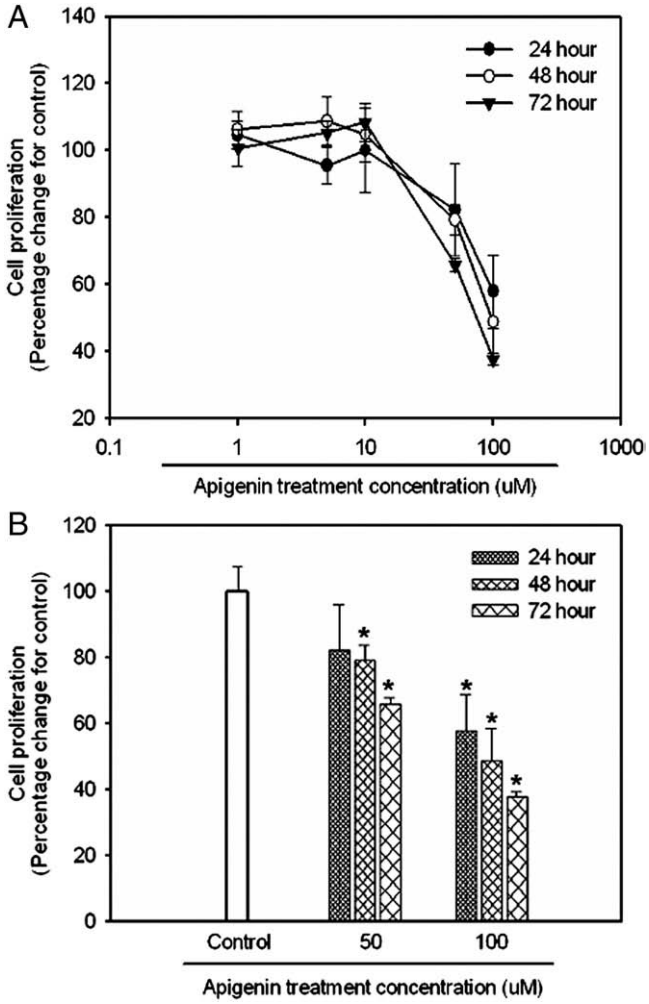

Fig. 1. Effect of apigenin on cell proliferation of SK-BR-3 cells. Cells were exposed to either vehicle (0.1% DMSO in medium) or apigenin (1–100 μM) and incubated for 24, 48 and 72 h. All data are reported as the percentage change in comparison with the vehicle-only group, which were arbitrarily assigned 100% viability. *P<.05, significantly different from the vehicle-only group (apigenin concentration="0").

2.5. Determination of cytotoxicity

Apigenin-induced cytotoxicity was evaluated by activity of lactate dehydrogenase (LDH), which was determined by formation of NADH (absorbance, 340 nm).

2.6. Statistical analyses

All data were expressed as percentage compared with vehicle-treated control cells, which were arbitrarily assigned

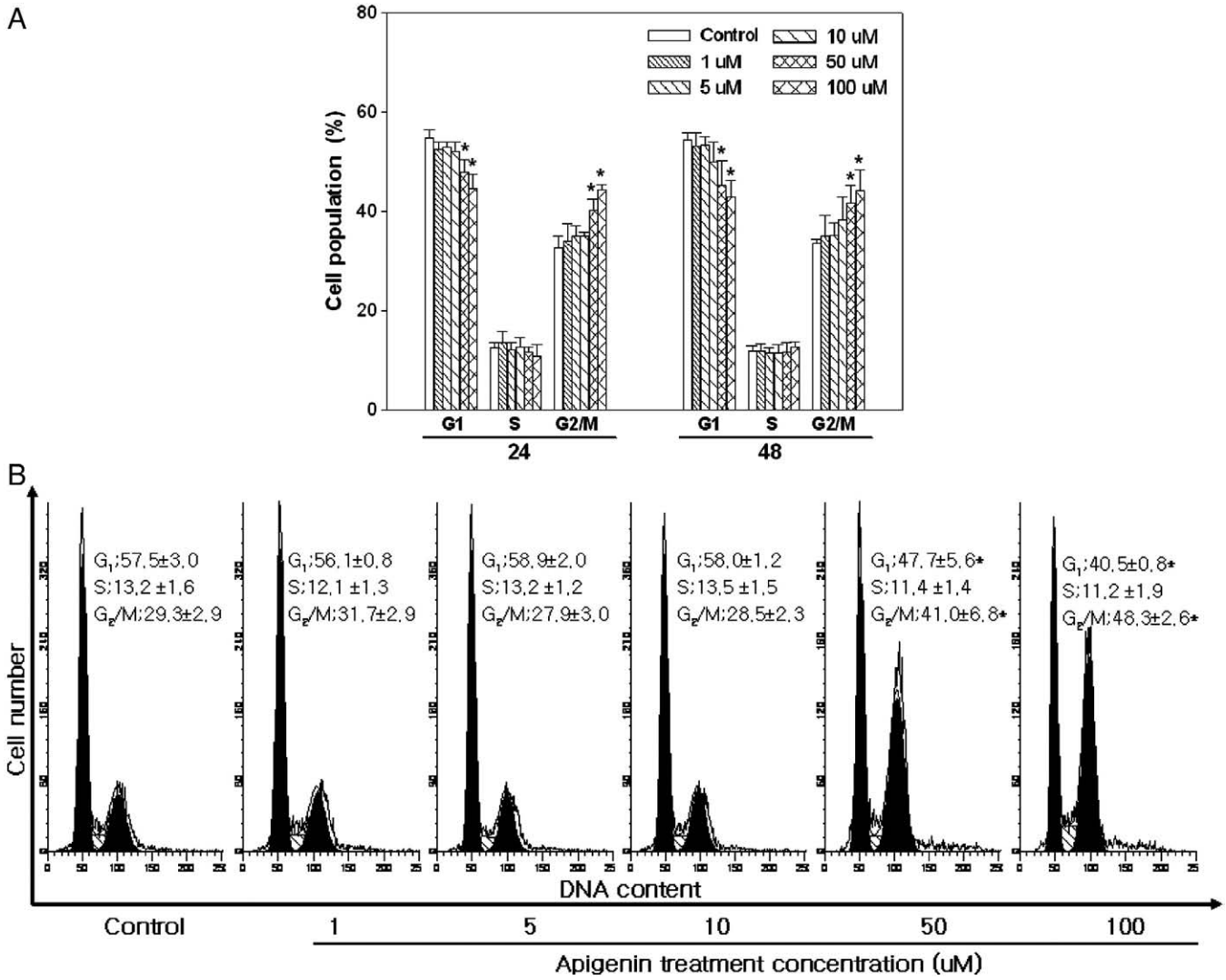

Fig. 2. Effect of apigenin on cell cycle distribution of SK-BR-3 cells. Cells were exposed to either vehicle (0.1% DMSO in medium) or apigenin (1–100 μM) and incubated for 24 and 48 h (A) and for 72 h (B). Values are expressed as percentage of the cell population in the G_1, S and G_1/M phase of cell cycle. *P<.05, significantly different from the vehicle-only group (apigenin concentration="0").

100%. Data were analyzed by one-way analysis of variance followed by Dunnett's multiple comparison test (Sigma Stat, Jandel, San Rafael, CA). For all comparisons, differences were considered statistically significant at P<.05.

3. Results

3.1. Apigenin inhibited cell proliferation

The effects of apigenin on cell proliferation were measured with the MTT assay, using human breast cancer SK-BR-3 cells exposed to between 1 and 100 μM apigenin for 24, 48 and 72 h. Apigenin did not affect cell proliferation at low concentrations of 1 to 10 μM, regardless of incubation time (Fig. 1A). Statistical differences in cell proliferation were first exhibited as inhibited cell proliferation at 100 μM after 24 h of apigenin treatment. At high concentrations of 50 and 100 μM, apigenin significantly decreased cell proliferation in a time-dependent manner (P<.05; Fig. 1B). Cell proliferation decreased up to 62.21% when treated with 100 μM apigenin for 72 h compared to the control.

3.2. Apigenin induced cell cycle arrest

We analyzed the cell cycle for SK-BR-3 cells exposed to apigenin for 24 and 48 h (Fig. 2A). Cell cycle arrest in SK-BR-3 cells exposed to apigenin was not observed at concentrations less than 10 μM but did occur in cells exposed to apigenin at high concentrations for 24 and 48 h. More pronounced results were obtained when the cells were treated with apigenin for 72 h (Fig. 2B). Compared to vehicle-treated SK-BR-3 cells, 50 μM apigenin caused an increase of 39.9% in the cell population in the G_2/M phase and 100 μM apigenin led to an increase of 64.8% after 72 h of treatment. The accumulation of the cell population in the G_2/M phase was

accompanied by a concomitant decrease in the cell population in the G_0/G_1 phase with 50 and 100 μM apigenin.

3.3. Apigenin affected the G_2/M-phase-related protein expression

We examined the specific regulatory proteins responsible for cell cycle arrest in SK-BR-3 cells exposed to high concentrations of apigenin (i.e., 50 and 100 μM) after 72 h of treatment by immunoblotting. Apigenin treatment resulted in a slight reduction in the expression of cyclin D and cyclin E compared to vehicle-treated SK-BR-3 cells, with no detectable change in CDK2 and CDK4 (Fig. 3A). However, CDK1, as well as cyclin A and cyclin B, which combine with CDK1 in the control of the G_2/M phase, markedly decreased, especially with 100 μM apigenin (Fig. 3A). These results imply that apigenin targets several components of the cell cycle regulatory apparatus. In addition, the expression of $p21^{Cip1}$ and $p27^{Kip1}$, which are inhibitors of cyclin-dependent kinase (CDK), increased in response to apigenin treatment (Fig. 3B). This suggests that apigenin induces apoptosis via $p21^{Cip1}$ activation, which is a CDK inhibitor (CKI) associated with the p53-dependent apoptotic pathway. The p53 expression increased gradually in a dose-dependent manner, and the expression of p53 downstream targets such as Bax and cytochrome *c* increased markedly with both 50 and 100 μM apigenin (Fig. 3C).

3.4. Apigenin induced cytotoxicity

The cytotoxicity of apigenin was determined by measuring the release of LDH. Apigenin significantly increased

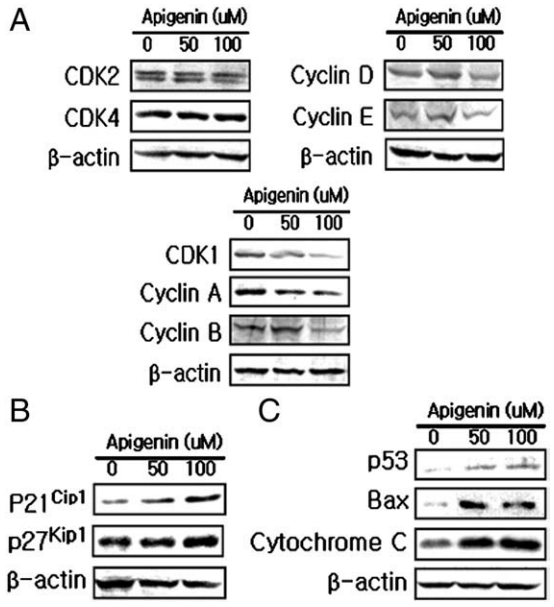

Fig. 3. Effect of apigenin on protein-expression-related cell cycle arrest (A), CKIs ($p21^{CIP1}$ and $p27^{Kip1}$) (B) and apoptosis (C) of SK-BR-3 cells. Cells were exposed to either vehicle (0.1% DMSO in medium) or apigenin (50 and 100 μM) and incubated for 72 h.

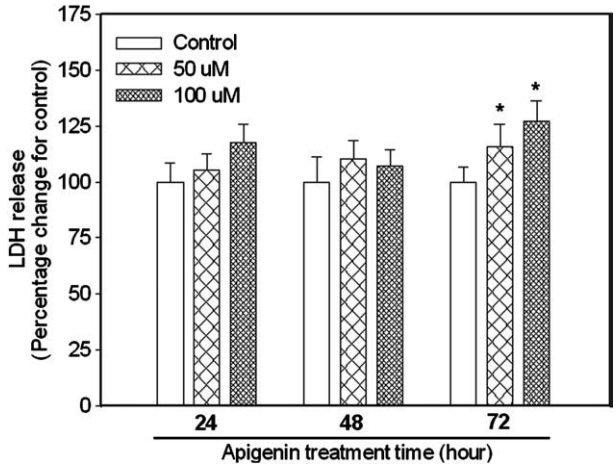

Fig. 4. Cytotoxicity of apigenin on the SK-BR-3 cells. Cells were exposed to either vehicle (0.1% DMSO in medium) or apigenin (50 μM and 100 μM) and incubated for 24, 48 and 72 h. All data are reported as the percentage change in comparison with the vehicle-only group, which were arbitrarily assigned 100% viability. *P<.05, significantly different from the vehicle-only group (apigenin concentration="0").

LDH release in SK-BR-3 cells exposed to high concentrations of apigenin (i.e., 50 and 100 μM), but neither 24 nor 48 h affected LDH release (Fig. 4).

4. Discussion

Recently, a great deal of attention has been focused on how flavonoids function in relation to their biological properties, especially in terms of their anticarcinogenic activity [16–18]. In particular, apigenin shows specific inhibitory activity for cancer cell growth [10,13], but the mechanisms underlying the effects of apigenin in the induction of cell cycle arrest and apoptosis in human breast cancer cells are still unknown. Although it has been reported that apigenin has weak estrogen activity [19], estrogen-negative SK-BR-3 cells were used because we wanted to investigate the estrogen-independent effects of apigenin.

We first examined the antiproliferative effect of apigenin on human breast cancer SK-BR-3 cells at various concentrations (1–100 μM) and exposure times (24, 48 and 72 h). Apigenin significantly decreased the proliferation of SK-BR-3 cells only at high concentrations, that is, 50 and 100 μM. This was inconsistent with previous reports stating that apigenin results in the cell cycle arrest of human colon, breast, skin and prostate carcinoma cells in a dose- and time-dependent manner [10,20,21] and that less than 10 μM apigenin has antiproliferative effects on human prostate cancer cells in a dose-dependent manner [12].

To further scrutinize these results, we analyzed the cell cycle of SK-BR-3 cells treated with apigenin using fluorescence-activated cell sorting under the same experimental conditions. Similar to data from our cell proliferation experiment, cell cycle arrest was not induced at low concentrations of apigenin, but significant G_2/M phase arrest

was induced at high concentrations. Many reports indicate that most flavonoids induce G_1 phase arrest in human cancer cells [22] and that apigenin inhibits the cell cycle at either the G/S or the G_2/M phase in various types of human cancer cells [15,19,23]. However, conflicting results have been reported with regard to the mechanisms underlying the cell cycle arrest caused by apigenin.

The cell cycle is tightly mediated through a complex network of positive and negative cell cycle regulatory molecules such as CDKs, CKIs and cyclins. G_1 progression and G_1/S transition are regulated by CDK2 and CDK4, which assemble with cyclin E and cyclin D. We found that apigenin caused a slight decrease in cyclin D and cyclin E expression, with no change in CDK2 and CDK4. In contrast, the down-regulation of CDK1 (Cdc2), as well as cyclin A and cyclin B, by apigenin may be the main cause of the G/M phase arrest. CDK1 is a catalytic subunit of the M-phase promoting factor, which is activated at the G_2/M transition and controls the onset of mitosis. Several investigators have shown that CDK1 in combination with cyclin A and cyclin B is critical in the G_2/M phase transition [24,25]. The decrease in the quantity of CDK1 protein observed as a result of apigenin treatment is consistent with arrest at the G_2/M phase because this protein is not expressed in resting cells.

In addition, $p21^{Cip1}$ and $p27^{Kip1}$, which are CKIs, were significantly up-regulated in apigenin-treated SK-BR-3 cells, although $p27^{Kip1}$ was only slightly elevated. These results imply that the activation of $p21^{Cip1}$ by apigenin may be a cause of apoptosis. It has been reported that p53 mediates cell cycle arrest and apoptosis by the direct induction of the CKI $p21^{Cip1}$ [26]. In addition, p53-dependent $p21^{Cip1}$ expression causes cell cycle arrest and apoptosis [27–29]. Treatment with high concentrations of apigenin (50 and 100 μM) for 72 h markedly induced p53 expression. The tumor suppressor gene p53 is regarded as a key factor in the balance between cell survival and cell death via the regulation of both the G_1 and G_2/M phases of the cell cycle [30]. The activation of p53 in response to DNA damage led to cell cycle arrest and the inhibition of cell proliferation [31–33]. Therefore, the up-regulation of p53 and $p21^{Cip1}$ expression may contribute to apigenin-mediated cell cycle arrest and apoptosis.

Apigenin increased the level of Bax, which is a major proapoptotic protein of the Bcl-2 family, to a level similar to that of p53. Bax is a downstream product of p53 and controls mitochondrial permeability and cytochrome *c* expression. The release of cytochrome *c* from mitochondria to the cytoplasm is a key step in the initiation of apoptosis.

We determined the cytotoxicity of apigenin at a treatment concentration high enough to induce cell cycle arrest and apoptosis using LDH release. One reported merit of apigenin is that it is nontoxic, unlike other flavonoids, such as quercetin [34,35]. Perhaps this is because flavonoids with hydroxyl groups at the 5-, 7- and 4′-positions, such as apigenin and genistein, are degraded significantly faster by human gut microorganisms than those with other structural motifs [36].

This study showed that apigenin increased LDH release up until 72 h, suggesting that apigenin might not be safe and free of side effects, although this depends on the treatment concentration and time. Moreover, there is a lot of evidence for the beneficial physiological effects of apigenin. In fact, most effective flavonoids have the potential to cause serious adverse effects in proportion to their effect. Some are used despite being toxic, depending on whether they are classified as nutritional supplements or pharmaceutical drugs, such as chemotherapy agents. Consequently, the anticancer activity of apigenin may be useful for developing anticancer medicine or adjuvants.

In conclusion, we suggest the existence of multiple pathways by which apigenin treatment results in G_2/M phase cell cycle arrest via up-regulated $p21^{Cip1}$ and down-regulated CDK1 and apoptotic cell death via modulation of the p53 pathway and up-regulation of cytochrome *c* expression. However, the effective dosage of apigenin must be considered carefully because the anticancer actions of cell cycle arrest and the induction of apoptosis were exhibited only at high concentrations, regardless of exposure time.

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References

[1] Sun SY, Hail Jr N, Lotan R. Apoptosis as a novel target for cancer chemoprevention. J Natl Cancer Inst 2004;96:662–72.
[2] Zhang Z, Li M, Rayburn ER, Hill DL, Zhang R, Wang H. Oncogenes as novel targets for cancer therapy (part IV): regulators of the cell cycle and apoptosis. Am J Pharmacogenomics 2005;5:397–407.
[3] Fesik SW. Promoting apoptosis as a strategy for cancer drug discovery. Nat Rev Cancer 2005;5:876–85.
[4] Yu J, Zhang L. Apoptosis in human cancer cells. Curr Opin Oncol 2004;16:19–24.
[5] Miean KH, Mohamed S. Flavonoid (myricetin, quercetin, kaempferol, luteolin, and apigenin) content of edible tropical plants. J Agric Food Chem 2001;49:3106–12.
[6] Soares R, Azevedo I. Apigenin: is it a pro- or anti-inflammatory agent? Am J Pathol 2006;168:1762–3.
[7] Fuchs J, Milbradt R. Skin anti-inflammatory activity of apigenin-7-glucoside in rats. Arzneimittelforschung 1993;43:370–2.
[8] Singh JP, Selvendiran K, Banu SM, Padmavathi R. Protective role of Apigenin on the status of lipid peroxidation and antioxidant defense against hepatocarcinogenesis in Wistar albino rats. Phytomedicine 2004;11:309–14.
[9] Romanova D, Vachalkova A, Cipak L, Ovesna Z, Rauko P. Study of antioxidant effect of apigenin, luteolin and quercetin by DNA protective method. Neoplasma 2001;48:104–7.
[10] Bektic J, Guggenberger R, Spengler B, Christiffel V, Pelzer A, Berger AP, et al. The flavonoid apigenin inhibits the proliferation of prostatic stromal cells via the MAPK-pathway and cell-cycle arrest in G1/S. Maturitas 2006;55s:s37–s46.
[11] Lindenmeyer F, Li H, Menashi S, Soria C, Lu H. Apigenin acts on the tumor cell invasion process and regulates protease production. Nutr Cancer 2001;39:139–47.

[12] Gupta S, Afag F, Mukhtar H. Selective growth-inhibitory, cell-cycle deregulatory and apoptotic response of apigenin in normal versus human prostate carcinoma cells. Biochem Biophys Res Commun 2001;287:914–20.
[13] Chiang LC, Ng LT, Lin IC, Kuo PL, Lin CC. Anti-proliferative effect of apigenin and its apoptotic induction in human Hep G2 cells. Cancer Lett 2006;237:207–14.
[14] Czeczot H, Tuek B, Kusztelak J, Szymczyk T, Dobrowolska B, Glinkowska G, et al. Isolation and studies of the mutagenic activity in the Ames test of flavonoids naturally occurring in medical herbs. Mutat Res 1990;240:209–16.
[15] Yin F, Giuliano AE, Law RE, Van Herle AJ. Apigenin inhibits growth and induces G2/M arrest by modulating cyclin-CDK regulators and ERK MAP kinase activation in breast carcinoma cells. Anticancer Res 2001;21:413–20.
[16] Ren W, Oiao Z, Wang H, Zhu L, Zhang L. Flavonoids: promising anticancer agents. Med Res Rev 2003;23:519–34.
[17] Lopez-Lazaro M. Flavonoids as anticancer agents: structure–activity relationship study. Curr Med Chem Anticancer Agents 2002;2: 691–714.
[18] Singh RP, Agarwal R. Natural flavonoids targeting deregulated cell cycle progression in cancer cells. Curr Drug Targets 2006;7:345–54.
[19] Breinholt V, Hossaini A, Svendsen GW, Brouwer C, Nielsen E. Estrogenic activity of flavonoids in mice. The importance of estrogen receptor distribution, metabolism and bioavailability. Food Chem Toxicol 2000;38:555–64.
[20] Wang W, Heideman L, Chung CS, Pelling JC, Koehler KJ, Birt DF. Cell-cycle arrest at G2/M and growth inhibition by apigenin in human colon carcinoma cell lines. Mol Carcinog 2000;28:102–10.
[21] Ford HL, Landesman-Bollag E, Dacwag CS, Stukenberg PT, Pardee AB, Seldin DC. Cell cycle-regulated phosphorylation of the human SIX1 homeodomain protein. J Biol Chem 2000;275: 22245–54.
[22] Birt DF, Hendrich S, Wang W. Dietary agents in cancer prevention: flavonoids and isoflavonoids. Pharmacol Ther 2001;90:157–77.
[23] Lepley DM, Li B, Birt DF, Pelling JC. The chemopreventive flavonoid apigenin induces G2/M arrest in keratinocytes. Carcinogenesis 1996; 17:2367–75.
[24] Morla AO, Draetta G, Beach D, Wang JY. Reversible tyrosine phosphorylation of cdc2: dephosphorylation accompanies activation during entry into mitosis. Cell 1989;58:193–203.
[25] Jessus C, Beach D. Oscillation of MPF is accompanied by periodic association between cdc25 and cdc2-cyclin B. Cell 1992;68:323–32.
[26] Lee JW, Moon MJ, Min HY, Chung HJ, Park EJ, Park HJ, et al. Induction of apoptosis by a novel indirubin-5-nitro-3¢-monoxime, a CDK inhibitor, in human lung cancer cells. Bioorg Med Chem Lett 2005;15:3948–52.
[27] Agrawal S, Agarwal ML, Chatterjee-Kishore M, Stark GR, Chisolm GM. Stat1-dependent, p53-independent expression of p21(waf1) modulates oxysterol-induced apoptosis. Mol Cell Biol 2005;22: 1981–92.
[28] Mahyar-Roemer M, Roemer K. p21 Waf1/Cip1 can protect human colon carcinoma cells against p53-dependent and p53-independent apoptosis induced by natural chemopreventive and therapeutic agents. Oncogene 2001;20:3387–98.
[29] Kachnic LA, Wu B, Wunsch H, MeKeel KL, DeFrank JS, Tang W, et al. The ability of p53 to activate downstream genes p21(WAF1/cip1) and MDM2, and cell cycle arrest following DNA damage is delayed and attenuated in scid cells deficient in the DNA-dependent protein kinase. J Biol Chem 1999;274:13111–7.
[30] Agarwal ML, Agarwal A, Taylor WR, Stark GR. p53 controls both the G2/M and the G1 cell cycle checkpoints and mediates reversible growth arrest in human fibroblasts. Proc Natl Acad Sci U S A 1995;92: 8493–7.
[31] Park M, Chae HD, Yun J, Jung M, Kim YS, Kim SH, et al. Constitutive activation of cyclin B1-associated cdc2 kinase overrides p53-mediated G2-M arrest. Cancer Res 2000;60:542–5.
[32] Lepik D, Jaks V, Kadaja L, Varv S, Maimet T. Electroporation and carrier DNA cause p53 activation, cell cycle arrest, and apoptosis. Anal Biochem 2003;318:52–9.
[33] Parker MA, Anderson JK, Corliss DA, Abraria VE, Sidman RL, Park KI, et al. Expression profile of an operationally-defined neural stem cell clone. Exp Neurol 2005;194:320–32.
[34] Fang J, Xia C, Cao Z, Zheng JZ, Reed E, Jiang BH. Apigenin inhibits VEGF and HIF-1 expression via PI3K/AKT/p70S6K1 and HDM2/p53 pathways. FASEB J 2005;19:342–53.
[35] Fang J, Zhou Q, Liu LZ, Xia C, Hu X, Shi X, et al. Apigenin inhibits tumor angiogenesis through decreasing HIF-1alpha and VEGF expression. Carcinogenesis 2007;28:858–64.
[36] Simons AL, Renouf M, Hendrich S, Murphy PA. Human gut microbial degradation of flavonoids: structure–function relationships. J Agric Food Chem 2005;53:4258–63.