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Wogonin induces apoptosis and down-regulates survivin in human breast cancer MCF-7 cells by modulating PI3K–AKT pathway

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ABSTRACT

Wogonin, one of flavonoid compounds isolated from Chinese herbal plants Scutellaria baicalensis Georgi, has been recognized as a potent anti-cancer agent acting through control of growth, differentiation and apoptosis. However, the underlying molecular mechanism of its anti-cancer activity remains to be further elucidated. In this study, we investigated the potential role of wogonin in the induced-apoptosis of human breast cancer cells MCF-7. Wogonin was found to inhibit the proliferation of MCF-7 in a concentration and time-dependent manner, notably wogonin could induce G1 phase arrest of MCF-7 cells. Wogonin-induced apoptosis was accompanied by a significant decrease of the Bcl-2 and survivin and increase of Bax and p53. Wogonin also increased active apoptosis forms of caspases-3, -8, -9 significantly. Z-DEVD-fmk, a specific caspase-3 inhibitor, significantly inhibited wogonin-induced cell apoptosis. Wogonin also suppressed the phosphorylation of PI3K/Akt and induced phosphorylation of ERK. PD98059, a specific ERK inhibitor, significantly blocked wogonin-induced apoptosis. On the other hand, LY294002, a specific PI3K inhibitor, significantly increased wogonin-induced cell apoptosis. Further study indicated that LY294002 not only down-regulated the expression of survivin alone, but also enhanced the inhibition of survivin expression combined with wogonin. In conclusion, the pro-apoptotic effect of wogonin is mediated through the activation of ERK and the activation of caspases, and is correlated with the block of the PI3K/Akt/survivin signal pathways in MCF-7 cells.

1. Introduction

Breast cancer is the most commonly occurring tumor among women worldwide. In America, approximately one in every ten women will develop the disease in their lifetime, and it is the second leading cause of cancer-related death in women [1]. Based on the cancer stage current treatment options include surgery, then perhaps radiation, hormonal therapy, and/or chemotherapy. Nonetheless, more than forty-thousand deaths from the disease were estimated during 2009 in the United States [2]. Therefore, searching for effective anti-cancer agents becomes more and more important for this disease.

Wogonin, a naturally occurring flavonoid, has been used as a potential agent for therapeutic purpose of anti-tumor and chemoprophylaxis [3]. Many studies had shown that wogonin could inhibit proliferation and induce apoptosis in many kinds of cancerous cells [4–7]. Some mechanisms of its pro-apoptotic effect have been hypothesized mainly including inhibition of NF-κB signaling pathway [8], inhibition of the cell cycle transit from G1 phase to G2 phase [9,10], inhibition of tumor angiogenesis via suppressing the phosphorylation of VEGFR-2 [11,12], inhibition of P-glycoprotein [13]. The exact mechanism of anti-tumor still remains poorly understood.

Apoptosis is a developmental phenomenon and processes such as deleting unneeded structures, sculpting structures and eliminating abnormal, damaged, redundant or harmful cells. It emerges as early as the blastocyst stage of development, during the formation of extraembryonic tissues, and continues throughout organogenesis [14]. Apoptosis has also come to be considered as a death way to eliminate precancerous and cancer cells [15]. Therefore, the induction of apoptosis is an important mechanism involved in the anti-cancer properties of many anti-cancer drugs. Apoptosis is a complicated process. The key molecular mechanism includes mitochondrion and the death receptor pathway [16]. The mitochondrion is the main site of action for members of the apoptosis-regulating protein family exemplified by Bcl-2. Bcl-2 proteins include three classes, of which one is anti-apoptotic and the others pro-apoptotic. Many Bcl-2 proteins are considered to associate with and regulate PT poreproteins, such as the voltage-dependent anion channel (VDAC) [17]. The caspases exist within the cell as inactive pro-forms or zymogens. These zymogens can be cleaved to form active enzymes such as caspase-3, following the induction of apoptosis. Survivin, a member of the inhibitor of apoptosis protein (IAP) family, is selectively expressed in the most common human tumors and related to clinical progression. In several cancer cells, the presence of survivin associates

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with anti-apoptosis and is correlated with increased malignancy [18]. Also, the family of serine–threonine protein kinases plays an important role in apoptosis. The PI3K/Akt and MAPK/ERK are important members of the family. PI3K activation activates Akt. Overactive PI3K/Akt pathway reduces apoptosis and promotes tumor cell growth [19]. MAPK (originally called ERK) communicates by adding phosphate groups to a neighboring protein, which acts as an "on" or "off" switch. Drugs that reverse the "on" or "off" switch has been investigated as cancer treatments. ERK is not only cytoprotective but also directly promotes hypertrophy [20]. The PI3K/Akt pathway plays an important role in the inhibition of apoptosis by inactivating some pro-apoptotic proteins, such as caspase-9 and BAD, meanwhile some anti-apoptotic proteins are also activated by this pathway [21,22]. Whether the two pathways are involved in the regulation of wogonin-induced MCF-7 cells apoptosis or not has not yet been determined.

In this study, we investigated whether wogonin-induced apoptosis was related to the expression of Bcl-2, P53, Bax, Survivin, caspase-3, MAPK/ERK and PI3K/Akt in MCF-7 cells. We found that wogonin-induced apoptosis was accompanied by the down-regulation of Bcl-2 and survivin, and activation of caspases-3. It was suggested that wogonin induce apoptosis through the activation of both the MAPK/ERK and the PI3K/Akt/survivin pathways.

2. Materials and method

2.1. Materials

Wogonin (Wog, purity > 98%) was purchased from Sigma (USA), dissolved in dimethyl sulfoxide (DMSO; Sigma, USA), and stored at −20 °C. Polyclonal antibodies against Bcl-2, Bax, survivin, caspase-3, Akt, PI3K/p85, and ERK were purchased from Boster Biological Technology Inc. (Wuhan, China). LY294002, PD98059, and the polyclonal antibodies against phospho-ERK1/2, phospho-Akt, phospho-PI3K/p85 were obtained from KeyGEN Biotech (Nanjing, China). Cleaved-caspase-3 antibody, horseradish peroxidase(HRP)-conjugated anti-rabbit immunoglobulin G (IgG) was purchased from Santa Cruz Biotechnology (Santa Cruz, California, USA).

2.2. Cell culture and proliferation assay

MCF-7 cells were cultured in Dulbecco's modified Eagle's medium (Gibco, USA) supplemented with 10% fetal bovine serum (Gibco, USA), and maintained in a humidified atmosphere of 5% CO_2 in air at 37 °C, two passages weekly. Cell proliferation was assessed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) staining as described by Matito [23]. Cells (7.5×10^3) were incubated in 96-well plates and incubated under various conditions as indicated. After each period of incubation, six wells were selected from each group of cells for MTT (50 mg/well) assay. After the cells were incubated at 37 °C for 4 h, the reaction was stopped by adding 150 ml/well of DMSO, and the cells were then incubated for another 10 min. The color reaction was quantified using an automatic plate reader (Bio-Tek Instrument Inc, USA) at 570 nm.

2.3. Cell cycle analysis

The cells (80% confluence) were serum-starved for 24 h to synchronize them in the same phase of the cell cycle, and then were treated with wogonin for 24 h. The cells were collected, washed twice with cold PBS, and centrifuged. The pellet was fixed in 70% (v/v) ethanol for 1 h at 4 °C.The cells were washed once with PBS and added RNaseA (0.1 mg/ml) in PBS (PH7.4) for 30 min at 37 °C. At last, the cells were resuspended in the dark in a cold PI solution (50 μg/ml) for 30 min at 4 °C.The foregoing experiment was accomplished by using cell cycle Detection Kit (KeyGEN, NanJing, China). Cells were analyzed by flow cytometry (Epicsxl, BECKMAN, USA), and the DNA curve fitting program Modfit LT (Becton, Dickinson).

2.4. Apoptosis assay

Apoptotic rates were determined by flow cytometry analysis using commercially available annexin V-fluorescence isothiocyanate (FITC)/propidium iodide (PI) kit (KeyGEN, Nanjing, China). The cells were exposed to wogonin alone, or combined wogonin with one of the inhibitors, either Z-DEVD-fmk, LY294002, or PD98059 at indicated concentration for 24 h. Later, the cells were collected, washed twice in cold PBS, and resuspended in PBS at room temperature. A suspension of 100 μl was taken and incubated with 5 μl propidium iodine (20 μg/ml) and 5 μl annexin V-FITC in the dark for 30 min at room temperature. Finally, 400 μl of cold PBS was added to each tube and analyzed by flow cytometry (Epicsxl, BECKMAN, USA).

2.5. RNA extraction and RT-PCR

The MCF-7 cells were collected after treated with various conditions of wogonin for 48 h. Total RNA was extracted by an Isogen RNA kit (Gibco-BRL, USA) according to the manufacture's manual. Briefly, 2 μg RNA was used to reverse mRNA into cDNA at 37 °C for 30 min with ologo(dT)18 primer and M-MLV (Promega, USA). The primer sequences for PCR were as follows: 5′-TG TGTGTGGAGAGCGTCAACC-3′ and 5′-TTCAGAGACAGCCAGGAGAAATC-3′ used for Bcl-2; 5′-TCAGGATGCGTC-CACCAA GAA-3′ and 5′-TCCCGGAGGAAGTCCAATGTC-3′ for Bax; 5′-CGACGTTGC CCCCTGCCTG-3′ and 5′-AAGGAAAGCGCAACCGGACGA-3′ for survivin; 5′-AGCG ATGGTCTGGCC CCTCC-3′ and 5′-GCGCCGGTCTC TCCCAGGA-3′ for p53; and 5′-ATTCAACGGCACAGTCAAGG-3′ and 5′-GCAGAAGGGGCGGAGATGA-3′ for glyceraldehyde-3-phosphate dehydrogenase internal control. Amplification was done at the temperature of 94 °C for 5 min 25 cycles of 94 °C for 30 s, 58 °C for 30 s and 72 °C for 45 s, and 72 °C for 10 min. The PCR products were separated on 1.2% agarose gel and visualized by SYBRGreenI staining. The DNA bands were analyzed with Quantity One software (Bio Rad, USA).

2.6. Western blotting

A total of 1×10^6 cells were collected and lysed for 15 min in ice-cold lyses buffer (29 mM Tris–HCl, pH 7.4, 137 Mm NaCl, 10% [w/v] glycerin, 1%[v/v]) Triton X-100, 2 mM EDTA, 1 mM PMSF). After removing the cell debris by centrifugation at 16,000 *g* for 15 min, equal amounts of proteins were separated on a 12% SDS-PAGE, transferred electrophoretically (Bio-Rad, USA) onto a nitrocellulose membrane (KeyGEN, Nanjing, China) and blocked

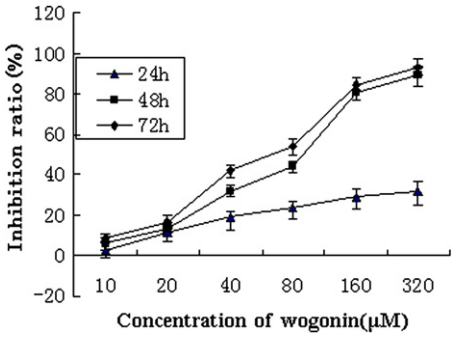
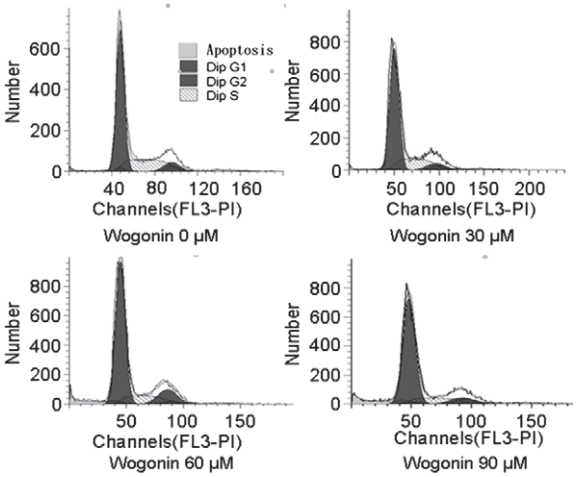


Fig. 1. Inhibition effect of wogonin on growth of MCF-7 cells. Cells were treated with various concentrations of wogonin for 24 h, 48 h and 72 h. The inhibition ratio was calculated from the loss of cell viability in cultures.

a



b

The results of cell cycle analysis。 *p<0.05 *vs* control group, n=3

Wogonin (μM)	G_0/G_1 (%)	S (%)	G2/M (%)	Apoptosis (%)
Control	64.84±2.5	26.74±1.4	8.42±1.2	2.19±0.21
30	68.68±3.1	23.65±2.1	7.66±1.3	5.04±0.56
60	69.82±2.6	15.72±1.6	14.46±1.1	8.52±0.74*
90	75.73±2.8*	15.25±1.7*	9.02±1.1	10.42±1.10*

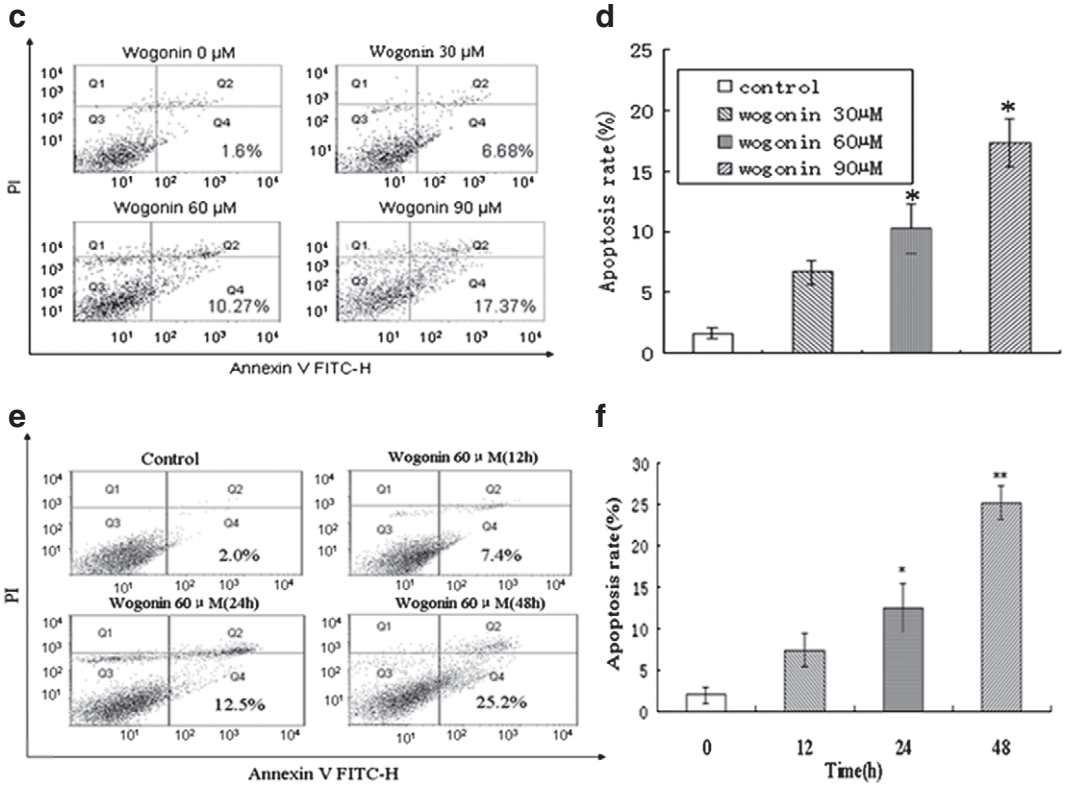


Fig. 2. Effect of wogonin on cell cycle and apoptosis in MCF-7 cells. (a) The cell cycle was assessed by flow cytometry. MCF-7 cells were collected and stained with PI after treated with various concentrations of wogonin for 24 h. (b) The results of cell cycle analysis. (c) Wogonin caused strong apoptosis in MCF-7 cells after treated with various concentrations of wogonin for 24 h. The cells were collected and stained with annexin V-FITC/PI. (d) The quantitative results of flow cytometry analysis. *p<0.05 *vs* control group, n=3. (e) Wogonin induced cell apoptosis after treated various times at 60 μmol/l. (f) The quantitative results of flow cytometry analysis. **p<0.01,*p<0.05 *vs* control group, n=3.

with 5% non-fat milk powder (w/v) in tris-buffer saline Tween-20 (TBST, 10 mM Tris, 100 mM NaCl, and 0.1% Tween-20) for 2 h at room temperature. The membranes were incubated with primary anti-human rabbit polyclonal antibodys (1:500) overnight at 4 °C, and with anti-β-actin rabbit polyclonal antibody (1:500) as a control. After washing with TBST five times, HRP-conjugated anti-rabbit secondary antibody (1:1000) were added and incubated at 37 °C for 1 h before another five-time washing. Labeled bands were developed with enhanced chemiluminescence detection system (Amersham Pharmacia, UK) and photographed with a Molecular Imager Gel Doc XR system (Bio-Rad, USA). Protein levels were quantified by density analysis using Quantity One software (Bio-Rad, USA). Relative protein expression levels were deduced from the ratio of the mean values of each band to that of β-actin.

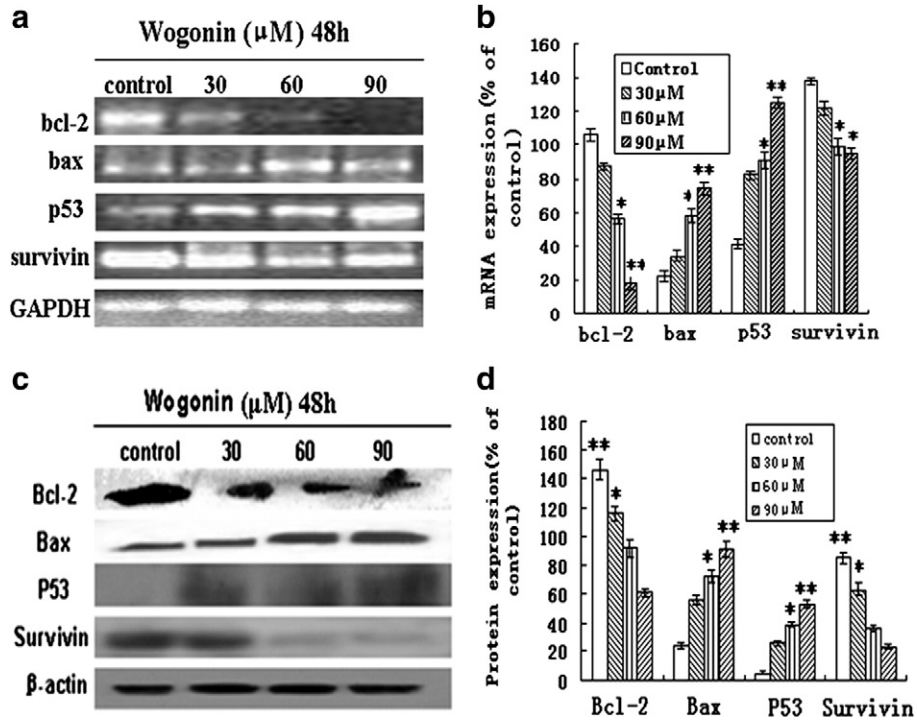


Fig. 3. Wogonin affects the mRNA and protein expression of anti- and pro-apoptosis proteins in MCF-7 cells. (a) The mRNA expression of Bcl-2, Bax, p53, survivin after different doses of wogonin treatment. (b) The quantitative results of each mRNA expression. The data are expressed as mean ± SD from three independent experiments. $^{*}p<0.05$, $^{**}p<0.01$ *vs* control group. (c) The protein expression of Bcl-2, Bax, p53, survivin after different doses of wogonin treatment. (d) The quantitative results of each protein expression. The data are expressed as mean ± SD from three independent experiments. $^{*}p<0.05$, $^{**}p<0.01$ *vs* control group.

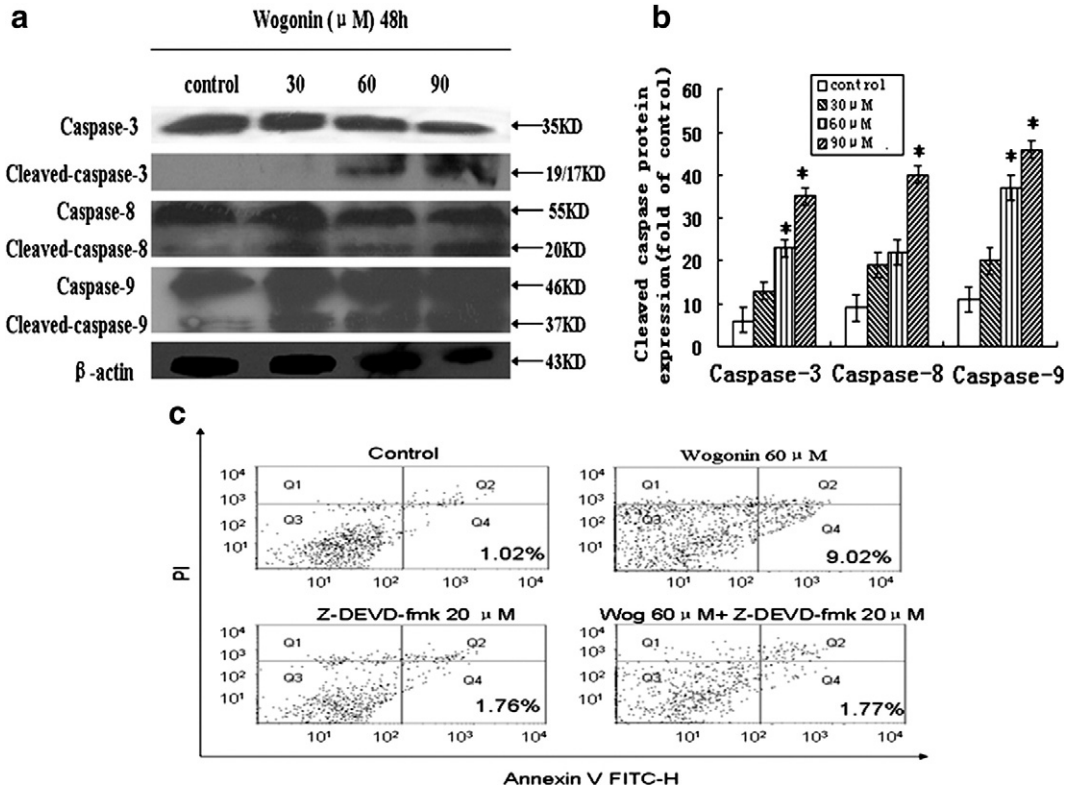
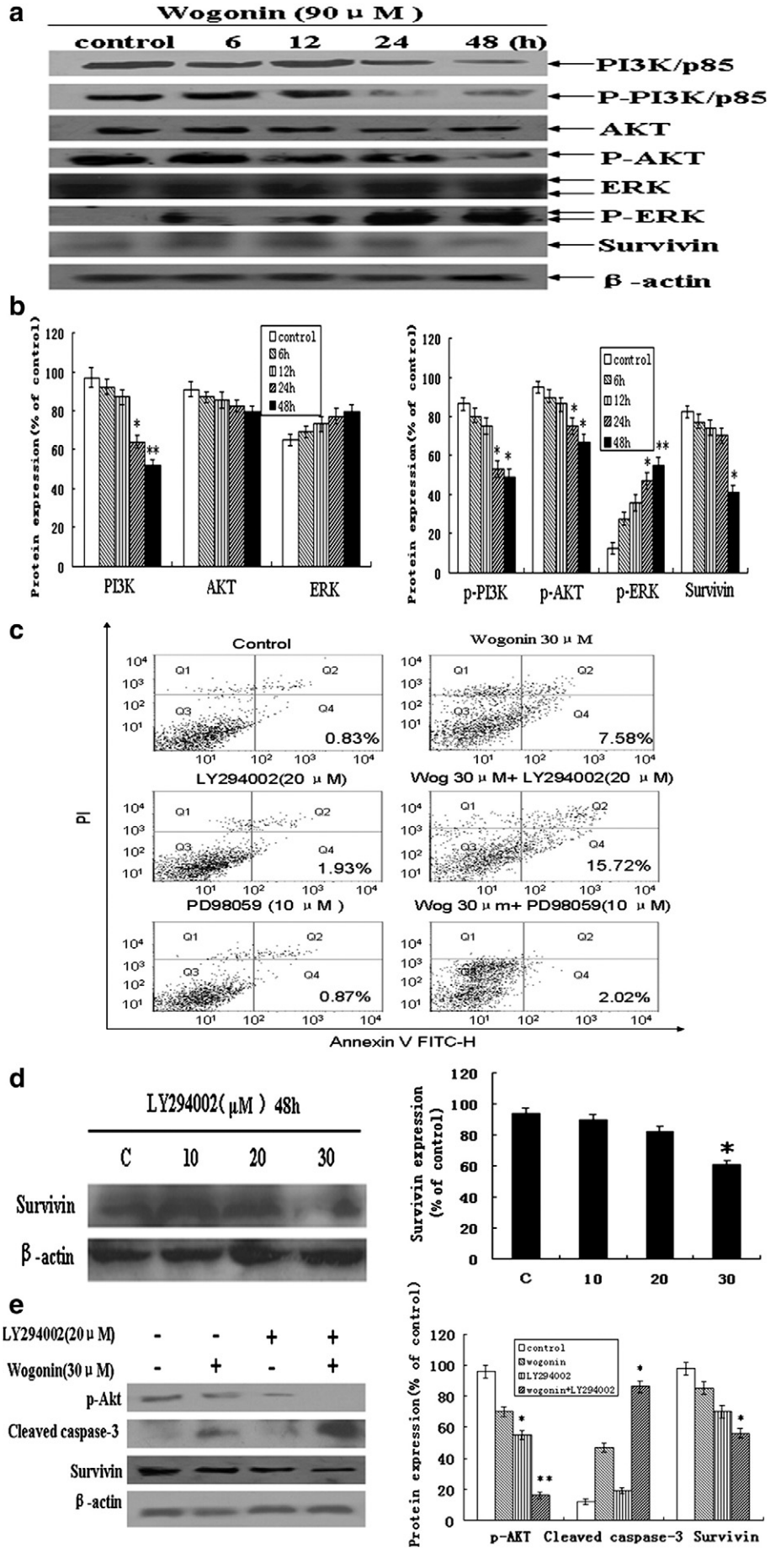


Fig. 4. Wogonin treatment induced caspases activation in MCF-7 cells. (a) The protein expression of caspase-3, caspase-8, caspase-9 after different doses of wogonin treatment for 48 h. The total cells were collected, and the lysates were subjected to western blotting with specific antibodies against caspase-3, caspase-8, caspase-9 respectively. β-actin was used as a loading control. (b) The quantitative analysis of cleavage of caspases after treatment with wogonin. Significant differences between control and wogonin-treated group analyzed by Dunnett's test are indicated by asterisks. The data are expressed as mean ± SD from three independent experiments. $^{*}p<0.05$ *vs* control group. (c) Caspases-3 specific inhibitor Z-DEVD-fmk inhibited the apoptosis induced by wogonin. Each group cells were treated with annexin V-FITC/PI and analyzed by flow cytometry.


a
Wogonin (90 μ M)
control 6 12 24 48 (h)
PI3K/p85
P-PI3K/p85
AKT
P-AKT
ERK
P-ERK
Survivin
β -actin
b
Protein expression(% of control)
control
6h
12h
24h
48h
PI3K
AKT
ERK
p-PI3K
p-AKT
p-ERK
Survivin
c
Control
Wogonin 30 μ M
LY294002(20 μ M)
Wog 30 μ M+ LY294002(20 μ M)
PD98059 (10 μ M)
Wog 30 μ m+ PD98059(10 μ M)
0.83%
7.58%
1.93%
15.72%
0.87%
2.02%
PI
Annexin V FITC-H
d
LY294002(μM) 48h
C 10 20 30
Survivin
β -actin
Survivin expression (% of control)
e
LY294002(20 μ M)
Wogonin(30 μ M)
p-Akt
Cleaved caspase-3
Survivin
β -actin
control
wogonin
LY294002
wogonin+LY294002
p-AKT Cleaved caspase-3 Survivin

3. Results

3.1. Wogonin inhibited the proliferation and induced the apoptosis of MCF-7 cells

Previous studies showed that wogonin had strong inhibitory effect against a series of cancer cell lines [4,24]. In this study, we first examined whether wogonin could inhibit the proliferation of MCF-7 cells. As shown in Fig. 1, wogonin inhibited the growth of MCF-7 cells in dose- and time-dependent manners. The median inhibition concentration (IC50) values for 48 h, and 72 h were 92.12 ± 3.47 μM and 71.32 ± 2.74 μM, respectively. To evaluate whether wogonin-induced inhibition of cell proliferation was due to cell cycle arrest and apoptotic death, we first examined the cell distribution in different cell cycles. As shown in Fig. 2a and b, cells in G_0/G_1 were significantly increased, and cells in the S phase were decreased by wogonin treatment. We next examined the effect of wogonin on apoptotic death of MCF-7 cells by flow cytometry analysis. Fig. 2c and d shows the percentages of apoptotic cell increase in a concentration-dependent manner. Wogonin induced a significant increase in the early and late apoptotic cells at the dose of 90 μM. The total percentage of the apoptotic cells was 17.37%. At the same time, we also examined if the apoptosis was induced by wogonin in a time-dependent manner. Fig. 2e and f shows as the time increased, the apoptotic rate increased significantly. All the data provided evidence for the induced apoptosis in wogonin-treated MCF-7 cells.

3.2. Modulation of apoptosis-related gene after treated with wogonin

The Bcl-2 and IAP protein families play regulatory roles in balancing of the anti- and pro-apoptotic processes and integrate a wide array of diverse upstream survival and death signals to determine the fate of the cells [19]. To examine the role of the Bcl-2 family in wogonin-mediated apoptosis, we analyzed the expression of anti-apoptotic Bcl-2, survivin and pro-apoptotic Bax and p53 by RT-PCR and western blotting. As shown in Fig. 3, the mRNA (Fig. 3a and b) and protein (Fig. 3c and d) levels of Bax were increased at 48 h in cells treated with 90 μM wogonin, whereas both levels of Bcl-2 decreased significantly. The Bax/Bcl-2 ratio was evidently higher than that of control at the concentration of 90 μmol/l wogonin. Treatment with wogonin also decreased the survivin mRNA and protein in a concentration-dependent manner. At the same time, we also found that oth levels of mRNA and protein of p53 significantly increased in the group receiving 90 μM wogonin.

3.3. Effect of wogonin on caspases

It has been reported that caspases are very important regulators of apoptosis [25]. In this study, we investigated the effect of wogonin on caspases. As shown in Fig. 4a and b, treatment of the cells with wogonin resulted in induction of cleaved form of caspases-3, -9 and -8 in a concentration-dependent manner. To confirm whether wogonin-induced apoptosis was caspase-3-dependent, Z-DEVD-fmk, a caspases-3 inhibitor, was used to inhibit the activation of caspases-3. Wogonin significantly increased the activity of caspase-3, and flow cytometry analysis indicated that Z-DEVD-fmk alone did not affect cell apoptosis, but strongly inhibited wogonin-induced apoptosis in MCF-7 cells. As shown in Fig. 4c, the apoptosis rate decreased to 1.77% as compared with that of the treatment of wogonin.

3.4. Effect of wogonin on PI3K/Akt and MAPK/ERK signal pathways

In order to confirm the specific apoptosis mechanism, we examined the effect of wogonin on the PI3K/Akt and MAPK/ERK signal pathways. Western blotting analysis showed that PI3K/Akt phosphorylated forms (p-PI3K/p-Akt) decreased after wogonin treatment for 24 h and further suppressed after 48 h treatment. The total PI3K protein levels decreased, while Akt protein remained constant during the course of wogonin treatment. The expression of survivin was significantly decreased after treatment for 48 h. The activation of ERK indicated by phosphorylated form p-ERK after treatment with wogonin was observed (Fig. 5a and b). To determine whether wogonin-induced apoptosis relates to PI3K/Akt signal pathway, the effect of LY294002, a PI3K inhibitor, on apoptosis was evaluated. Flow cytometry analysis indicated that the combination treatment of wogonin and LY294002 promoted the inhibitory effect; PD98059, an ERK specific inhibitor, significantly attenuated wogonin-induced apoptosis in MCF-7 cells (Fig. 5c). Western blotting results showed that the cleaved caspase-3 obviously increased in the combined treatment of wogonin and LY294002 (Fig. 5e). The previous research indicated wogonin could suppress survivin protein. To determine whether PI3K/Akt signal pathway is involved in regulating survivin, we investigated the effect of signal inhibitor on the expression of survivin. It was found that LY294002 suppressed the expression of survivin protein (Fig. 5d). Further study showed that wogonin could suppress the levels of p-PI3K, p-Akt, and survivin. Quantitative analyses suggested that LY294002 synergistically enhanced the suppression effect of wogonin on survivin level (Fig. 5e).

4. Discussion

Recently, traditional Chinese medicines have been researched as a new source of anti-cancer drugs [26]. So far, much effort has been devoted to search for agents that can significantly induce the apoptosis of cancer cells. Maximizing efficacy with minimizing side effects has become a major goal in the discovery of apoptosis inducers [27]. Many *in vivo* and *in vitro* studies have suggested that plant flavonoids possess potent anti-tumor and anti-inflammatory properties [28]. Wogonin, the principal active component of Scutellaria baicalensis Georgi, has attracted our attention because of its potent anti-tumor activity and low toxicity [29].

In this study, we firstly investigated whether wogonin induced apoptosis in MCF-7 cells. It was demonstrated that wogonin could time- and dose-dependently inhibit cell proliferation. Flow cytometry analysis indicated wogonin could induce cell apoptosis effectively in a time- and dose-dependent manner. Meanwhile, in response to the wogonin treatment, cell cycle was arrested in the G0/G1 phase. The results suggested that the wogonin-induced inhibition of cell proliferation involved cell cycle arrest and apoptosis.

There are two major apoptosis signaling pathways, mitochondrial and death receptor pathway. However Bcl-2 is an apoptosis inhibitor and Bax is an apoptotic protein. The anti-apoptotic protein Bcl-2 mainly inhibits the mitochondrial pathways. A change in the ratio of Bax/Bcl-2 is recognized to initiate caspase signaling [30,31]. Up-regulated P53 directly

Fig. 5. Wogonin induced apoptosis involving both PI3K/Akt/survivin and MAPK/ERK pathways in MCF-7 cells. (a) The MCF-7 cells were treated with 90 μM wogonin for 6, 12, 24 and 48 h, respectively. Meanwhile PI3K, p-PI3K, Akt, p-Akt, ERK, p-ERK and survivin were detected by western blotting analysis. (b) The quantitative results of related proteins after treatment with wogonin. The data are expressed as mean ± SD from three independent experiments. $^{*}p<0.05$, $^{**}p<0.01$ *vs* control group. (c) Effects of kinase inhibitors on wogonin-induced apoptosis in MCF-7 cells. The apoptosis rates were detected by flow cytometry after treat with wogonin, LY294002, wogonin + LY294002, PD98059, and wogonin + PD98059, respectively for 24 h.(d) PI3K inhibitor LY294002 reduced the expression of survivin. $^{*}p<0.05$ *vs* control group. (e) PI3K inhibitor LY294002 enhanced wogonin-induced reduction of p-Akt and survivin expression, and enhanced wogonin-induced activation of caspases-3. The MCF-7 cells were collected after the treatment of wogonin (30 μM), LY294002 (20 μM), or a combination of wogonin (30 μM) and LY294002 (20 μM). Lysates were subjected to western blotting with specific antibodies against p-Akt, cleaved caspase-3 and survivin respectively. The results analyzed by Dunnett's test are indicated by asterisks: $^{*}p<0.05$, $^{**}p<0.01$ *vs* control group, n = 3.

promotes Bax expression that destroys the integrity of the mitochondria to induce apoptosis [32]. Survivin has been known as a multifunctional protein that inhibits apoptosis and cell division [33]. Survivin could directly block the activating of effector caspase-3 and caspase-7, which act on a common down-stream of both apoptosis signaling pathways [34]. In this study, it was found that wogonin down-regulated the expression of Bcl-2 and survivin, and increased the expression of Bax and p53.

Caspases could be structurally divided on the basis of the presence or absence of an N-terminal prodomain: caspases containing a long prodomain are the first to be activated in response to apoptotic stimuli. The activated caspases would destroy the cellular architecture and ultimately resulting in cell death [35]. In this study, a significant increase on the expression and activity of caspases-3, -8, -9 were observed after wogonin treatment. Caspase-3 is the most important member among the caspases. A specific caspase-3 inhibitor z-DEVD-fmk was able to block wogonin-induced apoptosis, suggesting that caspase-3 played a critical role in wogonin-induced apoptosis. Taking all those into account, it indicated that the down-regulation of Bcl-2 and survivin might also lead to activation of caspase-3 and induced apoptosis in wogonin-mediated MCF-7 cells.

The PI3K/Akt is one of the most important signaling pathways in regulating cell proliferation, growth and apoptosis. Akt is a major downstream target of PI3K [36]. The PI3K/Akt signaling pathway regulates the progression and development of various cancers by increasing the activity of the anti-apoptotic action of Akt. Phosphorylation of Akt, which is required for suppression of cancer cell apoptosis and tumor progression, is commonly used as readout for the Akt activation [37]. The PI3K/Akt signaling pathway inhibits apoptosis by inactivating important members of the apoptotic cascade, including caspase-9, and pro-apoptotic Bad [38] and survivin [39]. It was suggested that ERK signaling pathway is preferentially activated in response to growth factors, and is generally known to promote cell survival [40]. However, more recent researches have found that the sustained activation of ERK is also involved in the apoptotic process. Robust ERK stimulation has been reported to repress the cell cycle and mediate the apoptosis via the up-regulation of Fas ligand expression [41,42]. Our data indicated that wogonin induced a down-regulation of PI3K/Akt and survivin, and an activation of ERK. Meanwhile, the expression of survivin was significantly decreased after the maximal down-regulation of p-PI3K and p-Akt. Further results showed that PI3K inhibitor LY294002 enhanced wogonin effects on suppression of survivin and increase of an activated form of caspases cleaved caspase-3. Our data suggested that the inhibition of survivin was mediated by inactivating PI3K/Akt in wogonin-treated MCF-7 cells. Moreover, wogonin-induced apoptosis was attenuated after the inhibition of MAPK/ERK pathway by PD98059.

In summary, this study demonstrated that wogonin induced apoptosis in human breast cancer MCF-7 cells, which was associated with the up-regulation of Bax, P53 and caspase-3 activation, down-regulation of Bcl-2 and survivin. Moreover, both PI3K/Akt and MAPK/ERK pathways played an important role on wogonin-induced apoptosis of MCF-7 cells. The inhibition of PI3K/Akt pathway by wogonin may be a means of down-regulating survivin expression, a downstream target of the PI3K/Akt pathway. Our data demonstrated the molecular mechanism of the anti-cancer activity of wogonin.

Acknowledgements

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