Original Article

Antitumor effects of concanavalin A and *Sophora flavescens* lectin *in vitro* and *in vivo*

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Aim: Proteins with legume lectin domains are known to possess a wide range of biological functions. Here, the antitumor effects of two representative legume lectins, concanavalin A (ConA) and *Sophora flavescens* lectin (SFL), on human breast carcinoma cells were investigated *in vitro* and *in vivo*.

Methods: Human breast carcinoma MCF-7 cells and human normal mammary epithelial MCF-10A cells were examined. Cell viability was detected using WST-1 and CCK-8 assays. Cell apoptosis was analyzed with Hoechst 33258 staining. Cell cycle was investigated using flow cytometry. The expression of relevant proteins was measured using Western blotting. Breast carcinoma MCF-7 bearing nude mice were used to study the antitumor effects *in vivo*. The mice were injected with ConA (40 mg/kg, ip) and SFL (55 mg/kg, ip) daily for 14 d.

Results: ConA and SFL inhibited the growth of MCF-7 cells in dose- and time-dependent manners (IC₅₀ values were 15 and 20 µg/mL, respectively). Both ConA and SFL induced apoptotic morphology in MCF-7 cells without affecting MCF-10A cells. ConA and SFL dose-dependently increased the sub-G₁ proportion in MCF-7 cells, while SFL also triggered the G₂/M phase cell cycle arrest. Both ConA and SFL dose-dependently increased the activities of caspase-3 and caspase-9 and release of cytochrome c from mitochondria into cytoplasm, up-regulated Bax and Bid, and down-regulated Bcl-2 and Bcl-X_L in MCF-7 cells. ConA reduced NF-κB, ERK, and JNK levels, and increased p53 and p21 levels, while SFL caused similar changes in NF-κB, ERK, p53, and p21 levels, but did not affect JNK expression. Administration of ConA and SFL significantly decreased the subcutaneous tumor mass volume and weight in MCF-7 bearing nude mice.

Conclusion: ConA and SFL exert anti-tumor actions against human breast carcinoma MCF-7 cells both *in vitro* and *in vivo*.

Keywords: legume lectin; concanavalin A; *Sophora flavescens* lectin; anticancer drug; breast carcinoma; MCF-7 cell; apoptosis, cell cycle; breast carcinoma xenograft nude mouse

Acta Pharmacologica Sinica (2014) 35: 248–256; doi: 10.1038/aps.2013.151; published online 23 Dec 2013

Introduction

Plant lectins are a class of highly diverse non-immune origin proteins that contain at least one non-catalytic domain for selective recognition and reversible cell agglutination. They contain at least one non-catalytic domain, which enables them to selectively recognize and reversibly bind to specific free sugars or glycans that are presented on glycoproteins and glycolipids without altering carbohydrate structure^[1]. Plant lectins have been divided into 12 families based on their tertiary structures and evolutionary statuses: *Amaranthin*, *Agaricus bisporus* agglutinin, Cyanovirin, Chitinase-related agglutinin,

Euonymus europaeus agglutinin, *Galanthus nivalis* agglutinin (GNA), hevein, jacalin, lysin motif, proteins with legume lectin domains, nictaba, and ricin-B families. Proteins with legume lectin domains have multiple significant biological functions such as anti-fungal, anti-viral, and most notably anti-tumor activities, which have given them much attention compared with the other plant lectins^[2,3].

Concanavalin A(ConA) is a long-studied representative legume lectin that reportedly diversifies human cancer cell death by targeting programmed cell death (PCD). PCD refers to apoptosis and autophagy, which are evolutionary conserved processes for maintaining homeostasis and eliminating harmful cells^[4]. Previous studies reported that ConA induced apoptosis in human melanoma A375 cells and murine macrophage PU5-1.8 cells. Moreover, ConA induced autophagic cell death in HeLa cells^[5–7]. Therefore, ConA bears notable

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Received 2013-07-15 Accepted 2013-09-18

apoptosis- and autophagy-inducing properties, which make it a potential anti-neoplastic agent for future cancer therapeutics.

Sophora flavescens lectin (SFL) is a mannose-binding lectin that was isolated from *Sophora flavescens* Ait roots, which have been used as a traditional Chinese medicine for thousands of years. SFL is also a member of the legume lectin family and has been considered to be a model system in which to study the molecular basis of protein-carbohydrate interactions for several decades. Previous findings have demonstrated that SFL has hemagglutinating and anti-fungal activities. Importantly, SFL can induce apoptosis in HeLa cells, thus functioning as an anti-tumor agent through a typical caspase-dependent pathway^[8-10].

The mechanisms by which ConA and SFL induce cancer cell death are only rudimentarily understood. In the current study, we report that ConA and SFL induced apoptotic cell death in MCF-7 cells. ConA induced apoptosis via NF- κ B, ERK and JNK down-regulation and p53 up-regulation in human breast carcinoma MCF-7 cells. SFL reduced NF- κ B and ERK expression and increased p53 and p21 expression. This shows that SFL initiates a G₂/M phase cell-cycle arrest via up-regulating p21 and down-regulating CDK1 and CDK2 expression. Both ConA and SFL only selectively induced MCF-7 cell death but displayed no significant cytotoxicity toward normal human mammary epithelial MCF-10A cells. Furthermore, anti-tumor effects of ConA and SFL were detected, and both lectins decreased subcutaneous tumor volume and weight *in vivo*. Together, these results may pave new roads for exploring the complicated molecular mechanisms of ConA and SFL-induced cancer cell apoptosis in future cancer therapeutics.

Materials and methods

Reagents

ConA was purchased from Sigma Chemicals (St Louis, MO, USA), and SFL was purified and maintained by our lab. Human breast adenocarcinoma MCF-7 cells were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). Fetal bovine serum (FBS) was purchased from Gibco BRL (Grand Island, NY, USA). MTT, z-VAD-fmk (pan-caspase inhibitor), z-DEVD-fmk (caspase-3 inhibitor) and z-LEHD-fmk (caspase-9 inhibitor) were purchased from Sigma Chemicals (St Louis, MO, USA).

Cell culture

Breast adenocarcinoma MCF-7 cells were cultured in RPMI-1640 media containing 10% FBS, 100 mg/mL streptomycin, 100 U/mL penicillin, and 0.03% *L*-glutamine and were maintained at 37°C with 5% CO₂ in a humidified atmosphere. MCF-10A cells were cultured in DMEM/F-12 media (Invitrogen) supplemented with 10% horse serum, 2 mmol/L glutamine, 100 mg/mL streptomycin (Invitrogen), 100 U/mL penicillin (Invitrogen), 0.25 μ g/mL ampicillin B, 100 ng/mL cholera toxin, 20 ng/mL epidermal growth factor (Upstate Biotechnology, Lake Placid, NY, USA), 0.5 μ g/mL hydrocortisone (Calbiochem) and 10 μ g/mL insulin. Both MCF-7 and MCF-10A cells were cultured at 37°C with 5% CO₂ in a humidified

atmosphere. To form an *in vivo* cancer model, the cultured human breast adenocarcinoma MCF-7 cell suspension (5.0×10^6 cells) was inoculated under the skin on the back of a 3-month-old female nude mouse.

Cell proliferation and viability assays

WST-1 and cell counting kit-8 (CCK-8) assays were used to determine the effects of different ConA and SFL concentrations on MCF-7 cell viability after 12, 24, 36, and 48 h of treatment. WST-1 assays (BioVision Research Products, Milpitas, CA, USA) and CCK-8 assays (Dojindo Molecular Technologies Inc, Kumamoto, Japan) were performed according to the manufacturer's instructions.

To determine the effects of ConA and SFL on MCF-7 cell proliferation, 30% confluent MCF-7 cells were incubated in RPMI-1640 media with different FBS concentrations (0%, 5%, and 10%) in the presence or absence of 15 μ g/mL ConA and 20 μ g/mL SFL for 48 h. After incubation, cultures were trypsinized, and the cell numbers were determined with an Invitrogen CountessH Automated Cell Counter (Carlsbad, CA, USA).

Observed cell morphology changes

MCF-7 and MCF-10A cells were seeded into 96-well plates and cultured for 24 h. Control groups were treated with PBS, and ConA groups were treated with 50 and 100 μ g/mL ConA, while SFL groups were treated with 50 and 100 μ g/mL ConA SFL. After another 24 h incubation with PBS, ConA and SFL, MCF-7 and MCF-10A cell morphology was observed using phase contrast microscopy (Leica, Wetzlar, Germany)^[11]. Hoechst 33258 staining was applied to further detect MCF-7 cells apoptotic nuclear morphology changes. Cells were fixed with 4% paraformaldehyde for 30 min at room temperature after 24 h incubation with or without 50 and 100 μ g/mL ConA and 50 and 100 μ g/mL SFL, and the cells were then washed twice with PBS. Hoechst 33258 (5 μ g/mL) was added, and the cells were stained for 15 min. Then, the cells were washed and analyzed immediately with fluorescence microscopy (Olympus, Tokyo, Japan).

Cell cycle measurement and sub-G₁ cells

MCF-7 and MCF-10A cells were treated with increasing ConA or SFL concentrations (both from 0 to 100 μ g/mL) or PBS at 37°C for 24 h and then were harvested. FACSscan flow cytometry assays were performed as previously described^[12]. The percentages of the cells at different cell cycle phases or those that were undergoing apoptosis were evaluated by Calibur FACSscan flow cytometry (Becton Dickinson, Franklin Lakes, NJ, USA).

Caspase assay

Apoptosis was assessed by measuring caspase-3 activity using the caspase-3 activity kit (Beyotime Institute of Biotechnology, Haimen, China) following the manufacturer's instructions. Cell lysates were prepared after treatment with various ConA and SFL concentrations for 24 h. In total, 10 μ L cell lysate

combined with 80 μ L reaction buffer [1% NP-40, 20 mmol/L Tris-HCl (pH 7.5), 137 mmol/L NaCl and 10% glycerol] along with 10 μ L caspase-3 substrate (Ac-DEVD-pNA, 2 mmol/L) was incubated in 96-well microtiter plates at 37°C for 4 h. Samples were then measured by ELISA, and the absorbance was read at 405 nm.

Western blot analysis

MCF-7 cells were treated with increasing ConA and SFL concentrations (both from 0 to 100 μ g/mL) for 24 h, and both adherent and floating cells were collected. The cell pellets were resuspended with lysis buffer and lysed at 4°C for 1 h. The lysis buffer consisted of 50 mmol/L Hepes, pH 7.4, 1% Triton X-100, 2 mmol/L sodium orthovanadate, 100 mmol/L sodium fluoride, 1 mmol/L acetic acid, 1 mmol/L PMSF, 10 mg/L aprotinin, and 10 mg/L leupeptin (Sigma, MO, USA). After 12 000 $\times$ g centrifugation for 15 min, the supernatant protein content was determined using the Bio-Rad DC protein assay (Bio-Rad Laboratories, Hercules, CA, USA). Equal amounts of total protein were separated by 12% SDS-PAGE and transferred to nitrocellulose membranes, and the membranes were soaked in blocking buffer (5% skim milk). The following antibodies were purchased from Santa Cruz Biotech: caspase 3 (#sc-7148), caspase 9 (#sc-8355), cytochrome c (#sc-7159), Bax (#sc-493), Bid (#sc-6538), Bcl-2 (#sc-492), Bcl-X_L (#sc-8392), NF- κ B (#sc-114), ERK (#sc-154), p53 (#sc-126), and β -actin (#sc-47778). Antibodies including cdk1 (ab18), cdk2 (ab6538), p21 (ab7960), and JNK (ab4821) were from Abcam. Proteins were visualized using horseradish peroxidase (HRP)-conjugated anti-rabbit or anti-mouse IgG and 3,3'-diaminobenzidine tetrahydrochloride (DAB) as the HRP substrate.

In vivo study design

In total, 40 3-month-old female nude mice were randomly divided into four groups: the blank control group (administered PBS after MCF-7 cell injection), 40 mg/kg ConA group, 55 mg/kg SFL group and the positive control group (administered cisplatin after MCF-7 cell injection). The mice were injected with PBS, ConA, SFL and cisplatin intraperitoneally, and the therapy lasted for two weeks. Animal handling was in accordance with the Ethics Committee of Sichuan University, and all of the animals were kept in 12-h light/dark cycles with free access to water and food, which is consistent with Sichuan University IVC requirements.

Relative tumor volume, survival rate, inhibitory rate and body weight determination

Tumor volume was determined from caliper measurements according to the formula, $T_{vol} = \text{length} \times \text{width} \times \text{depth} \times 0.5$. Tumor volume inhibitory rate = $(V_{\text{control}} - V_t) / V_{\text{control}} \times 100\%$. After 14 d of treatment, the mice were killed by cervical dislocation, and the subcutaneous tumor mass was determined. Tumor weight inhibitory rate = $(W_{\text{control}} - W_t) / W_{\text{control}} \times 100\%$.

Statistical analysis

All of the results presented here were confirmed in at least

three independent experiments. These data were expressed as the mean $\pm$ SEM. Statistical comparisons were made by Student's *t*-test and two-way ANOVA. $P < 0.05$ was considered to be statistically significant.

Results

Cytotoxic effects of ConA and SFL on MCF-7 cells

Both ConA and SFL induced MCF-7 cell death in a dose- and time-dependent manner, and 0 to 100 μ g/mL ConA and SFL exerted potent inhibitory effects on MCF-7 cell growth (Figure 1A). The WST-1 (Figure 1Ba) assay demonstrated that after 24 h incubation with 18 μ g/mL ConA and 27 μ g/mL SFL, the MCF-7 cell inhibitory rate reached nearly 50%. While the ConA and SFL IC₅₀ values detected by the CCK-8 assay were 15 and 20 μ g/mL, respectively (Figure 1Bb). All of these results indicated that ConA has a more potent growth inhibitory activity toward MCF-7 cells than SFL.

Observation of cellular morphology

Marked apoptotic morphological changes such as membrane blebbing, cell volume reduction and rounding were obvious in ConA- and SFL-treated MCF-7 cells by phase contrast microscopy (Figure 2A). Hoechst 33258 staining demonstrated that ConA- and SFL-treated MCF-7 cells presented manifest fragmented nuclear DNA, while in the control group nuclear DNA was round and homogeneously stained (Figure 2B). These results suggested that both ConA and SFL induced cell death in MCF-7 cells. However, ConA and SFL did not result in apoptotic morphology in the non-cancerous MCF-10A cells (Figure 2C), which were observed under phase contrast microscopy. These results indicated that both ConA and SFL selectively induced MCF-7 apoptosis but could not induce MCF-10A cell apoptosis.

ConA and SFL induced apoptosis in MCF-7 cells

As demonstrated in Figure 3, both ConA (Figure 3A) and SFL (Figure 3B) markedly increased MCF-7 cell sub-G₁ proportions in a dose-dependent manner, indicating that both ConA and SFL induced apoptosis in MCF-7 cells. Moreover, as demonstrated in Figure 3B, SFL also triggered G₂/M phase cell-cycle arrest (control group: 34.37% $\pm$ 1.55%; 12.5 μ g/mL group: 39.1% $\pm$ 1.06%; 25 μ g/mL group: 41.07% $\pm$ 0.9%; 50 μ g/mL group: 42.13% $\pm$ 0.69%; 100 μ g/mL group: 43.23% $\pm$ 1.51%). However, both ConA and SFL did not enhance sub-G₁ proportion enhancement or G₂/M phase cell-cycle arrest in MCF-10A cells (Figure 3C and 3D).

ConA and SFL induced apoptosis in a mitochondrial-mediated, caspase-dependent manner

As demonstrated in Figure 4A, ConA (Figure 4Aa) and SFL (Figure 4Ab) significantly enhanced caspase-3 activity in a dose-dependent manner. Caspase-3 activity was also significantly inhibited after exposure to 200 μ mol/L caspase-3 inhibitor z-DEVD-fmk (Figure 4Ac), indicating both ConA and SFL induced apoptosis occurs by activation of common apoptotic proteins such as caspase-3. Moreover, Western blot

Figure 1. The proliferative effects of ConA and SFL on MCF-7 cells. (A) MCF-7 cells were supplied with increasing amounts of serum for 24 h followed by treatment with PBS, 15 $\mu\text{g/mL}$ ConA and 20 $\mu\text{g/mL}$ SFL, and then the cell numbers were counted. (B) The MCF-7 cell inhibitory ratios with different ConA and SFL concentrations were detected with WST-1 (a) and CCK-8 assays (b). Mean $\pm$ SEM. $n=3$. ^b $P<0.05$, ^c $P<0.01$ vs control.

analyses demonstrated that caspase-3 and caspase-9 expression were increased with enhanced concentrations (0, 12.5, 25, 50, and 100 $\mu\text{g/mL}$) after treatment with these two lectins, suggesting that both ConA and SFL increased caspase-3 and caspase-9 activities in a dose-dependent manner (Figure 4B). Mitochondrial cytochrome *c* was also decreased, but cytoplasmic cytochrome *c* was increased, suggesting that cytochrome *c* was released from the mitochondria (Figure 4B) into the cytoplasm. Pro-apoptotic proteins Bax and Bid were up-regulated and anti-apoptotic Bcl-2 and Bcl-X_L were down-regulated after SFL and ConA treatment (Figure 4B). All of these results clearly indicate that both ConA and SFL induced apoptosis in MCF-7 cells in a mitochondrial-mediated, caspase-dependent pathway.

Analyses of cell cycle arrest- and apoptosis-related proteins

Western blot data demonstrated that treatment of MCF-7 cells with ConA resulted in NF- κ B, ERK, and JNK protein level down-regulation and p53 up-regulation (shown in Figure 5A). Because p53 is an important regulator of p21, p21 levels were further detected. As demonstrated in Figure 8B, p21 was also increased with the enhanced p53 concentration after ConA treatment. However, SFL treatment resulted in NF- κ B and ERK down-regulation (demonstrated in Figure 5C), and JNK expression remained the same throughout various time points (data not shown). p53 and p21 expression was up-regulated after SFL interference (demonstrated in Figure 5C). Moreover, because of the SFL-induced G₂/M phase cell cycle arrest, cell cycle-related proteins were detected. As demonstrated in Figure 5B, CDK1 and CDK2 expression was reduced after treat-

ment with various concentrations of SFL, indicating that SFL induced G₂/M phase cell cycle arrest via p53 up-regulation and CDK1 and CDK2 down-regulation.

Tumor volume and body weight detection

Acute toxicity testing indicated that ConA and SFL doses were 40 and 55 mg/kg, respectively. Tumor volumes were determined in three dimensions with vernier calipers, and the volume and weight inhibitory ratios were calculated. After 14 d of ConA and SFL treatment, all of the mice were sacrificed, and subcutaneous tumors were peeled off and weighed. As demonstrated in Table 1, after 14 d of 40 mg/kg ConA and 55 mg/kg SFL treatment, tumor volume decreased to 0.20 ± 0.13 cm³ and 0.31 ± 0.09 cm³, respectively, which was a 67.21% and 49.18% reduction compared with the blank control group, while the cisplatin-treated inhibitory volume ratio reached 88.52%. Meanwhile, tumor weights after treatment with 40 mg/kg ConA and 55 mg/kg SFL decreased to 0.37 ± 0.21 g and

Table 1. Inhibitory of ConA and SFL on MCF-7-bearing mice at the 14th d of treatment ($n=10$).

Group	Volume (cm ³)	Weight (g)	Inhibitory ratio of volume (%)	Inhibitory ratio of weight (%)
Blank control	0.61 ± 0.25	0.86 ± 0.47		
55 mg/kg SFL	0.31 ± 0.09	0.53 ± 0.31	49.18	38.37
40 mg/kg ConA	0.20 ± 0.13	0.37 ± 0.21	67.21	56.98
Positive control	0.07 ± 0.03	0.18 ± 0.09	88.52	79.07

Figure 2. Morphologic observations of ConA and SFL on MCF-7 and MCF-10A cells. (A) MCF-7 cells were treated with PBS or 15 µg/mL ConA and 20 µg/mL SFL for 24 h, and the morphology was detected under phase contrast microscopy and (B) fluorescent microscopy (200×). (C) After treating MCF-10A cells with PBS or 15 µg/mL ConA and 20 µg/mL SFL for 24 h, the morphologic varieties were detected using phase contrast microscopy.

0.53±0.31 g, respectively, which was a reduction of 56.98% and 38.37%, respectively, whereas the positive control group inhibitory ratio was 79.07%. After MCF-7 cell inoculation,

the tumor gradually formed and the mouse body weight gradually increased. Treating the mice with ConA and SFL resulted in gradual body weight reduction (Table 2).

Figure 3. Effects of ConA and SFL on cell cycle progression. MCF-7 cells were treated with various ConA (A) and SFL (B) concentrations for 24 h, and different cell phase percentages were measured by flow cytometry. Cell cycle percentages were represented by a bar diagram (mean±SD, $n=3$). MCF-10A cells were treated with various concentrations of ConA (C) and SFL (D) for 24 h, and different cell phase percentages were measured by flow cytometry and represented by a bar diagram. Mean±SEM. $n=3$. ^b $P<0.05$, ^c $P<0.01$ vs control.

Figure 4. ConA and SFL induce apoptosis through a caspase-mediated mitochondrial pathway. (A) Effects of ConA (a), SFL (b), and z-DEVD-fmk added (c) on caspase-3 activation. (B) Various concentrations of ConA- and SFL enhanced caspase-3 and caspase-9 expression, and cytochrome c was released from mitochondria into the cytosol. Pro-apoptotic proteins Bax and Bid were up-regulated, and anti-apoptotic proteins Bcl-2 and Bcl-X_L were down-regulated. β-Actin was used as an equal loading control. Mean±SEM. $n=3$. ^b $P<0.05$, ^c $P<0.01$ vs control. ^f $P<0.01$ vs ConA or SFL treatment group.

Table 2. Body weight varieties of ConA and SFL on MCF-7-bearing mice ($n=10$).

Group	D 0 (g)	D 3 (g)	D 6 (g)	D 9 (g)	D 12 (g)	D 14 (g)
Blank control	25.78±0.56	25.88±0.36	25.93±0.62	26.04±0.47	26.15±0.49	26.19±0.59
40 mg/kg ConA	25.69±0.64	25.51±0.48	25.38±0.43	25.25±0.70	25.19±0.55	25.04±0.45
55 mg/kg SFL	25.65±0.53	25.47±0.60	25.31±0.59	25.19±0.38	25.06±0.48	24.92±0.55
Positive control	25.75±0.49	25.60±0.65	25.43±0.58	25.26±0.50	24.93±0.65	24.78±0.51

Figure 5. The molecular mechanisms of ConA- and SFL-induced apoptosis and G₂/M phase cell cycle arrest. (A) MCF-7 cells were treated with increasing ConA concentrations, which resulted in NF-κB, ERK, and JNK protein level down-regulation as well as p53 up-regulation. β-Actin was used as an equal loading control. (B) p21 expression after ConA treatment in MCF-7 cells was also detected, and β-Actin was used as an equal loading control. (C) Increased SFL concentrations down-regulated NF-κB and ERK and up-regulated p53 and p21. Additionally, SFL triggered G₂/M phase cell cycle arrest via CDK1 and CDK2 down-regulation. β-Actin was used as an equal loading control.

Discussion

In the past few decades, a great number of plant lectins with *in vivo* and *in vitro* anti-proliferative effects against cancer cells have been well established. Among the 12 lectin families, legume lectins have gained much attention from the scientific community because of their remarkable anti-proliferative activities and potential applications in cancer therapeutics.

ConA was the first reported legume lectin. It can induce mitochondrial apoptosis, p73-Foxo1a-Bim apoptosis and

BNIP-mediated mitochondrial autophagy, eventually causing cancer cell death^[13, 14]. Additionally, ConA induced leukemic cell death and promoted apoptosis with DNA fragmentation, mitochondrial depolarization and increased ROS production^[5]. SFL reportedly induced HeLa cell apoptosis via caspase-dependent pathways, and its molecular mechanisms might involve the death receptor pathway^[10]. Furthermore, subsequent studies demonstrated that other proteins with legume lectin domains possess significant anti-proliferative and apoptosis-inducing activities towards a variety of cancer cell types. For instance, Phaseolus coccineus lectin, a legume lectin family member with specificity towards sialic acid, possessed marked cytotoxicity and induced murine fibrosarcoma L929 cell apoptosis^[15]. A further study reported that French bean agglutinin induced apoptosis *via* the death receptor-mediated pathway in MCF-7 cells^[16].

Both the WST-1 and CCK-8 assays indicated that the ConA IC₅₀ value was lower than SFL, suggesting that ConA has more potent anti-tumor activities towards MCF-7 cells. Both ConA and SFL selectively induced MCF-7 cell death but displayed no significant cytotoxicity toward normal human mammary epithelial MCF-10A cells. ConA- and SFL-induced apoptosis are featured by marked apoptotic morphology including blebbing, nuclear fragmentation and cell volume reduction. Moreover, apoptosis was further evaluated by measuring cell number in the Sub-G₁ region. Both ConA and SFL did not enhance Sub-G₁ proportions or G₂/M phase cell-cycle arrest in MCF-10A cells. Together, all of the abovementioned results indicated that both lectins selectively induce apoptosis in MCF-7 cells but not in MCF-10A cells. In this work, ConA induced apoptosis *via* NF-κB, ERK and JNK down-regulation and p53 and p21 up-regulation in human breast carcinoma MCF-7 cells. p21 expression is tightly controlled by p53. Previous findings have presented that p21 is induced by both p53-dependent and -independent mechanisms following stress and that p21 induction may cause cell cycle arrest^[17]. Although p21 is important in regulating cell cycle arrest, p21 did not initiate cell cycle arrest in ConA-treated MCF-7 cells. We could infer that p21 was only involved in ConA-induced apoptosis. SFL treatment decreased NF-κB and ERK and enhanced p53 and p21 expression. SFL was first reported to trigger G₂/M phase cell-cycle arrest by up-regulating p21 expression and down-regulating CDK1 and CDK2 expression.

Apoptosis is the major type of cell death that controls tumor suicide and can be regulated by numerous molecular signaling pathways. In total, two core pathways (the death receptor

and the mitochondrial pathways) induce apoptosis^[7]. p53 is a key tumor suppressor protein that has numerous functions, and loss of p53 in many cancers causes genomic instability, impaired cell cycle regulation, and apoptosis inhibition. NF- κ B is a well-known nuclear transcription factor that regulates expression of a great number of genes that are involved in apoptosis, tumorigenesis, and inflammation^[18, 19]. p53 induction also reportedly activates NF- κ B, which correlates with the ability of p53 to induce apoptosis. Inhibition or loss of NF- κ B activity abrogated p53-induced apoptosis, indicating that NF- κ B is essential for p53-mediated cell death^[20]. p21 is also known as cyclin-dependent kinase inhibitor 1, and it is induced by both p53-dependent and p53-independent mechanisms. p21 induction may cause cell cycle arrest^[21, 22]. p21 expression is tightly controlled by p53. Moreover, p21 can protect against apoptosis in response to some stimuli such as growth factor deprivation, p53 overexpression or monocytic differentiation^[23]. The mitogen-activated protein kinase (MAPK) family, including ERK and JNK, are involved in various cellular processes, and Ras and Raf are usually upstream of ERK. Both Ras and Raf act as proto-oncogenes by targeting cell proliferation, transformation, and apoptosis. The Ras-Raf-MEK-ERK pathway usually promotes cell survival in cancer^[22].

Here, the *in vivo* effects of ConA and SFL were also detected, and both lectins visibly reduced subcutaneous tumor mass volumes and weights. After 14 d of treatment with the highest SFL (55 mg/kg) dosage, tumor volume and weight decreased nearly 49.18% and 38.37%. The highest ConA dose (40 mg/kg) resulted in a 67.21% and 56.98% decrease in tumor volume and weight, respectively. According to above descriptions, high ConA dosage had similar anti-tumor effects to cisplatin, which is a widely effective anti-tumor agent. These abovementioned findings suggest that lectins can inhibit MCF-7 cell growth *in vivo*.

However, it was not determined whether NF- κ B, ERK, JNK, p53, and p21 proteins were also involved in ConA and SFL-induced apoptosis *in vivo* because of poor experimental techniques and environments. Subsequent intricate molecular mechanisms that have been implicated in ConA- and SFL-induced apoptosis *in vivo* were urgently investigated, but more investigations and preliminary experiments are still necessary.

In summary, we report for the first time that ConA and SFL induce apoptotic cell death in human breast carcinoma MCF-7 cells but not in MCF-10A cells. ConA induced apoptosis via NF- κ B, ERK, and JNK down-regulation and p53 and p21 up-regulation in human breast carcinoma MCF-7 cells. While SFL triggers reduced NF- κ B and ERK expression and increased p53 expression. Additionally, SFL triggers G₂/M phase cell-cycle arrest by up-regulating p21 expression and down-regulating CDK1 and CDK2 expression. ConA and SFL also have anti-cancer and cytotoxic effects *in vivo*, and they decreased subcutaneous tumor volume and weight. However, the above-mentioned findings on ConA and SFL anti-neoplastic activities are still in their infancy, and further pre-clinical and clinical studies on these legume lectins are urgently needed.

By understanding the molecular mechanisms of ConA and SFL-induced anti-tumor properties both *in vitro* and *in vivo*, the legume lectin family can become a potential antineoplastic agent in future cancer therapeutics.

Acknowledgements

We are grateful to Dr Jian LI (Ohio University) and Wen-wen LI (University College London) for providing constructive suggestions. This work was supported in part by the National Natural Science Foundation of China (No 81173093, 30970643, J1103518, 81373311, and 31300674) and the Special Program for Youth Science and the Technology Innovative Research Group of Sichuan Province, China (No 2011JTD0026).

Author contribution

Zheng SHI and Jin-ku BAO designed the research study; Jie CHEN and Chun-yang LI performed the experiments; Na AN, Zi-jie WANG, Shu-lin YANG, and Kai-feng HUANG contributed to data analysis; Zheng SHI, Chun-yang LI, and Jin-ku BAO wrote the paper.

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