Physalin F Induces Cell Apoptosis in Human Renal Carcinoma Cells by Targeting NF-kappaB and Generating Reactive Oxygen Species

Szu-Ying Wu¹, Yann-Lii Leu², Ya-Ling Chang¹, Tian-Shung Wu³,⁴,⁵, Ping-Chung Kuo³, Yu-Ren Liao³, Che-Ming Teng¹*, Shiow-Lin Pan⁶*

¹Department of Pharmacology, National Taiwan University, Taipei, Taiwan, ²Graduate Institute of Natural Products, College of Medicine, Chang Gung University, Taoyuan, Taiwan, ³Department of Chemistry, National Cheng Kung University, Tainan, Taiwan, ⁴Department of Pharmacy and Chinese Medicine Research and Development Center, China Medical University, Taichung, Taiwan, ⁵Department of Pharmacy, China Medical University, Taichung, Taiwan, ⁶Institute of Biotechnology and Pharmaceutical Research, National Health Research Institutes, Miaoli County, Taiwan

Abstract

Background: The aim of this study was to determine the molecular mechanisms of physalin F, an effective purified extract of Physalis angulata L. (Solanacae), in renal carcinoma A498 cells.

Methodology/Principal Findings: Physalin F was observed to significantly induce cytotoxicity of three human renal carcinoma A498, ACHN, and UO-31 cells in a concentration-dependent manner; this was especially potent in A498 cells. The physalin F-induced cell apoptosis of A498 cells was characterized by MTT assay, nuclear DNA fragmentation and chromatin condensation. Using flow cytometry analysis, physalin F induced A498 cell apoptosis as demonstrated by the accumulation of the sub-G1 phase in a concentration- and time-dependent manner. Moreover, physalin F-mediated accumulation of reactive oxygen species (ROS) caused Bcl-2 family proteins, Bcl-2, and Bcl-xL degradation, which led to disruption of mitochondrial membrane potential and release of cytochrome c from the mitochondria into the cytosol. These effects were associated with induction of caspase-3 and caspase-9 activity, which led to poly(ADP-ribose) polymerase cleavage. However, the antioxidant N-acetyl-L-cysteine (NAC) and glutathione (GSH) resulted in the inhibition of these events and reversed physalin F-induced cell apoptosis. In addition, physalin F suppressed NF-κB activio and nuclear translocation of p65 and p50, which was reversed by NAC and GSH.

Conclusion: Physalin F induced cell apoptosis through the ROS-mediated mitochondrial pathway and suppressed NF-κB activation in human renal cancer A498 cells. Thus, physalin F appears to be a promising anti-cancer agent worthy of further clinical development.

Introduction

Among urological cancers, the incidence of renal cell carcinoma (RCC) is third in frequency after prostate and bladder cancers. In contrast to many other malignancies, RCC is generally resistant to chemotherapy, radiotherapy and hormone therapy. In addition, up to half of RCC patients will eventually develop metastatic RCC (mRCC), with a 5-year survival rate of only 9%. More recent attempts to treat this disease have used growth inhibitory and other immunotherapeutic methods such as cytokine-based regimens; however, they have had only of marginal benefit [1]. Therefore, novel approaches to the treatment of RCC are needed.

Reactive oxygen species (ROS) mediate many biological and pathological processes that are very important in normal and cancer cells. ROS include: superoxide anions (O₂⁻), hydroxyl radicals (·OH), hydrogen peroxide (H₂O₂), singlet oxygen (¹O₂), and highly reactive hydroxyl radicals. Mitochondria serve as electron transport chains that generate adenosine triphosphate (ATP) to supply energy to cells; as a result of this process, reactive oxygen species are also produced. Many prior studies have reported on natural compounds that induce the generation of ROS, directly in tumor cells and then go on to cause cell apoptosis. These natural compounds that induce the generation of ROS, could be combined with other anti-cancer drugs to potentiate their apoptotic effects. [2].

Natural products are a source of compounds that have important pharmacological activity in humans; scientists work to isolate the active ingredients from herbal medicines that may be developed into effective drugs for the treatment of human disease. Physalin F, the focus of this study, is an ethanolic extract from the plant Physalis angulata L. (Solanacae). Physalis angulata L. present in the tropics as well as sub-tropical areas, including Taiwan. Physalis angulata L.
Physalin F Induces Renal Carcinoma Cell Apoptosis

Materials and Methods

Drugs and Chemicals

The plants of Physalis angulata L. was obtained from Tainan District Agriculture Improvement Station, Taiwan. Physalin F was extracted and purified in Professor T.S. Wu’s Lab. (Department of Chemistry, National Cheng Kung University, Taiwan). Minimum Essential Medium (MEM), RPMI 1640 medium, fetal bovine serum (FBS), penicillin, and streptomycin were obtained from Gibco BRL Life Technologies (Grand Island, NY). EGTA, EDTA, leupeptin, dithiothreitol, phenylmethylsulfonyl fluoride (PMSF), L-glutathione (GSH), and RNase (100 U/mL)/streptomycin (100 μg/mL). DNA content was determined in a humidified incubator at 37°C in 5% CO₂/95% air.

Cytotoxicity Assay

Cells were incubated in 96-well plates (5,000 cells per well) in complete media with presence or absence of physalin F. The assay was terminated and cell survival was measured by MTT assay described in the previous study [20]. In brief, 100 μL MTT solution (0.5 mg/mL in phosphate-buffered saline; PBS) was added to each well. After 1 hr incubation at 37°C, MTT solution was removed and DMSO was added to dissolve dye. Absorbance at 550 nm was measured using a microplate reader, using medium as a blank.

Sulfonhodamine B Assay

Cells were seeded into 96-well plates in medium with 5% FBS. After 24 hr, cells were fixed with 10% trichloroacetic acid (TCA) to represent cell population at the time of physalin F addition. After additional incubation of vehicle or physalin F for 48 hr, this assay was terminated and cell growth was measured by microplate reader at a wavelength of 515 nm.

In situ Labeling of Apoptotic Cells

In situ detection of apoptotic cells was carried out using TUNEL apoptosis detective methods. Cells were cultured in chamber slides for 24 hr and then treated with physalin F (1, 3 and 10 μg/mL). After 24 hr treatment, cells were washed twice with PBS and fixed for 10 min with an ice-cold 1% paraformaldehyde solution. Staining was carried out according to the protocol provided by Promega (Madison, WI). Apoptosis was also assessed by nuclear morphology after staining with 4′,6-diamidino-2-phenylindole (DAPI), a DNA-binding fluorescent dye. After being fixed and permeabilized, the cells were stained with DAPI (1 μg/mL) for 15 min at room temperature. Finally, photomicrographs of TUNEL and DAPI staining were visualized and captured at ×400 magnifications with a fluorescence microscope (Nikon).

Cell Death Detection Assay

The Roche Cell Death Detection ELISAPLUS kit (Roche Diagnostics) is designed for quantitative detection of mono- and oligonucleosomal DNA fragmentation when cells are undergo apoptotic death in vitro. Protocol was applied from Roche and data was calculated and compared with control group.

FACScan Flow Cytometric Analysis

After the treatment of vehicle with 0.1% DMSO or compound for the indicated time courses, the cells were harvested by trypsinization, fixed with 70% (v/v) alcohol at 4°C for 60 min and washed with PBS. Then, the cells were centrifuged and resuspended with 0.5 mL propidium iodide solution containing Triton X-100 (0.1%), v/v, RNase (100 μg/mL) and propidium iodide (80 μg/mL). DNA content was determined in a humidified incubator at 37°C in 5% CO₂/95% air.
analyzed with the FACSScan and CellQuest software (Becton Dickinson, Mountain View, CA).

**Western Blot Analysis**

Total cell pellet was lysed with lysis buffer as previously described [21]. Cell homogenates were diluted with loading buffer and boiled for 10 min for detecting phosphorylation, expression, and cleavage of proteins. For Western blot analysis, proteins (30–60 μg) were separated by electrophoresis in a 10% polyacrylamide gel and transferred to a polyvinylidene fluoride membrane. After incubation at room temperature in PBS/5% nonfat milk for 1 hr, the membrane was washed three times with PBS/1% Tween 20. Then the membrane was immunoreacted with primary antibodies for overnight at 4°C. After washing with PBS/1% Tween 20, horseradish peroxidase (HRP)-conjugated goat anti-mouse or anti-rabbit IgGs were applied to the membranes for 1 hr at room temperature. Finally, the membranes were visualized with an enhanced chemiluminescence kit (Amersham, Buckinghamshire).

**Preparation of Cytosolic and Mitochondrial Fractions**

After the treatment of cells with vehicle (0.1% DMSO) or compound for the indicated time courses, proteins of the cytosolic fractions were isolated using the Cytochrome c Releasing Apoptosis Assay Kit (Catalog #K257-100, BioVision, Inc.). The levels of cytochrome c in the cytosolic fractions were detected by Western blot analysis.

**Preparation of Nuclear Fractions**

After cells were treated with DMSO or compound for the indicated time courses, proteins of the nuclear fractions were isolated as previously described [22]. Briefly, after removing medium, cells were washed by PBS and resuspended in buffer A (10 mM Hepes [pH 7.9], 10 mM KCl, 1.5 mM MgCl2, 0.2 mM PMSF, and 0.5 mM DTT). After incubation on ice for 15 min, cells were centrifuged at 3,000 rpm for 5 min, and the supernatants were the cytosolic fraction. Then the pellets were resuspended in buffer C (20 mM Hepes, 420 mM NaCl, 25% glycerol, 1.5 mM MgCl2, 0.2 mM EDTA, 0.5 mM DTT, and 0.2 mM PMSF). After incubation on ice for 20 min, cells were centrifuged at 13,000 rpm for 10 min. The supernatants were nuclear extract and the level of p65/p50 were detected by Western blot analysis.

**Measurement of the Change of Mitochondrial Membrane Potential (ΔΨm)**

Cells were treated with or without the indicated agent. Thirty minutes before the termination of incubation, the rhodamine 123 solution (final concentration of 5 μM) was added to the cells and incubated for the last 30 min at 37°C. The cells were finally harvested and the mean fluorescence was determined using FACScan flow cytometric analysis.

**Measurement of reactive oxygen species (ROS)**

Cells were incubated in the absence or presence of the indicated agents for 0.5, 1 or 3 hr. Thirty minutes before the termination of incubation period, DCF-DA (final concentration of 10 μM) was added to the cells and incubated for the 30 min at 37°C. Then, the cells were harvested for the detection of ROS accumulation using FACScan flow cytometric analysis.

**Electrophoretic Mobility Shift Assay (EMSA)**

Cells were incubated in the absence or presence of the indicated agents for 6 hr, and nuclear extracts were prepared by Nuclear Extraction Kit (AY2002, Panomics). An EMSA kit from Panomics was utilized to determine the binding activities of NF-κB p65. Treated or untreated nuclear extracts (4 μg) were incubated with biotin-labeled p65 probe, and protein-DNA complexes were separated on polyacrylamide gel. The gel was transferred to nylon membrane and detected by using horseradish peroxidase (HRP)-based chemiluminescence. The bands were visualized after exposure to film.

**Statistical Analysis**

All experiments were performed at least three times. Data were presented as the mean ± SE for the indicated number of separate experiments. Statistical analysis of data was done with Student’s t test. P values <0.05 were considered significant.

**Results**

**Physalin F Inhibited Cell Viability in Human Renal Cancer Cells**

To investigate the anti-cancer effects of physalin F, three renal carcinoma cell lines (A498, ACHN, and UO-31) were treated with various concentrations of physalin F (0, 0.3, 1, 3, and 10 μg/mL) for 24 hr and the cell viability was determined by MTT assay (Fig. 1A). Physalin F inhibited cell viability in human renal cancer cell lines A498, ACHN, and UO-31 in a concentration-dependent manner with an IC50 of 1.40 μg/mL, 2.10 μg/mL, and 2.81 μg/mL, respectively. However, the most potent cytotoxic effect was in A498 cells after physalin F treatment. In addition, physalin F inhibited cell growth (GI50 = 2.48 μg/mL) (Fig. 1B) in a concentration-dependent manner in A498 cells based on the results of the SRB assay. Also, physalin F had influences on inducing nuclear DNA fragmentation and chromatin condensation in A498 cells, according to the TUNEL assay and DAPI staining, in a concentration-dependent fashion (Fig. 1C and 1D). Finally, the results of the cell death Detection ELISA kit showed that physalin F significantly triggered A498 cell apoptosis in a concentration-dependent manner (Fig. 1E).

**Effects of Physalin F on the Cell Cycle Progression**

The DNA cell content was measured by flow cytometry to investigate the proportion of cells in different phases of the cell cycle, after treatment with physalin F. After treatment with physalin F, the population of sub-G1 cells was increased in a concentration- and time-dependent manner (Fig. 2A and 2B). These data suggested that physalin F inhibited A498 cell viability through the apoptosis pathway.

**Physalin F Induced Cell Apoptosis via a Mitochondria-mediated Pathway**

The mitochondria play a crucial role in cell apoptosis pathway. Loss of the mitochondrial membrane potential during apoptosis results in the release of cytochrome c from the mitochondria to the cytosol, which leads to induction of caspase activity and triggering of cell apoptosis. As shown in Figure 3A, treatment with physalin F caused loss of the mitochondrial membrane potential in a time-dependent manner. In addition, physalin F induced the release of cytochrome c into the cytosol (Fig. 3B). Furthermore, the Bcl-2 protein family regulated mitochondrial permeability and the release of cytochrome c to control cell apoptosis. As shown in the Figure 3C, the expression of the Bcl-2 protein family, including Bcl-2, and Bcl-xL was reduced after treatment with physalin F. However, physalin F had no significant effect on the expression of Bax (Fig. 3C). These results suggested that physalin F-induced
apoptosis through a mitochondria-dependent pathway in A498 cells.

Effects of Physalin F on Apoptosis-related Protein Expression in A498 Cells

To determine the mechanism associated with Physalin F induced apoptosis, the expression of apoptosis-related proteins was investigated. Poly (ADP-ribose) polymerase (PARP) specific proteolytic cleavage, by caspases, is considered to be one of the characteristics of apoptosis; therefore, cleavage of PARP and the associated caspases were evaluated. As shown in Figure 4A, physalin F induced significant PARP and caspase-3 cleavage, accompanied by a decrease in proform levels of caspase-8 and 9.

It has been reported that p53 mediates transcriptional regulation of the cyclin-dependent kinase inhibitor p21 that plays an important role in cell apoptosis. The findings of this study showed that physalin F induced p53 and p21 in a time-dependent manner (Fig. 4B). These results indicated that physalin F-induced apoptosis via induction of p53 and p21 proteins, and subsequently cleaved caspase-8/-9/-3 and PARP.

Figure 1. Effect of physalin F on viability in human renal cancer cells. Renal cancer cell lines (A498, ACHN, and UO-31) were treated with various concentrations (0.3, 1, 3, and 10 μg/mL) for 24 hr for MTT assay (A) and for 48 hr for SRB assay (B) in A498 cells. A498 cells were treated with vehicle or physalin F at the indicated concentration (1, 3, and 10 μg/mL) for 24 hr and viewed by fluorescence microscope followed by TUNEL (C) and DAPI (D) staining (magnification × 400). (E) To quantitatively evaluate cell death after treatment of physalin F for 24 hr, we used cell death ELISA kit to detect. Data are expressed as the mean percentage of control ± S.D. of three independent experiments. **, ##, & & P < 0.01 and ###, &&& P < 0.001 compared with the control group.

doi:10.1371/journal.pone.0040727.g001

Figure 2. Effect of physalin F on cell cycle distribution in A498 cells. (A) Cells were incubated with vehicle or various concentrations (1, 3, and 10 μg/mL) of physalin F for 24 hr. (B) Cells were incubated with vehicle or 10 μg/mL of physalin F for indicated time period. The cell cycle analysis and cell apoptosis were determined using FACS analysis as described in Methods and Materials. * P < 0.05, ** P < 0.01, and *** P < 0.001 compared with the 0-time point group in sub-G1 phase. # P < 0.05 compared with the 0-time point group in G0/G1 phase.

doi:10.1371/journal.pone.0040727.g002
Physalin F-induced Cell Apoptosis through Reactive Oxygen Species Generation

Lee et al. reported that oxidative stress was involved in Physalis angulata extract-induced apoptosis in human oral cancer cells [23] and that the reactive oxygen species (ROS) induced mitochondrial-dependent apoptosis in many human cancer cells. In this study, physalin F-induced apoptosis mediated by ROS generation was evaluated. The results showed that ROS production was increased in a time-dependent manner after treatment with 10 μg/mL of physalin F in A498 cells (Fig. 5A). Next, four types of ROS scavengers (vitamin C, NAC, trolox, and GSH) were studied to determine which antioxidant could rescue the cells from physalin F-induced cell death. Notably, NAC and GSH significantly reversed physalin F-induced cell death according to the results of the MTT assay (Fig. 5B). These two ROS scavengers also blocked physalin F-induced ROS generation for 3 hr (Fig. 5C) and significantly reversed the physalin F-induced disruption of the mitochondrial membrane potential (Fig. 5D). In addition, the downstream signals, such as caspase-9, caspase-3, PARP, p53, and p21 were restored by NAC and GSH (Fig. 5E). These results suggested that the cell death induced by physalin F, in the A498 cells, was mediated by the accumulation of ROS and the mitochondria-dependent apoptotic pathway.

Figure 3. Effect of physalin F on reduction of ΔΨm and release of cytochrome c. A498 cells were incubated in the absence or presence of physalin F (10 μg/mL) for indicated time, and cells were harvested and prepared for detection (A) mitochondria membrane potential by using FACScan analysis, (B) release of cytochrome c in cytosol (The fractions were collected by using the protocol of preparation of cytosolic and mitochondrial fractions as mentioned in Materials and Methods.), and (C) Bcl-2, Bcl-xL and Bax expression by using Western blotting analysis. * P<0.05 compared with the 12 hr-time point control group. † P<0.05 compared with the 18 hr-time point control group. ‡ P<0.01 compared with the 24 hr-time point control group.

doi:10.1371/journal.pone.0040727.g003
Physalin F Inhibited NF-κB Activation

NF-κB, a family of transcription factors, plays a pivotal role in inflammation, immunity, angiogenesis, cell migration, cell proliferation, and apoptosis [24,25]. In normal state, cytosolic NF-κB is bound to inhibitory IκB protein. Upon signal activation, IκBα becomes phosphorylated, ubiquitinated, and subsequently degraded by proteasomes. The data showed that physalin F decreased the expression of IκBα and phosphorylated IκBα (Fig. 6A) and inhibited nuclear translocation of p65/p50 in A498 cells (Fig. 6B). Because phosphorylation of p65 at serine 536 by IKK is required for transcriptional activity of NF-κB, as seen in Figure 6B, we found that physalin F inhibited the phosphorylation of p65 (Ser536) in the nuclear fraction. Furthermore, to investigate whether physalin F inhibited NF-κB activity, we performed electrophoretic-mobility shift assay to observe the interaction between NF-κB and its specific recognition sequence. As shown in Figure 6C, physalin F repressed NF-κB DNA binding, and the observed signals disappeared in the presence of the cold NF-κB competitor, which indicated these signals were NF-κB specific. In addition, physalin F abrogated the nuclear translocation of p65/p50 subunits that were reversed by both ROS scavengers, NAC and GSH (Fig. 6D). These data suggested that the NF-κB/ROS pathway was involved in physalin F-induced A498 cell apoptosis.

Discussion

Renal cell carcinoma (RCC) is the most common type of kidney cancer in adults; it is also the most deadly cancer among urological tumors. Approximately 30% of all patients with RCC will develop metastatic lesions [26]. Because of metastatic RCC (mRCC) is resistant to radiation therapy and chemotherapy, novel therapeutic treatment strategies are needed.

Physalin F is purified from Physalis angulata L., which has been reported to have anti-cancer activity in many cancer cell types. In this study, physalin F triggered A498 cell growth inhibition and apoptosis in a concentration- and time-dependent manner. Physalin F-induced cell apoptosis was also mediated by disruption of the mitochondria membrane potential leading to release of cytochrome c into the cytosol, and then activation of caspase-3/-8/-9. When cytochrome c is released from the mitochondria, it mediates the allosteric activation of apoptosis-protective activating factor 1 (Apaf-1). Cytochrome c and Apaf-1 come together to form the apoptosome. The apoptosome recruits caspase-9 and results in the proteolytic cleavage and activation of caspase-9 and caspase-3 [27].

The mitochondria are the major site of production of ROS [28]. The results of this study showed that treatment with physalin F largely increased ROS production in A498 cells. ROS is critical for the metabolic and signal transduction pathways associated with
Figure 5. Effect of physalin F on cellular ROS accumulation in A498 cells. A498 cells were incubated in the absence or presence of physalin F (10 μg/mL) for indicated time. The fluorescent intensity of DCFH-DA was detected by flow cytometric analysis. ** P<0.01 compared with the 0.5 hr-time point control group. * P<0.05 compared with the 1 hr-time point control group. *** P<0.001 compared with the 3 hr-time point control group. (B) Different ROS scavengers were preincubated for 30 min and cell viability was determined by MTT assay. *** P<0.001 compared with the control group. # P<0.001 compared with the physalin F-treated group. Treatment of NAC and GSH inhibited physalin F-induced ROS generation (C, * P<0.05 and ** P<0.01 compared with the physalin F-treated group.) and the loss of ΔΨm (D, *** P<0.001 compared with the control group. # P<0.05 compared with the physalin F-treated group.) in A498 cells. (E) NAC (10 mM) and GSH (3 mM) reduced the apoptosis-related results in physalin F-treated cells for 24 hr.

doi:10.1371/journal.pone.0040727.g005
cell growth and apoptosis. However, excessive production of ROS leads to oxidative stress and cell damage [29]. Therefore, the generation of ROS and pro-oxidation therapy, as a treatment for cancer, has attracted great interest. The results of this study demonstrated that physalin F-induced ROS accumulation was significantly reversed by NAC and GSH. In addition, NAC and GSH rescued the decrease of the mitochondria membrane potential and restored apoptosis-related proteins, such as caspase-9, caspase-3, PARP, p53, and p21, after physalin F treatment in A498 cells. Because NAC is a precursor of GSH, it has been reported to increase intracellular levels of glutathione, which is important to the prevention of oxidative stress in cells [30]. In addition, NAC and GSH might protect cells from damage caused by conjugation with electrophiles [30,31]. These results show that ROS production plays an important role in physalin F-induced A498 cell apoptosis.

Physalis angulata L. has been commonly used as an herbal medicine to treat various inflammatory disorders, especially rheumatism, dermatitis, and cancer. These observations imply that the transcriptional factor, nuclear factor-κB (NF-κB) heterodimer, plays a key role in regulating cell function after exposure to physalin F. Physalin F, extracted from Witheringia solanacea, was also reported to have anti-inflammatory activity by mediating NF-κB inhibition and apoptosis [32]. NF-κB is recognized to be a major transcription factor involved in cell proliferation, development, and oncogenesis. The constitutive activation of NF-κB has been reported in many human solid tumors [33]. There are emerging reports suggest that NF-κB plays a pivotal role in the development of RCC [34,35]. Moreover, the activity of progression, invasion, and metastases of RCC has been shown to correlate with the increased activation of NF-κB [36]. In our study, we examined the basal NF-κB activity in three RCC cell lines (Fig. S1). Our results

![Figure 6. Effect of physalin F on NF-κB.](image-url)
showed that NF-κB was constitutive active in A498 and ACHN, but not in UO-31. In addition, physalin F is more potent in A498 cells, it suggests that inhibiting NF-κB activity by physalin F is important in A498 cells. Moreover, in our study, both of the ROS scavengers, NAC and GSH, could significantly reverse physalin F suppressed p63/p50 translocation into the nucleus, suggesting that the ROS/NF-κB pathway is involved in the cell apoptosis induced by physalin F.

Taken together, the results of this study showed that physalin F induced ROS generation and caused cell apoptosis. Because of the loss of the mitochondria membrane potential, cytochrome c was released into the cytosol and induced caspase activation resulting in apoptosis. In addition, NAC and GSH reversed the generation of the ROS, the disruption of mitochondria membrane potential and death of the A498 cells. Moreover, the phosphorylation of IκBα was inhibited and prevented NF-κB nuclear translocation in A498 cells with physalin F treatment. Furthermore, these phenomena were reversed by NAC and GSH. As measured by EMSA, physalin F blocked NF-κB activation in A498 cells. These findings suggest that NF-κB was involved in the physalin F-induced A498 cell apoptosis pathway. These results further the understanding of physalin F and its potential use for cancer therapy. Additional studies are needed to investigate the molecular mechanisms associated with cancer cells.

Supporting Information

Figure S1  NF-κB activity was evaluated by EMSA in three RCC cell lines. The nuclear extracts of three cell lines (A498, ACHN, and UO-31) were incubated with a hot NF-κB probe (lane 2–4) or cold probe (lane 1, indicate “cold”) and demonstrate the specificity of the bands obtained on EMSA. P indicates positive nuclear extract.

(TIFF)

Author Contributions

Conceived and designed the experiments: S-YW C-MT S-LP. Performed the experiments: S-YW. Analyzed the data: S-YW C-MT S-LP. Contributed reagents/materials/analysis tools: Y-LJ Y-LC T-SW P-CK Y-RL. Wrote the paper: S-YW.

References

1. Rohrmann K, Staehler M, Haeck N, Bachmann A, Stief CG, et al. (2005) Immunomodulation in metastatic renal cell carcinoma. World Journal of Urology 23: 196–201.
2. Hosseinizadeh L, Behravan J, Mosaelf F, Bahrami G, Bahrami A, et al. (2011) Curcumin potentiates doxorubicin-induced apoptosis in H9c2 cardiac muscle cells through generation of reactive oxygen species. Food and Chemical Toxicology 49: 1102–1109.
3. Ankrn A-N, Nyarko AK, Addo PGA, Ofosuhene M, Dzokoto C, et al. (2003) Evaluation of efficacy and safety of a herbal medicine used for the treatment of malaria. Phytotherapy Research 17: 697–701.
4. Lin YS, Chiang HC, Kan WS, Hene E, Shih SJ, et al. (1992) Immunomodulatory activity of various fractions derived from Physalis angulata L extract. Am J Chin Med 20: 233–243.
5. Soares MBP, Bellintani MC, Ribeiro IM, Tomassini TCB, Ribeiro dos Santos R (2003) Inhibition of macrophage activation and lipopolysaccharide-induced death by seco-steroids purified from Physalis angulata L. European Journal of Pharmacology 459: 107–112.
6. Chiang HC, Jaw SM, Chen CF, Kan WS (1992) Antitumor agent, physalin F from Physalis angulata L. Anticancer Res 12: 837–843.
7. Chiang HC, Jaw SM, Chen PM (1992) Inhibitory effects of physalin B and physalin F on various human leukemia cells in vitro. Anticancer Res 12: 1155–1162.
8. Issail N, Alam M (2001) A novel cytotoxic flavonoid glycoside from Physalis angulata. Fitoterapia 72: 676–679.
9. Wu SY, Pan SL, Chen TH, Liao CH, Huang DY, Goh JH, et al. (2010) Moscattin repressed lipopolysaccharide-induced HIF-1alpha accumulation and NF-kappaB activation in murine RAW264.7 cells. Shock 33: 70–75.
10. Kuo P-C, Kuo T-H, Damu AG, Su C-R, Lee EJ, et al. (2006) Physanolide A, a novel cytotoxic flavonol glycoside from Physalis peruviana. Journal of Natural Products 69: 1552–1555.
11. Wu S-J, Ng L-T, Chen C-H, Lin D-L, Wang S-S, et al. (2004) Antihepatoma activity of Physalis angulata and P. peruviana extracts and their effects on drug-resistant HepG2 cells. Life Sciences 74: 2061–2073.
12. Lin YS, Chiang HC, Kan WS, Hone E, Shih SJ, et al. (1992) Immunomodulatory activity of various fractions derived from Physalis angulata L extract. Am J Chin Med 20: 233–243.
13. Hsu C-C, Wu Y-C, Farh L, Du Y-C, Tseng W-K, et al. (2012) Physalin B from Physalis angulata. Journal of Pharmacy and Pharmacology 58: 235–241.
14. Panzo NB, Moraes TC, Carvalho KMB, Silva CR, Andrade-GM, et al. (2010) Topical anti-inflammatory potential of Physalin E from Physalis angulata on experimental dermatitis in mice. Phytotherapy Research 24: 740–743.
15. Soares MBP, Brustolim D, Santos LA, Bellintani MC, Paiva FP, et al. (2006) Physalin B, F and G, seco-steroids purified from Physalis angulata L, inhibit lymphocyte function and allergenic transection. International Immunopharmacology 6: 408–414.
16. Antoun MD, Abramson D, Tyson RL, Chang CJ, McLaughlin JL, et al. (1981) Structures, and Structure-Cytotoxic Activity Relationships of Withanolides and Physalins from Witheringia solanacea as Modulators of the NF-κB Cascade. J Nat Prod 44: 579–585.
17. Lee HZ, Liu WZ, Hsieh WT, Tang FY, Chung JG, et al. (2009) Oxidative stress involvement in Physalis angulata-induced apoptosis in human oral cancer cells. Food and Chemical Toxicology 47: 561–570.
18. Pinto NB, Morais TC, Carvalho KMB, Silva CR, Andrade-GM, et al. (2010) Topical anti-inflammatory potential of Physalin E from Physalis angulata. Journal of Natural Products 70: 1146–1152.
19. Panzo NB, Morais TC, Carvalho KMB, Silva CR, Andrade-GM, et al. (2010) Topical anti-inflammatory potential of Physalin E from Physalis angulata on experimental dermatitis in mice. Phytotherapy Research 24: 740–743.
20. Pan SL, Goh JH, Chang YL, Kuo SC, Lee FY, et al. (2004) YC-1 prevents sodium nitroprusside-mediated apoptosis in vascular smooth muscle cells. Cardiovasc Res 61: 152–159.
21. Wu SY, Pan SL, Chen TH, Liao CH, Huang DY, Goh JH, et al. (2009) Moscattin repressed lipopolysaccharide-inducted HIF-1alpha accumulation and NF-kappaB activation in murine RAW264.7 cells. Shock 33: 70–75.
22. Lee HZ, Liu WZ, Hsieh WT, Yang FY, Chung JG, et al. (2009) Oxidative stress involvement in Physalis angulata-induced apoptosis in human oral cancer cells. Food and Chemical Toxicology 47: 561–570.
23. Greten FR, Karin M (2004) The IKK/NF-κB activation pathway—a target for prevention and treatment of cancer. Cancer Letters 206: 193–199.
24. Karin M, Cao Y, Greten FR, Li Z-W (2002) NF-kappaB in cancer: from innocent bystander to major culprit. Nat Rev Cancer 2: 301–310.
25. van der Veldt AA, Haanen JB, van der Eertwegh AJ, Boven E (2010) Targeted therapy for renal cell carcinoma: future perspectives. Cancer Med 10: 394–405.
26. Garrido C, Galahiz L, Brunet M, Puig PE, Didelet C, et al. (2006) Mechanisms of cytochrome c release from mitochondria. Cell Death Differ 13: 1423–1433.
27. Pinto NB, Morais TC, Carvalho KMB, Silva CR, Andrade-GM, et al. (2010) Topical anti-inflammatory potential of Physalin E from Physalis angulata on experimental dermatitis in mice. Phytotherapy Research 24: 740–743.
28. Jezek P, Hlavata´ L (2005) Mitochondria in homeostasis of reactive oxygen species in cell, tissues, and organ. The International Journal of Biochemistry & Cell Biology 37: 2478–2505.
29. Hsu C-C, Wu Y-C, Farh L, Du Y-C, Tseng W-K, et al. (2012) Physalin B from Physalis angulata L. Chemistry & Biodiversity 4: 443–449.
30. Buttiatti-Angelo A, Rivas-Flores L, Carmona MI, Ferrer D, Parra RA, et al. (2004) Immunomodulatory activity of Physalis angulata and P. peruviana extracts and their effects on apoptosis in human Hep G2 cells. Life Sciences 74: 2061–2073.
31. Kuo P-C, Kuo T-H, Damu AG, Su C-R, Lee EJ, et al. (2006) Physanolide A, a Novel Steroid Ketone, and Other Cytotoxic Principles from Physalis angulata. Organic Letters 8: 2953–2956.
32. Damu AG, Kuo P-C, Su C-R, Kuo T-H, Chen T-H, et al. (2007) Isolation, Constitutive activation of nuclear factor-kappaB prevents TRAIL-induced apoptosis in renal cell carcinoma. Carcinogenesis 24: 377–384.
33. An J, Sun Y, Fisher M, Rettig MB (2004) Maximal apoptosis of renal cell carcinoma by the proteasome inhibitor bortezomib is nuclear factor-kappaB dependent. Molecular Cancer Therapeutics 3: 727–736.
34. Oya M, Ohitsubo M, Takayanagi A, Tachibana M, Shimura N, et al. (2001) Constitutive activation of nuclear factor-kappaB prevents TRAIL-induced apoptosis in renal cancer cells. Oncogene 20: 3893–3896.