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Isoliquiritigenin induces apoptosis by depolarizing mitochondrial membranes in prostate cancer cells☆

Jae In Jung[a], Soon Sung Lim[a,b], Hyun Ju Choi[a], Han Jin Cho[b], Hyun-Kyung Shin[a,b], Eun Ji Kim[b], Won-Yoon Chung[c], Kwang-Kyun Park[c], Jung Han Yoon Park[a,b,*]

[a]*Department of Food Science and Nutrition, Hallym University, Chuncheon 200-702, South Korea*
[b]*Silver Biotechnology Research Center, Hallym University, Chuncheon 200-702, South Korea*
[c]*Department of Oral Biology, College of Dentistry, Yonsei University, Seoul 120-752, South Korea*

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Abstract

Isoliquiritigenin (ISL), a simple chalcone derivative, 4,2′,4′-trihydroxychalcone, found in licorice, shallot and bean sprouts, has been reported to have chemoprotective effects. To examine the effects of ISL on the growth of prostate cancer cells, we cultured MAT-LyLu (MLL) rat and DU145 human prostate cancer cells with various concentrations (0–20 μmol/L) of ISL. Treatment of the cells with increasing concentrations of ISL led to dose-dependent decreases in the viable cell numbers in both DU145 and MLL cells ($P<.05$). Hoechst 33258 dye staining of condensed nuclei and annexin V binding to surface phosphatidylserine revealed increased numbers of apoptotic cells after ISL treatment. Western blot analysis revealed that ISL increased the levels of membrane-bound Fas ligand (FasL), Fas, cleaved casapse-8, truncated Bid (tBid), Bax and Bad in DU145 cells ($P<.05$). Isoliquiritigenin increased the percentage of cells with depolarized mitochondrial membranes, in a concentration-dependent manner ($P<.05$). Isoliquiritigenin induced the release of cytochrome *c* and Smac/Diablo from the mitochondria into the cytoplasm ($P<.05$). Isoliquiritigenin dose-dependently increased the levels of cleaved caspsase-9, caspase-7, caspase-3 and poly(ADP-ribose) polymerase ($P<.05$). The present results indicate that ISL inhibits prostate cancer cell growth by the induction of apoptosis, which is mediated through mitochondrial events, which are associated with an evident disruption of the mitochondrial membrane potential, and the release of cytochrome *c* and Smac/Diablo, and the activation of caspase-9.

Keywords: Bcl-2; Caspase; Apoptosis; Cytochrome *c*; Prostate cancer cells

1. Introduction

Prostate cancer is the second leading cause of cancer-related deaths in the United States [1], and the incidence is also rapidly increasing in Asian countries. Androgen ablation has been the most frequently used treatment for disseminated prostate cancer, and most patients with a metastatic disease encounter an initial regression in response to this treatment. However, the disease eventually relapses in most patients and progressed to an androgen-independent state, which no longer responds to conventional therapy [2]. In the United States, prostate cancer is mainly found in men aged over 55 years, with the average age of patients at the time of diagnosis of 70 years. For this reason, it is necessary to increase efforts to gain a better understanding of and develop novel treatment and chemopreventive approaches for this disease.

Studies to identify agents with potentially preventive and therapeutic roles in cancer are increasing rapidly. Flavonoids, polyphenolic compounds ubiquitously present in plants, have drawn considerable interest due to their apparent ability to act as highly effective chemopreventive and chemotherapeutic agents [3]. Many of these compounds seem to act on multiple target signaling pathways in cancer cells [4].

Abbreviations: ISL, isoliquiritigenin; MLL, MAT-LyLu; PARP, poly-(ADP-ribose) polymerase.

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* Corresponding author. Department of Food Science and Nutrition, Hallym University, Chunchon 200-702, South Korea. Tel.: +82 33 248 2134; fax: +82 33 256 0199.
E-mail address: jyoon@hallym.ac.kr (J.H.Y. Park).

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The ability of tumor cell populations to increase in number is determined not only by the rate of cell proliferation, but also by the rate of cell death. Because deregulated inhibition of apoptosis lies at the heart of all tumor development, it presents an obvious target for preventive and therapeutic intervention in all cancers [5]. Apoptosis is controlled by two major pathways, including the mitochondrial [6] and membrane death receptor pathways [7]. The great majority of cell death signals engage the mitochondrial pathway, where death signals lead to alterations in the mitochondrial membrane permeability, with the subsequent release of pro-apoptotic factors, such as cytochrome *c* and Smac/Diablo [6]. Cytosolic cytochrome *c* recruits and activates caspase-9 [8,9] by forming the macromolecular complex, apoptosome, which then processes and activates other caspases to induce apoptosis [10]. The Smac/Diablo released from the mitochondria during apoptosis functions to promote caspase activation by competing with caspases for binding of the inhibitor of apoptosis protein (IAP) family, thereby relieving the inhibitory effects of IAPs on caspases [11,12].

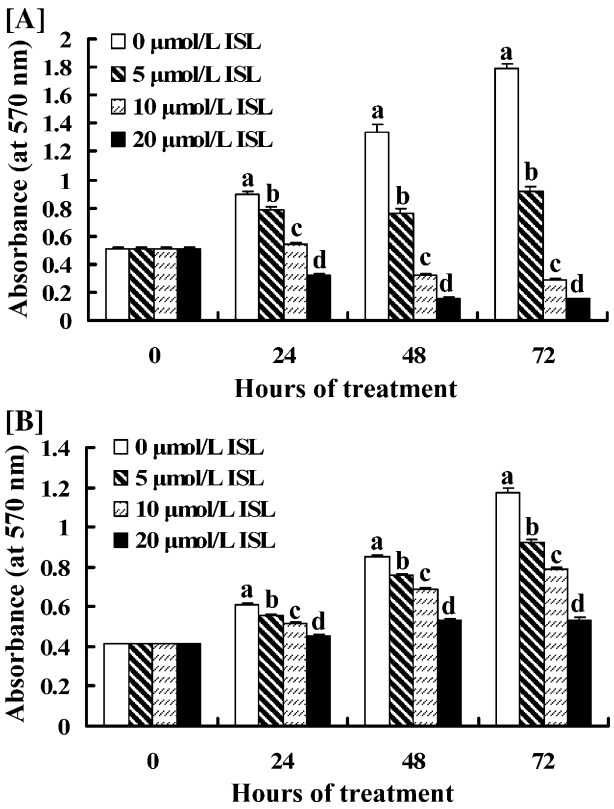

Fig. 1. Isoliquiritigenin decreases viable cell numbers in prostate cancer cells. (A) MAT-LyLu cells were plated at a density of 20,000 cells/well in 12-well plates with DMEM/F12 supplemented with 10% FBS. Forty-eight hours after plating, the monolayers were serum-starved with DMEM/F12 supplemented with 5 mg/L transferrin, 5 μg/L selenium, 2.25 mg/L glutamine and 0.1 g/L BSA (serum-free medium) for 24 h. (B) DU145 cells were plated at a density of 50,000 cells/well in 24-well plates with DMEM/F12 supplemented with 10% FBS. Twenty-four hours after plating, the monolayers were serum-starved for 24 h. Both cell lines were then incubated for 24, 48 or 72 h in serum-free medium containing 0, 5, 10 or 20 μmol/L ISL. Cell numbers were estimated by the MTT assay. Each bar represents the mean±S.E.M. ($n=3$ or 6). Means at a time without a common letter differ, $P<.05$.

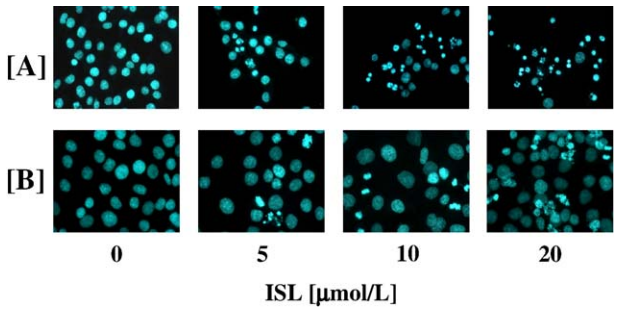

Fig. 2. Detection of apoptosis in prostate cancer cells by Hoechst Dye 33258. MAT-LyLu (A) and DU145 (B) cells were plated in chamber slides at 20,000 cells/well in DMEM/F12 supplemented with 10% FBS. After serum starvation, the monolayers were incubated for 24 h (MLL) and 48 h (DU145) in serum-free medium containing 0, 5, 10 or 20 μmol/L ISL. Cells were fixed and stained with a DNA specific dye, Hoechst 33258.

The key regulatory factors of mitochondria-mediated apoptosis are the Bcl-2 family of proteins, which can either promote cell survival or induce apoptosis [13,14]. For example, Bcl-2 appears to preserve the integrity of the mitochondrial outer membrane, thereby preventing the release of pro-apoptotic factors, whereas Bax promotes cytochrome *c* and Smac/Diablo release from mitochondria [15,16].

Isoliquiritigenin (ISL), a flavonoid with a chalcone structure, found in licorice, shallot and bean sprouts, has been reported to exhibit anticarcinogenic effects. Isoliquiritigenin has been shown to suppress 7,12-dimethylbenz[*a*]anthracene-induced and TPA-promoted skin papilloma formation [17] and to inhibit the induction of preneoplastic aberrant crypt foci in azoxymethane-treated F344 rats [18]. In vitro studies have shown that ISL induces apoptosis in hepatoma [19], gastric [20] and melanoma cancer cells [21]. However, the mechanisms by which ISL induces apoptosis in cancer cells have not been well characterized. In addition, to our knowledge, the effects of ISL on prostate cancer have not been studied in detail, with the exception of a study that reported a decreased growth and increased expression of GADD153 mRNA in LNCaP and DU145 prostate cancer cells in the presence of ISL [18].

The present study examined whether ISL induces apoptosis of prostate cancer cells and investigated the underlying mechanisms using MAT-LyLu (MLL) rat and DU145 human prostate cancer cells.

2. Materials and methods

2.1. Materials

The following antibodies were purchased from their respective sources. Cytochrome *c* and caspase-8 (BD PharMingen, Franklin Lake, NJ); cleaved caspase-9, cleaved

caspase-7, cleaved caspase-3, cleaved Bid, cleaved PARP and Bad (New England Biolabs, Beverly, MA); and Bcl-2, Bax, Fas, Fas ligand (FasL) and Smac/Diablo (Santa Cruz Biotechnology, Santa Cruz, CA).

2.2. Cell culture

The intestinal epithelial cell line 6 (IEC-6), MLL and DU145 cells were obtained from the American Type Culture Collection (Manassas, VA) and maintained in DMEM/F12, containing 100 ml/L fetal bovine serum (FBS), with 100,000 U/L penicillin and 100 mg/L streptomycin (Gibco BRL, Gaithersburg, MD). To examine the effect of ISL (Sigma, St. Louis, MO), we plated cells in multiwell plates with DMEM/F12 containing 100 ml/L FBS. Prior to ISL treatment, the cell monolayers were rinsed and serum-starved for 24 h, using DMEM/F12 supplemented with 5 mg/L transferrin, 0.1 g/L bovine serum albumin (BSA) and 5 μg/L selenium (serum-free medium). After serum starvation, fresh serum-free medium, containing the indicated concentrations of ISL, was replaced. Viable cell numbers were estimated by the MTT assay, as described previously [22].

2.3. Detection of apoptotic cell death

To examine whether ISL induces apoptosis in prostate cancer cells, MLL and DU145 cells were plated in four-well chamber slides, at 20,000 cells/slide, and treated with increasing concentrations of ISL for 24 and 48 h, respectively. The cells were fixed with 40 g/L PBS-buffered formaldehyde for 20 min, after which staining with Hoechst 33258 (10 mg/L) was performed in the dark for 30 min at 37°C. Slides were then examined using fluorescence microscopy. Apoptotic cells were defined on the basis of their nuclear morphology changes, such as chromatin condensation and fragmentation.

To estimate the number of apoptotic cells, cells were plated in 24-well plates and treated with increasing concentrations of ISL. The cells were trypsinized and then incubated in the dark with phycoerythrin (PE)-conjugated annexin V (annexin V-PE) (BD Pharmingen) and 7-amino-actinomycin (7-AAD) for 15 min at room temperature. Apoptotic cells were analyzed by flow cytometry, utilizing FACScan (Becton Dickinson, Franklin Lake, NJ). The data were analyzed using the ModFit v. 1.2 software.

2.4. Western blot analysis

Total cell lysates were prepared as described previously [23]. Cytosolic and mitochondrial proteins were separated, as described by Eguchi et al. [24], and the purity of the fractions estimated by immunoblotting with an antibody raised against the mitochondrial heat shock protein 60 [25]. The proteins in total cell lysates, mitochondrial and cytoplasmic fractions were resolved on a sodium dodecyl sulfate–4% to 20% polyacrylamide gel and transferred onto polyvinylidene fluoride membrane (Millipore, Bedford, MA). The blots were incubated for 1 h with either anti-cytochrome *c* (1:1000), caspase-8 (1:1000), cleaved caspase-9 (1:1000), cleaved caspase-7 (1:1000), cleaved caspase-3 (1:1000), cleaved Bid (1:1000), cleaved PARP (1:1000), Bad, (1:1000), Bcl-2 (1:1000), Bax (1:1000), Fas (1:1000), FasL (1:500), Smac/Diablo (1:1000) or anti-β-actin (1:2000) in 50 g/L nonfat dry milk TBS-T (20 mmol/L Tris–Cl, pH 7.5, 150 mmol/L NaCl and 1 ml/L Tween 20). The blots were then incubated with antimouse, antigoat or antirabbit horseradish peroxidase-conjugated antibodies. Signals were detected using the enhanced chemiluminescence method, with the Supersignal West Dura Extended Duration Substrate (Pierce). The relative abundance of each band was measured by densitometric scanning of the exposed films, using the Bio-profile Bio-1D application (Vilber-Lourmat, France).

2.5. Flow cytometry measurement of mitochondrial membrane potential

To measure the mitochondrial membrane potential, the dual-emission potential-sensitive probe 5,5′,6,6′-tetra-chloro-1,1′,3,3′-tetraethyl-imidacarbocyanine iodide (JC-1, Sigma) was used. JC-1 is a green-fluorescent monomer at low membrane potential, with the membrane potential of energized mitochondria promoting the formation of red-fluorescent J aggregates. The ratio of red to green

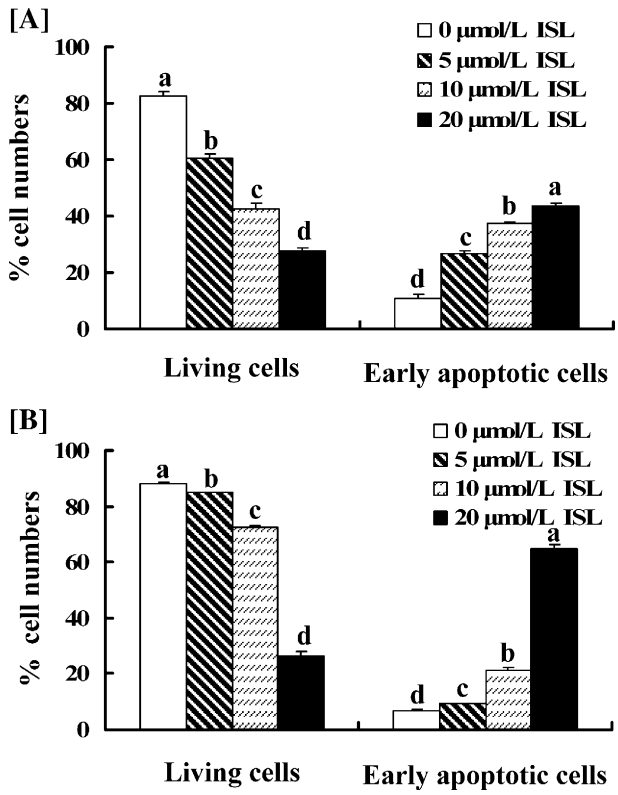

Fig. 3. ISL increases apoptotic cell numbers in prostate cancer cells. MAT-LyLu (A) and DU145 (B) cells were treated with ISL for 24 and 48 h, respectively, as described in Fig. 1. Cells were trypsinized, loaded with 7-aminoactinomycin D and annexin V, and then analyzed by flow cytometry. The number of living cells and early apoptotic cells is expressed as a percentage of total cell number. Each bar represents the mean±S.E.M. ($n=6$). Means at a time without a common letter differ, $P<.05$.

fluorescence of JC-1 depends only on the membrane potential, with a decrease being indicative of membrane depolarization [26,27]. DU145 cells treated with ISL were harvested, loaded with 2 mg/L JC-1 at 37°C for 20 min and then analyzed using a FACScan flow cytometer (Becton Dickinson).

2.6. Statistical analysis

For all studies, three to six independent experiments were performed with separate batches of cells. The results were expressed as the mean±S.E.M. and analyzed using a one-way or two-factor repeated measurements analysis of variance. Differences between the treatment groups were analyzed using Duncan's multiple range test. Statistical analyses were conducted utilizing the SAS system for Windows v. 8.12 (SAS Institute, Cary, NC).

3. Results

3.1. Isoliquiritigenin inhibits growth and induces apoptosis of prostate cancer cells

In the present study, the effects of three concentrations (5, 10 and 20 μmol/L) of ISL on the growth of rat (MLL) and human (DU145) prostate cancer cells were investigated by measuring the total number of viable cells. Treatments of cells with increasing concentrations of ISL led to dose-dependent decreases in the number of viable cells in both MLL and DU145 cells ($P<.05$; Fig. 1). To examine whether ISL was toxic to normal intestinal epithelial cells, we similarly treated IEC-6 cells with ISL, but no effect on their growth was found (data not shown).

The induction of apoptosis in prostate cells treated with ISL was associated with a decreased rate of cell growth. Hoechst 33258 dye staining displayed ISL-induced chromatin condensation, showing an intense pycnotic bluish-white fluorescence in their nuclei, in a concentration-dependent manner in both MLL and DU145 cells (Fig. 2). To estimate the early apoptotic cell number, we stained ISL-treated MLL and DU145 cells with annexin V and 7-AAD, and then analyzed them by flow cytometry. There were dose-dependent increases in both annexin V-positive/7AAD-negative cells when MLL and DU145 cells were treated with increasing concentrations of ISL for 24 and 48 h, respectively ($P<.05$; Fig. 3).

3.2. Isoliquiritigenin increases the levels of cleaved caspases and PARP in prostate cancer cells

As a first step to exploring the mechanisms by which ISL induces apoptosis, the activation of caspases was examined by determining their cleaved levels. Western blot analysis of total cell lysates revealed that ISL increased the levels of cleaved caspase-9, -7 and -3, in dose-dependent manners, in both MLL (Fig. 4A) and DU145 (Fig. 4B) cells. The cleaved PARP levels were also increased in both cell types, in ISL dose-dependent manners ($P<.05$; Fig. 4).

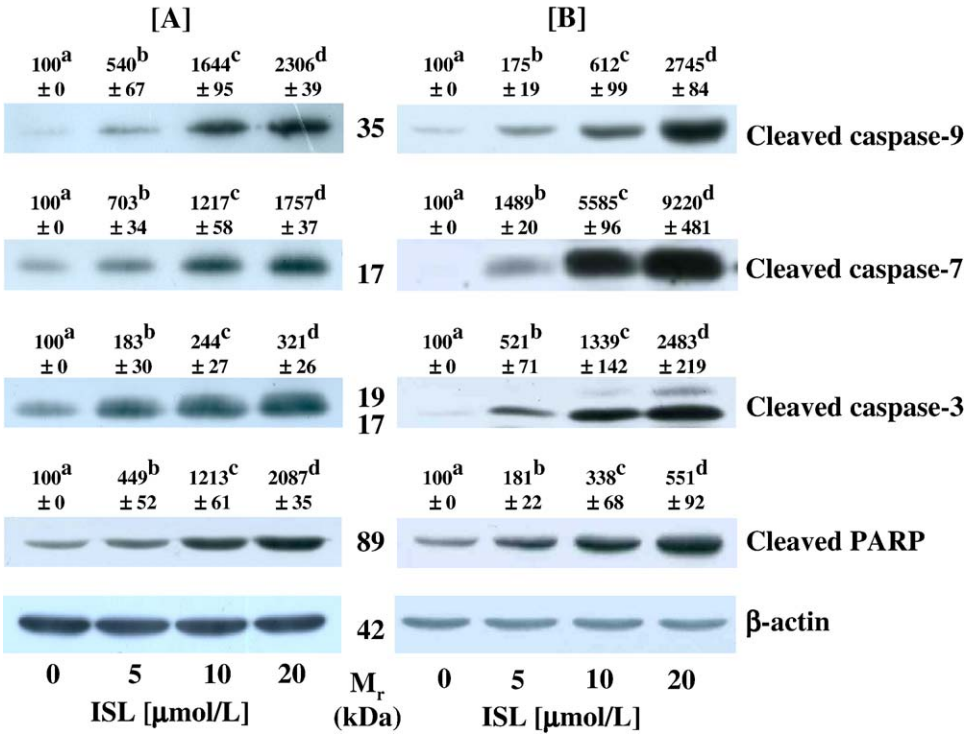

Fig. 4. ISL increases the protein levels of cleaved caspases and PARP in prostate cancer cells. MAT-LyLu (A) and DU145 (B) cells were treated with ISL as described in Fig. 1. Cell lysates were analyzed by Western blotting with the indicated antibodies. Photographs of the chemiluminescent detection of the blots, which were representative of three independent experiments, are shown. The relative abundance of each band to its own β-actin was quantified, and the control levels were set at 100%. The adjusted mean±S.E.M. ($n=3$) of each band is shown above each blot. Means without a common letter differ, $P<.05$.

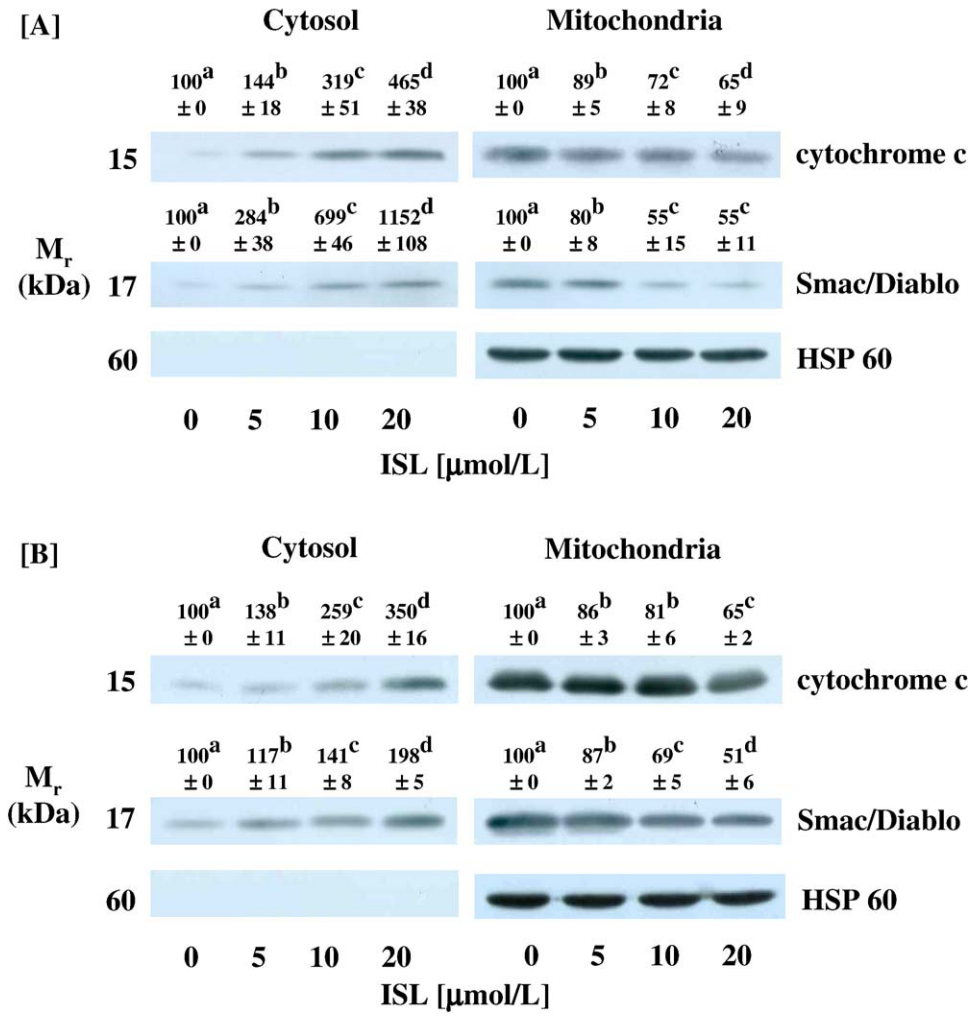

Fig. 5. ISL increases the release of cytochrome *c* and Smac/Diablo from mitochondria in prostate cancer cells. MAT-LyLu (A) and DU145 (B) cells were treated with ISL as described in Fig. 1 and subjected to subcellular fractionation. The resulting cytosolic and mitochondrial fractions were analyzed by Western blotting with antibodies raised against cytochrome *c*, Smac/Diablo or HSP 60. Photographs of the chemiluminescent detection of the blots, which were representative of three independent experiments, are shown. The relative abundance of each band was quantified, and the control levels were set at 100%. The adjusted mean±S.E.M. ($n=3$) of each band is shown above each blot. Means without a common letter differ, $P<.05$.

3.3. Isoliquiritigenin increases the release of cytochrome c and Smac/Diablo from mitochondria in prostate cancer cells

Cytosolic cytochrome *c* activates procaspase-9 by binding to Apaf1, which leads to caspase-9 activation, with the subsequent activation of the downstream executioner caspases, caspase-3 and caspase-7 [10]. Upon mitochondrial stress, Smac/Diablo is also released from mitochondria, which competes with caspases for binding of IAPs [11,12]. Because ISL induces activation of caspase-9, whether ISL alters the levels of cytochrome *c* and Smac/Diablo in the cytoplasm and mitochondria was then examined. Isoliquiritigenin-dependent increases in the levels of cytochrome *c* and Smac/Diablo in the cytoplasm, with concomitant decreases in these protein levels in mitochondria, were observed in both MLL (Fig. 5A) and DU145 (Fig. 5B) cells ($P<.05$).

3.4. Isoliquiritigenin increases the levels of Bax, Bad and truncated Bid in DU145 cells

The Bcl-2 family of proteins localize (or can be targeted) to mitochondria, which regulate the permeability of the mitochondrial outer membrane to cytochrome *c* and other apoptotic proteins [28]. Western blot analysis of DU145 cell lysates revealed that ISL increased the Bax levels in a concentration-dependent manner, but had no effect on the Bcl-2 levels (Fig. 6). However, the Bad levels increased with treatment of 10 μmol/L ISL and the truncated Bid (tBid) levels with treatment only at 20 μmol/L ISL ($P<.05$).

3.5. Isoliquiritigenin induces mitochondrial membrane depolarization in DU145 cells

To examine whether ISL induces caspase-9 activation in a time-related manner, we treated DU145 cells with 0 or

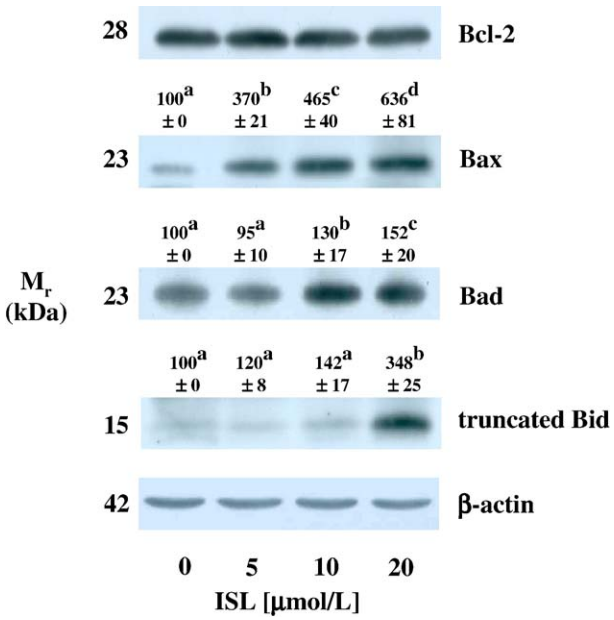

Fig. 6. Effect of ISL on Bcl-2 family protein levels in DU145 cells. DU145 cells were treated with isoliquiritigenin as described in Fig. 1. Cell lysates were subjected to Western blotting with antibodies raised against Bad, Bcl-2, Bax, tBid or β-actin. Photographs of the chemiluminescent detection of the blots, which were representative of three independent experiments, are shown. The relative abundance of each band to its own β-actin was quantified, and the control levels were set at 100%. The adjusted mean±S.E.M. ($n=3$) of each band is shown above each blot. Means without a common letter differ, $P<.05$.

20 μmol/L ISL for various periods. The ISL was observed to increase the cleaved form of caspase-9 within 6 h in DU145 cells (data not shown). Whether ISL induces mitochondrial membrane depolarization within 6 h was then examined. The ISL-treated cells were stained with JC-1, and the percentages of cells with green-positive and red-negative fluorescence were scored as depolarized cells. The number of cells with

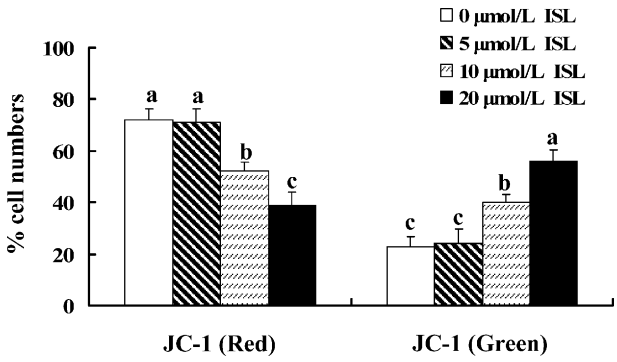

Fig. 7. ISL induces mitochondrial membrane depolarization in DU145 cells. Cells were cultured and treated for 6 h in serum-free medium containing 0, 5, 10 or 20 μmol/L ISL as described in Fig. 1. Cells were loaded with JC-1 and then analyzed by flow cytometry. The number of cells with normal polarized mitochondrial membranes (red) and cells with depolarized mitochondrial membranes (green) is expressed as a percentage of total cell number. The number of cells with intact mitochondria and depolarized mitochondrial membranes is expressed as a percentage of total cell number. Each bar represents the mean±S.E.M. ($n=6$). Means without a common letter differ, $P<.05$.

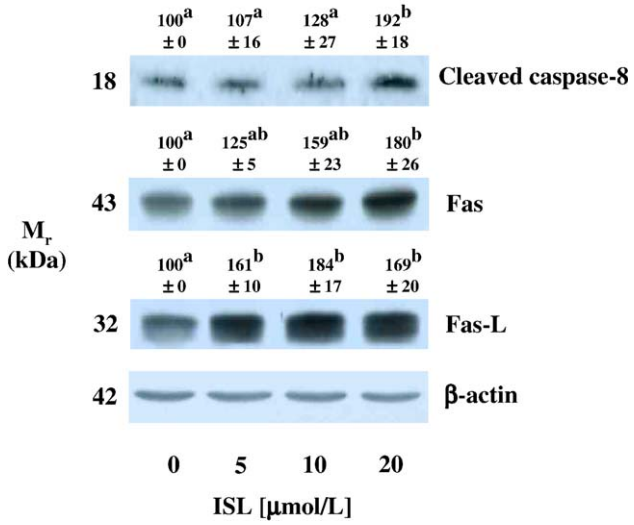

Fig. 8. ISL increases cleaved caspase-8 levels in DU145 cells. Cells were treated with ISL as described in Fig. 7. Cell lysates were analyzed by Western blotting with anti-cleaved caspase-8, Fas or FasL antibodies. Photographs of the chemiluminescent detection of the blots, which were representative of three independent experiments, are shown. The relative abundance of each band to its own β-actin was quantified, and the control levels were set at 100%. The adjusted mean±S.E.M. ($n=3$) of each band is shown above each blot. Means without a common letter differ, $P<.05$.

depolarized mitochondrial membranes was found to increase in an ISL concentration-dependent manner (Fig. 7).

3.6. Isoliquiritigenin increases the levels of cleaved caspase-8 and Fas in DU145 cells

Because ISL increased the tBid levels at a concentration of 20 μmol/L (Fig. 6), whether ISL increases the levels of cleaved caspase-8, membrane-bound FasL and Fas proteins was next examined using Western blotting of cell lysates. Treatment of cells with 20 μmol/L ISL was found to significantly increase the levels of Fas and cleaved caspase-8. However, the levels of membrane-bound FasL were increased in cells treated with only 5 μmol/L ISL (Fig. 8).

4. Discussion

Isoliquiritigenin has been reported to have potent anti-tumorigenic activity, including inhibition of colon carcinogenesis [29], skin papilloma formation [17] and pulmonary metastasis of mouse renal cell carcinomas [30]. To our knowledge, the effects of ISL on prostate cancer have not been studied in detail, with the exception of a study by Kanazawa et al. [31], who reported decreased growth and increased expression of GADD153 in LNCaP and DU145 cells in the presence of ISL. The present results confirmed those of Kanazawa et al. in that ISL inhibited prostate cancer cell growth at concentrations between 1 and 20 μmol/L. The strong in vitro effectiveness of ISL in androgen-insensitive DU145 prostate cancer cells suggests that this compound might be useful in the treatment of hormone-refractory prostate carcinomas.

Numerous studies have recently been performed to assess the mechanisms whereby ISL prevents cancer, including induction of cell-cycle arrest, apoptosis and antiproliferation, etc. One of the hallmarks of cancer is the deregulation of apoptotic or programmed cell death mechanisms usually found in normal cells, which allow for damaged cells to undergo cellular suicide. Isoliquiritigenin has been reported to induce apoptosis in hepatoma [19], gastric [20] and melanoma cancer cells [21]. Our present results demonstrated that ISL inhibits prostate cancer cell growth by the induction of apoptosis.

The morphological and biochemical features of apoptosis result from cellular degradation triggered by intracellular cysteine proteases (caspases), which cleave substrates after specific aspartate residues. These proteases are present in healthy cells as zymogens, which become fully activated in response to apoptotic signals [32]. The abundant nuclear enzyme, PARP, catalyses the synthesis of poly(ADP-ribose) from nicotinamide adenine dinucleotide (NAD+) [33], which is important for cells to maintain viability. This protein is one of the chief cleavage targets of caspase-3 [34], with cleavage of this enzyme facilitating cellular disassembly, serving as an indicator of cells undergoing apoptosis [35]. In the present study, ISL was found to increase the levels of cleaved caspase-9, caspase-7, caspase-3 and PARP, in dose-dependent manners, indicating that activation of caspases is one of the mechanisms by which ISL induces apoptosis in prostate cancer cells.

The vast majority of cell death signals engage the mitochondrial pathway, where the cysteine protease, caspase-9, is recruited and activated [8,9]. Activation of caspase-9 is mediated by the formation of the macromolecular complex, apoptosome, with the release of cytochrome *c* from mitochondria [10]. The present results indicate that ISL increases the release of cytochrome *c* from mitochondria, which, in turn, results in activation of caspase-9, leading to activation of caspases-3 and -7. Activation of the mitochondrial pathway leads to the release of Smac/Diablo, which removes the IAP blockage of caspase activation [16]. The IAP family, which includes X-linked IAP and survivin, functions by binding to and inhibiting several caspases [36]. Our results, which showed increases in Smac/Diablo in the cytoplasm, with corresponding decreases in mitochondria, suggest that the release of Smac/Diablo from mitochondria into the cytoplasm may have contributed to the ISL-induced apoptosis of prostate cancer cells. It remains to be determined whether Smac/Diablo in the cytosol actually increases the activities of caspases in prostate cancer cells.

The release of cytochrome *c* and Smac/Diablo from mitochondria due to ISL treatment was closely associated with mitochondrial depolarization, as observed from the JC-1 staining (Fig. 7). Evidence indicates that Bcl-2 acts to preserve the mitochondrial membrane, preventing the release of cytochrome *c* from mitochondria [37,38], whereas Bax and Bad promote the release of cytochrome *c* [15,16]. In addition, Bax induces Smac/Diablo release from mitochondria [16]. In the present study, ISL was found to increase the levels of Bax and Bad, but without changes in the Bcl-2 levels in DU145 cells. These changes may have increased the mitochondrial depolarization, leading to the release of cytochrome *c* and Smac/Diablo.

Bid, a BH3-only proapoptotic member of the Bcl-2 family, undergoes proteolysis due to caspase-8 activation by cell surface death receptors, such as Fas and TNF receptors [39,40]. The resulting tBid translocates to mitochondria and binds to Bax, leading to a conformational change of Bax and to the release of cytochrome *c* from mitochondria [40,41]. The cytochrome *c*-releasing activity of Bid has been reported to be antagonized by Bcl-2 [40]. Isoliquiritigenin increased tBid in DU145 cells at a concentration of 20 μmol/L (Fig. 6), suggesting that the increased tBid may be one of the reasons for the depolarization of mitochondrial membranes that leads to the release of cytochrome *c* and Smac/Diablo in ISL-treated cells.

Caspase-8 is a 55-kDa cytosolic protein produced as a proenzyme, which is proteolytically cleaved into smaller subunits of 43/41 (doublet) and 28/26 kDa upon activation [42]. Subsequent cleavage of the receptor-bound p43 results in formation of the prodomain, p26, and the release of the active site-containing fragment, p18 [43]. Our results, which showed increased levels of Fas, FasL, p18-cleaved caspase-8 and tBid in DU145 cells treated with ISL, suggest that a change in the Fas–FasL pathway may have contributed to ISL-induced apoptosis of prostate cancer cells. However, the changes in Fas, caspase-8 and tBid only occurred at a concentration of 20 μmol/L ISL, suggesting that the Fas–FasL pathway was activated only at higher concentrations of ISL.

Taken together, these data support a model in which ISL induces apoptosis by inducing mitochondrial membrane depolarization, thereby leading to caspase activation. The present data suggest that ISL may have efficacy, if delivered to the prostate at concentrations between 5 and 20 μmol/L, to treat prostate cancer.

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