Serenoa repens induces growth arrest and apoptosis of human multiple myeloma cells via inactivation of STAT 3 signaling

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Abstract. *Serenoa repens*, a palm species native to the Southeastern United States, is one of the widely used phytotherapeutic agents in benign prostatic hyperplasia. In this study, we found for the first time that *Serenoa repens* induced growth arrest of a variety of human leukemia cells including U266 and RPMI 8226 multiple myeloma cells as measured by mitochondrial-dependent conversion of the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide assay. TUNEL assays showed that *Serenoa repens* induced apoptosis of U266 cells in a time- and dose-dependent manner. *Serenoa repens* also increased the expression of cleaved-PARP or p27 protein in different human leukemia cell lines. In addition, we found that *Serenoa repens* down-regulated basal level of phosphorylated form of signal transducer and activator of transcription 3 (STAT 3) and Interleukin-6 induced level of phosphorylated form of STAT 3 and extracellular signal-related kinase (ERK) were also reduced after *Serenoa repens* treatment in U266 cells. Furthermore, we found that inhibition of STAT 3 signaling by *Serenoa repens* or Janus family of tyrosine kinase (JAK) inhibitor of AG490 enhanced the ability of docetaxel to inhibit the growth of U266 and RPMI 8226 cells, as measured by trypan blue exclusion test. These results indicate that *Serenoa repens* might be useful for the treatment of individuals with multiple myeloma.

Introduction

Multiple myeloma (MM) is a malignant plasma cell disorder that accounts for ~10% of all hematologic cancers and it is the second most common hematologic malignancy in the United States (1,2). The characteristic findings in MM are lytic bone disease, renal insufficiency, anemia, hypercalcemia and immunodeficiency (3). Despite previous new insights into the pathogenesis of MM, it remains incurable with a median survival of ~3 to 4 years with conventional chemotherapies and 4 to 5 years with high-dose chemotherapy followed by autologous stem-cell transplantation (4). Therefore, novel treatment strategies for MM are urgently needed.

Signal transducers and activators of transcription (STATs) were originally discovered as latent cytoplasmic proteins with roles as signal messengers and transcription factors that participate in normal cellular responses to cytokines and growth factors (5-7). STATs are activated by phosphorylation of tyrosine and serine residues by upstream kinases (8). This signaling has been demonstrated to play a major role in tumor formation and aberrant signaling of STAT 3 is found in many types of malignancies including multiple myeloma, head and neck cancer, breast cancer and prostate cancer (9-12).

Phytotherapy have been used for many centuries for treatment of cancer (13,14). Use of these therapies has been dramatically rising in previous years in the United States (15). One of the phytotherapeutic agents frequently used by individuals with benign prostatic hyperplasia is *Serenoa repens* (also named Saw Palmetto) and it has also been shown to possess anti-tumor activity (16-18). For example, *Serenoa repens* was shown to inhibit growth and induce apoptosis of prostatic cancer cells in studies *in vitro* (19-21). *Serenoa repens* effectively induced a dose-dependent anti-proliferative effect on human breast cancer cells (22). However, the mechanism of *Serenoa repens* for inhibiting growth of cancer cells remains to be fully elucidated.

In this study, we found that *Serenoa repens* inhibited the proliferation of a variety of human leukemia cells including U266 and RPMI 8226 multiple myeloma cells. We also found that *Serenoa repens* inhibited basal level of phosphorylated form of STAT 3 and Interleukin-6 (IL-6) induced level of phosphorylated form of STAT 3 in U266 cells suggesting that *Serenoa repens* may induce growth arrest and apoptosis of human multiple myeloma cells through inactivation of STAT 3 signaling. *Serenoa repens* might be useful for treatment of individuals with human multiple myeloma.

Materials and methods

Cell line. Human acute leukemia cell lines (HL-60, NB4, Jurket) and multiple myeloma cell lines (U266, RPMI 8226)

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Abbreviations: BPH, benign prostatic hyperplasia; ERK, extracellular signal-related kinase; IL-6, Interleukin-6; JAK, Janus family of tyrosine kinase; MM, multiple myeloma; PARP, poly (ADP-ribose) polymerase; SR, *Serenoa repens*; STAT 3, signal transducer and activator of transcription 3

Key words: *Serenoa repens*, multiple myeloma, apoptosis, signal transducer and activator of transcription 3

were grown in RPMI-1640 supplemented with 10% fetal bovine serum (heat inactivated at 56˚C for 45 min) and an appropriate amount of penicillin/streptomycin in a 37˚C incubator with a humidified, 5% CO_2 atmosphere.

Chemical. Serenoa repens was extracted from the fruit of *Serenoa repens* and one capsule of *Serenoa repens* contains 400 mg of powered herbal extracts. Stock solutions of *Serenoa repens* were prepared by exposing these herbal extracts to 70% ethanol (one capsule/1 ml of 70% ethanol).

MTT assays. Cells (10^4/ml) were incubated with a variety of concentrations of *Serenoa repens* (0-2 μl/ml) for 3 days in 96-well plates (Flow Laboratories, Irvine, CA). After culture, cell numbers and viability were evaluated by measuring the mitochondrial-dependent conversion of the tetrazolium salt, MTT (Sigma), to a colored formazan product, as previously described (23).

Trypan blue exclusion test. MM cells ($2x10^5$/ml) were incubated with various concentrations of *Serenoa repens* (0-2 μl/ml) for 3 days in 96-well plates (Flow Laboratories). After culture, cell numbers and viability were evaluated by staining with trypan blue and counting using light microscopy.

Flow cytometry assay. The DNA content of cultured cells was analyzed by flow cytometry assay. Apoptotic cells have a lower DNA content than normal cells and appear as a pre-G_1 peak on a DNA cell-cycle histogram and cells were harvested to quantitate the amount of apoptosis present after various treatments. In brief, cells were trypsinized and washed with cold phosphate-buffered saline (PBS). Cells were then fixed with 70% ethanol and stored overnight at -20˚C. Propidium iodide (10 μg/ml; Sigma) was added to stain cells in the presence of RNase (Promega, Madison, WI) at 37˚C for 10 min and cells were then analyzed on a FACscan flow cytometer with manual gating using CellQuest software.

Assessment of apoptosis. Apoptotic cell death was examined by terminal deoxyribonucleotide transferase-mediated dUTP nick end-labeling (TUNEL) method using the In Situ Cell Death Detection kit (Roche Molecular Biochemicals, Germany), as previously described (24). For quantification three different fields were counted under the microscope and at least 300 cells were counted in each field. All experiments were performed at least twice.

Western blotting. Lysates were made by standard methods, as previously described (25). Equal amounts of proteins were resolved by 9-15% sodium dodecyl sulfate (SDS)-polyacrylamide gel, transferred to an immobilon polyvinylidene difuride membrane (Amersham Corp., Arlington Heights, IL), and probed sequentially with antibodies. Anti-PARP (Santa Cruz Biotechnology, Santa Cruz, CA), anti-Mcl-1 (sc-819, Santa Cruz Biotechnology), anti-$p27^{kip1}$ (sc-527, Santa Cruz Biotechnology), anti-STAT 3 (Santa Cruz Biotechnology), anti-p-STAT 3 (Cell Signaling, Beverly, MA), anti-ERK (Santa Cruz Biotechnology), anti-p-ERK (Cell Signaling), anti-ß-actin antibodies (Santa Cruz Biotechnology) were used. The blots were developed using the enhanced chemiluminescence kit (Amersham Corp.).

Statistical analysis. Statistical analysis was performed to assess the difference between two groups under multiple conditions by one-way analysis of variance (ANOVA) using PRISM statistical analysis software (GraphPad Software, Inc., San Diego, CA).

Results

Effect of Serenoa repens on proliferation of MM cells. To explore the anti-proliferative effects of *Serenoa repens* on MM cells, U266 and RPMI 8226 MM cell lines were cultured in the presence of a various concentrations of *Serenoa repens* (0-2 μl/ml). *Serenoa repens* effectively inhibited the growth of U266 and RPMI 8226 cells with the effective dose that inhibited 50% growth (ED_{50s}) of ~0.7 and 1.2 μl/ml, respectively as measured by MTT assay on the third day of culture (Fig. 1A).

Serenoa repens induced apoptosis of MM cells. Next, flow cytometry was performed to analyze changes in cell death of MM cells in response to *Serenoa repens* treatment. The cells with DNA content smaller than G_0/G_1 were considered to be apoptotic cells. *Serenoa repens* showed dose-dependent apoptotic in U266 and RPMI 8226 cells, respectively ($p<0.05$) (Fig. 1B and C). A TUNEL assay was utilized to further test the pro-apoptotic effect of *Serenoa repens*. After 24 h culture, 0.5 and 1.0 μl/ml of *Serenoa repens* induced a mean 9.0±0.7 and 18.4±3.2% of U266 cells to become apoptotic and which increased to a mean of 30.0±3.9 and 45.3±5.0% of U266 cells to become apoptotic, respectively, after another 24 h culture showing that *Serenoa repens* induced apoptosis of these cells in a dose- and time-dependent manner ($p<0.05$) (Fig. 1D).

Effect of Serenoa repens on expression of apoptotic-related protein in MM cells. Furthermore, the proapoptotic effect of *Serenoa repens* was explored by examining for the cleavage of poly (ADP-ribose) polymerase (PARP) which is thought to be a late event in apoptosis. As shown in Fig. 1E, U266 and RPMI 8226 cells were exposed to *Serenoa repens* (1.0 μl/ml, 24 h) and Western blot analysis of cellular lysates showed cleaved of PARP band in RPMI 8226 cells (Fig. 1E). Other apoptotic-related proteins such as Mcl-1 and $p27^{kip1}$ were also detected in these two cell lines. For example, exposure of U266 cells to *Serenoa repens* (1 μl/ml, 24 h) decreased the level of Mcl-1 by 60.0% compared with that in control cells. Whereas, *Serenoa repens* (1 μl/ml, 24 h) did not modulate level of Mcl-1 in RPMI 8226 cells (Fig. 1E). Level of the $p27^{kip1}$ protein was negligible in RPMI 8226 cells and exposure of U266 cells to *Serenoa repens* (1 μl/ml, 24 h) resulted in a dramatic increased on $p27^{kip1}$ protein level (Fig. 1E). These results indicate that *Serenoa repens* induces growth arrest and apoptosis of human MM Cells.

Effect of Serenoa repens on proliferation and apoptosis of human leukemia cells. The effects of *Serenoa repens* on other malignant hematopoietic cells apart from its role in

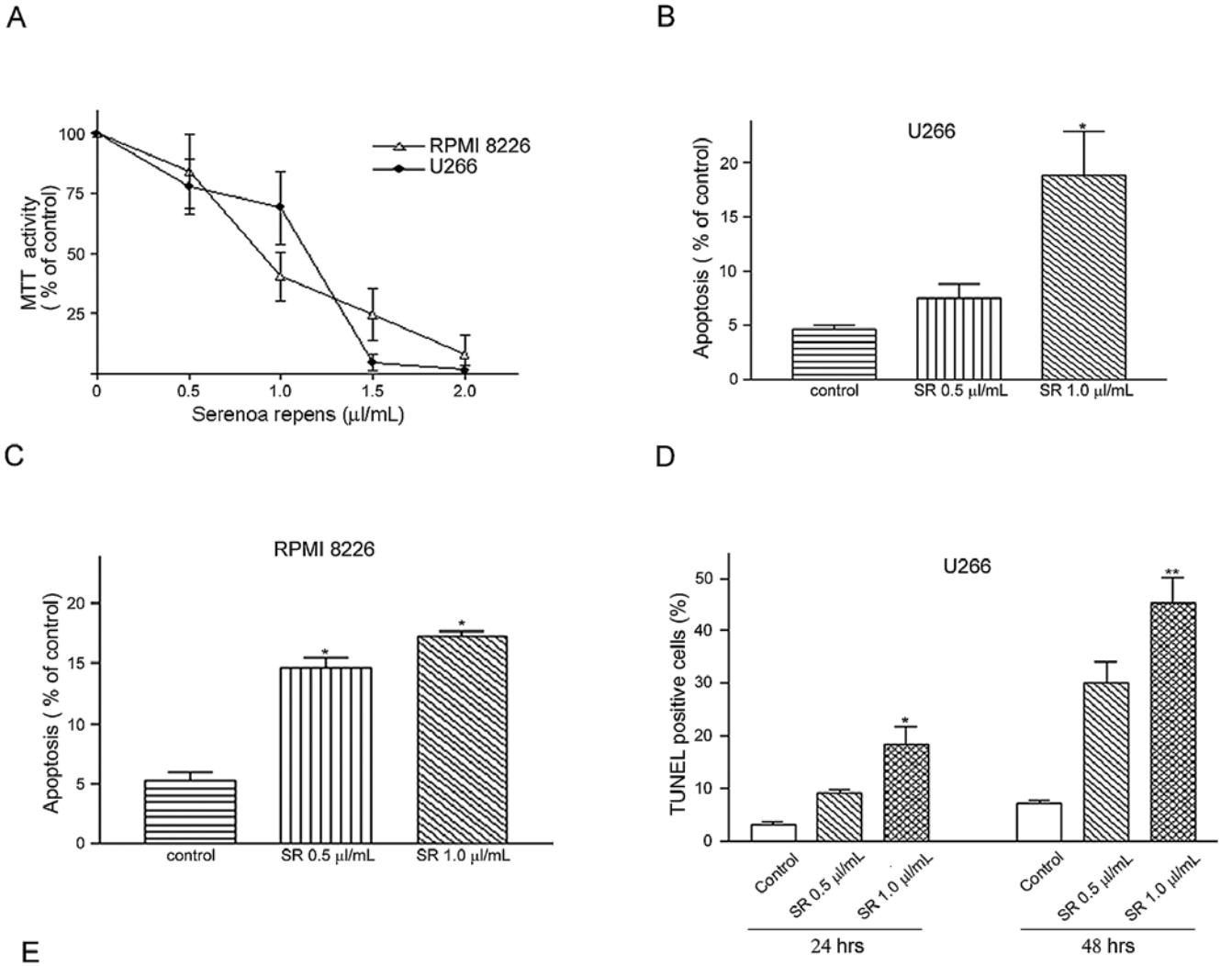

Figure 1. Effect of *Serenoa repens* on proliferation and apoptosis of MM cells. (A) MTT assay. U266 and RPMI 8226 cells (10^5/ml) were plated in a 96-well plate and cultured with various concentrations of *Serenoa repens* (0-2.0 μl/ml). After 48 h, the cells were treated with MTT for 4 h at 37˚C and MTT activity was measured. Results represent the mean ± SD of three experiments performed in triplicate. (B and C) Flow cytometry assay. U266 (B) or RPMI 8226 (C) cells were incubated for 24 h either with or without *Serenoa repens* (0.5 or 1 μl/ml) and then harvested, fixed with 70% methanol, stained with propidium iodide, and subjected to flow cytometric analysis to determine the percentages of cells in pre-G_1 phase (represent apoptotic cells). Results represent mean ± SD of three experiments performed in duplicate. *P<0.05. (D) TUNEL assay. U266 cells were plated in a 24-well plate and cultured either with or without *Serenoa repens* (0.5 or 1 μl/ml); after 24 or 48 h, apoptosis was measured by TUNEL assay. Results represent the mean ± SD of two experiments performed in triplicate. *P<0.05, **P<0.005. (E) Western blot analysis. U266 and RPMI 8226 cells were cultured with either *Serenoa repens* (1 μl/ml) or control diluent. After 24 h, cells were harvested and subjected to Western blot analysis. Membrane was sequentially probed with anti-PARP, Mcl-1, -$p27^{kip1}$ and -ß-actin antibodies. For Western blotting assay, ß-actin is shown as a loading control. Protein bands were scanned by phosphorimaging and the relative band intensities were normalized to each ß-actin band. The band intensity of untreated sample was set as 1. The numerical value of each sample represents the percentage of band intensity relative to that of untreated sample.

the proliferation and apoptosis of human MM cells were explored in the human acute myelogenous leukemia (HL-60, NB4) and acute lymphoblastic T-cell leukemia cells (Jurkat) in this study. As shown in Fig. 2A, *Serenoa repens* effectively inhibited the growth of NB4 and Jurkat cells with the effective dose that inhibited 50% growth (ED_{50s}) of ~1.3 and 0.8 μl/ml, respectively as measured by MTT assay on the third day of culture (Fig. 2A). *Serenoa repens* partly inhibited the growth of HL-60 cells although the inhibiting effect was weaker than the other two cell lines (Fig. 2A).

Next, apoptotic-related proteins such as PARP and $p27^{kip1}$ were detected in these cells. As shown in Fig. 2B, the

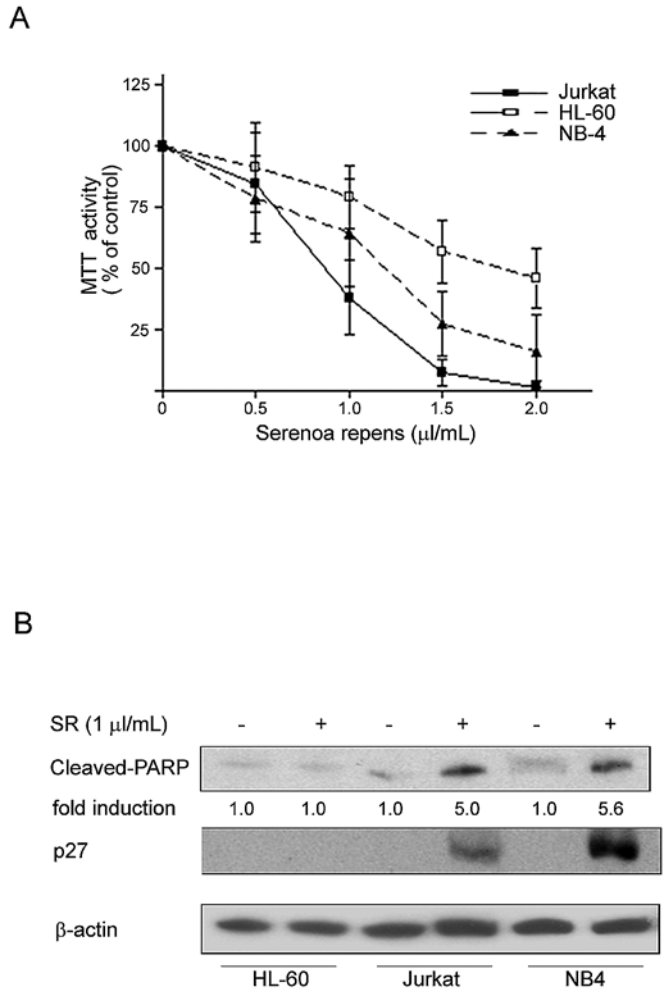

Figure 2. Effect of *Serenoa repens* on the proliferation and apoptosis of human leukemia cells. (A) MTT assay. Human leukemia HL-60, NB4 and Jurkat cells (10^5/ml) were plated in a 96-well plate and cultured with a various concentrations of *Serenoa repens* (0-2.0 μl/ml). After 48 h, the cells were treated with MTT for 4 h at 37˚C and MTT activity was measured. Results represent the mean ± SD of three experiments performed in triplicate. (B) Western blot analysis. HL-60, NB4 and Jurkat cells were cultured with either *Serenoa repens* (1 μl/ml) or control diluent. After 24 h, cells were harvested and subjected to Western blot analysis. Membrane was sequentially probed with anti-PARP, -p27^{kip1} and -ß-actin antibodies. For Western blot assay, ß-actin is shown as a loading control. Protein bands were scanned by phosphorimaging and the relative band intensities are shown.

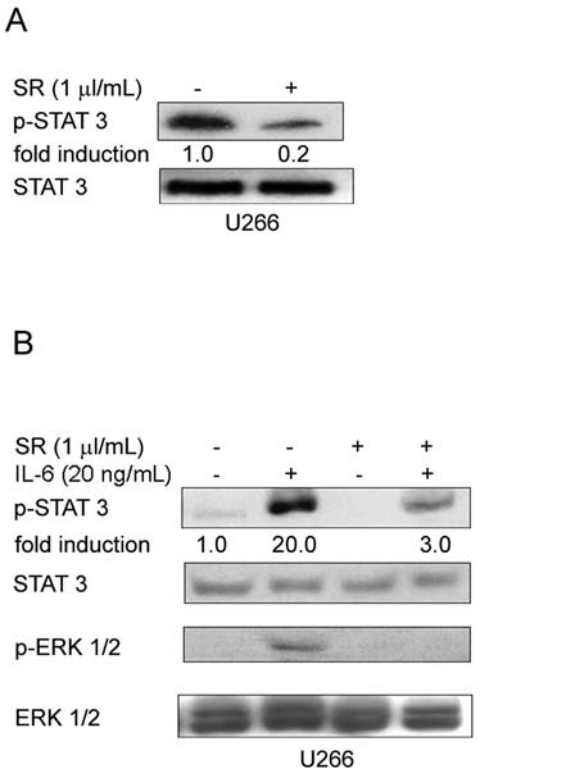

Figure 3. Effect of *Serenoa repens* on IL-6-stimulated phosphorylation of STAT 3 and ERK in MM cells. (A) Western blot analysis. U266 cells were cultured with either *Serenoa repens* (1 μl/ml) or control diluent for 24 h and then harvested to Western blot analysis. Membrane was sequentially probed with anti-p-STAT 3, -STAT 3 antibodies. (B) Western blot analysis. U266 cells were cultured with either *Serenoa repens* (1 μl/ml) or control diluent. After 3 h, cells were exposed to IL-6 (20 ng/ml) for 30 min, harvested, subjected to Western blot analysis and membrane was sequentially probed with anti-p-STAT 3, -p-ERK, -STAT 3 and -ERK antibodies. For each Western blot assay, STAT 3 or ERK is shown as a loading control. Protein bands were scanned by phosphorimaging and the relative band intensities are shown.

cleaved bands of PARP were increased by 5.0- and 5.6-fold, respectively, in Jurkat and NB4 cells after *Serenoa repens* (1.0 μl/ml, 24 h) treatment. Exposure of Jurkat and NB4 cells to *Serenoa repens* (1 μl/ml, 24 h) resulted in a dramatic increase of p27^{kip1} protein level (Fig. 2B). On the other hand, no matter the level of cleaved PARP, p27^{kip1} was increased in HL-60 cells (Fig. 2B). These findings were consistent with the resultant proliferation as determined by MTT assay showing that *Serenoa repens* has an effect on proliferation and apoptosis of human acute leukemia cells (Fig. 2A).

Effect of Serenoa repens on IL-6-stimulated phosphorylation of STAT 3 and ERK in MM cells. A recent study showed that *Serenoa repens* induced growth arrest and apoptosis of prostate cancer cells via inactivation of STAT 3 signaling (25). We next explored the effect of *Serenoa repens* on STAT 3 signaling in U266 cells. U266 cells were exposed to *Serenoa repens* (1.0 μl/ml, 24 h) and then harvested for Western blot analysis. As shown in Fig. 3A, U266 cells constitutively expressed the phosphorylated form of STAT 3 and the expression of phosphorylated form of STAT 3 was reduced by 80.0% after *Serenoa repens* treatment (Fig. 3A). U266 cells were serum-starved for 24 h and exposure of these cells to IL-6 (20 ng/ml, 30 min) dramatically increased the level of the phosphorylated form of STAT 3 by 20.0-fold, and pretreatment (3 h) of these cells with 1.0 μl/ml of *Serenoa repens* blocked the IL-6-induced phosphorylation of STAT 3 by 85.0% (Fig. 3B). In addition, we explored the effect of *Serenoa repens* on phosphorylation of ERK mediated by IL-6 in U266 myeloma cells. Levels of the phosphorylated form of ERK 1/2 were negligible in control U266 cells which were serum-starved for 24 h. Addition of IL-6 (20 ng/ml, 30 min) to these cells induced phosphorylation of ERK and pre-treatment of these cells with 1.0 μl/ml of *Serenoa repens* for 3 h almost completely blocked the IL-6-induced phosphorylation of ERK1/2 (Fig. 3B). These data indicate that *Serenoa repens* may reduce the expression of phosphorylation of STAT 3 or ERK mediated by IL-6 in MM cells.

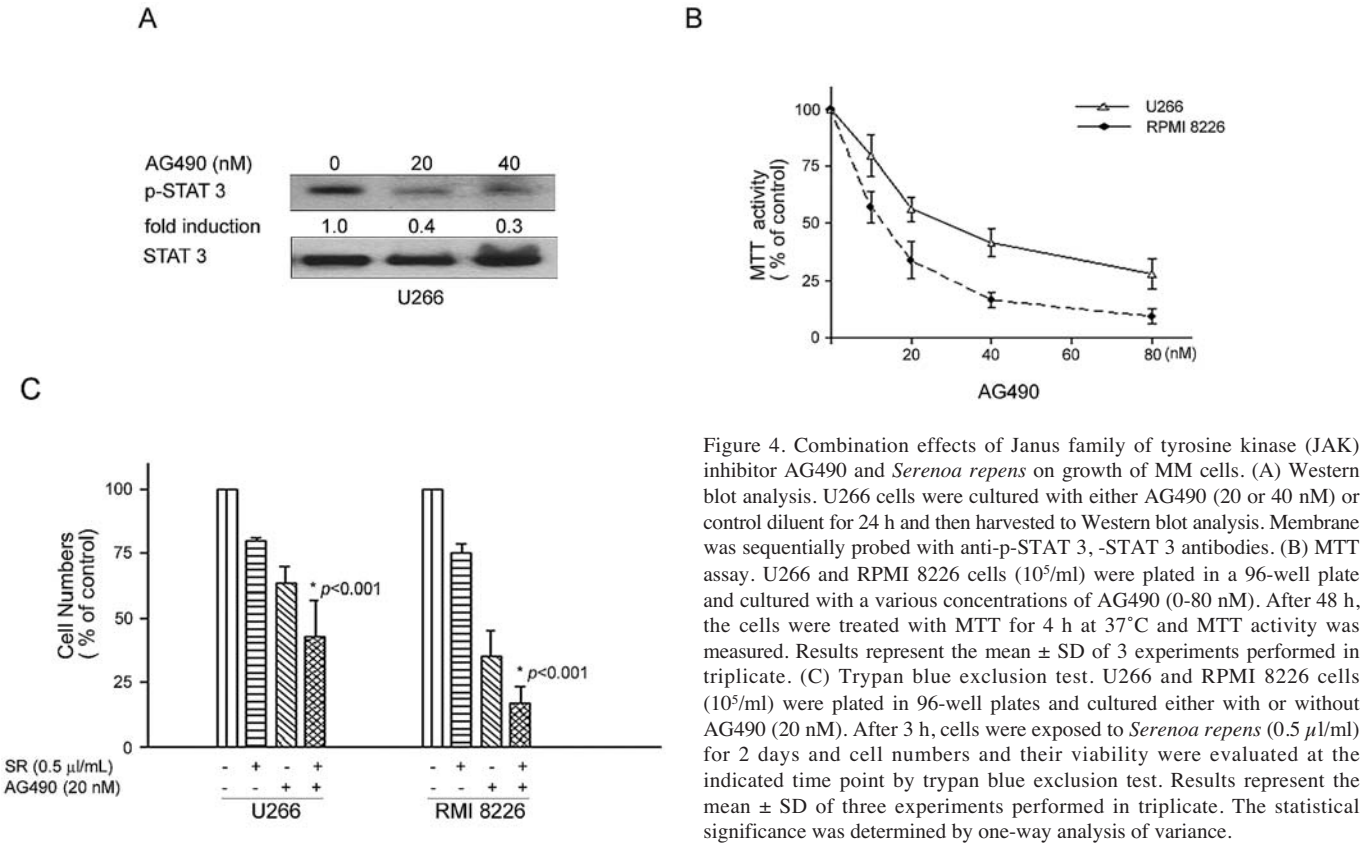

Figure 4. Combination effects of Janus family of tyrosine kinase (JAK) inhibitor AG490 and *Serenoa repens* on growth of MM cells. (A) Western blot analysis. U266 cells were cultured with either AG490 (20 or 40 nM) or control diluent for 24 h and then harvested to Western blot analysis. Membrane was sequentially probed with anti-p-STAT 3, -STAT 3 antibodies. (B) MTT assay. U266 and RPMI 8226 cells (10^5/ml) were plated in a 96-well plate and cultured with a various concentrations of AG490 (0-80 nM). After 48 h, the cells were treated with MTT for 4 h at 37˚C and MTT activity was measured. Results represent the mean ± SD of 3 experiments performed in triplicate. (C) Trypan blue exclusion test. U266 and RPMI 8226 cells (10^5/ml) were plated in 96-well plates and cultured either with or without AG490 (20 nM). After 3 h, cells were exposed to *Serenoa repens* (0.5 μl/ml) for 2 days and cell numbers and their viability were evaluated at the indicated time point by trypan blue exclusion test. Results represent the mean ± SD of three experiments performed in triplicate. The statistical significance was determined by one-way analysis of variance.

Combination effects of Janus family of tyrosine kinase (JAK) inhibitor AG490 and Serenoa repens on growth of MM cells. To study the role of STAT 3 signaling in survival of MM cells, we blocked this pathway using a Janus family of tyrosine kinase (JAK) inhibitor AG490. As shown in Fig. 4A, the expression of phosphorylated form of STAT 3 was effectively reduced after AG490 (20-40 nM, 48 h) treatment. AG490 significantly inhibited the proliferation of U266 and RPMI 8226 cells in a dose-dependent manner with ED_{50s} of ~28.45 and 13.06 nM, respectively as measured by MTT assay (Fig. 4B). We next studied the interaction of AG490 and *Serenoa repens* on growth of MM cells. As shown in Fig. 4C, U266 and RPMI 8226 cells were pre-incubated with AG490 (20 nM) for 3 h and then exposed to *Serenoa repens* (0.5 μl/ml) and cultured for 48 h. For example, *Serenoa repens* or AG490 alone inhibited the growth of U266 cells by 79.7±1.1 and 67.2±6.1%, respectively (Fig. 4C). The combination of AG490 and *Serenoa repens* inhibited the growth of U266 cells by 42.9±12.0% comparing with that of control cells showing that Janus family of tyrosine kinase inhibitor AG490 significantly enhances the ability of *Serenoa repens* to inhibit the growth of MM cells ($p<0.001$).

Inhibition of STAT 3 signaling by Serenoa repens or AG490 sensitized MM cells to anti-proliferative effect of docetaxel. Docetaxel has activity against multiple malignancies including MM (26). Therefore, we explored whether inhibition of STAT 3 signaling by either *Serenoa repens* or AG490 sensitized MM cells to docetaxel-mediated growth inhibition. The U266 and RPMI 8226 cells were pre-incubated with *Serenoa repens* (0.5 μl/ml) for 3 h and exposed to docetaxel (10^{-9} M). After 48 h, cell numbers were assessed by trypan blue exclusion test (Fig. 5A). Either *Serenoa repens* (0.5 μl/ml) or docetaxel (10^{-9} M) alone inhibited the growth of U266 cells by 79.7 or 90.1%, respectively. The combination of *Serenoa repens* and docetaxel inhibited the growth of these cells by 51.6% compared to control cells (Fig. 5A, left panel). Statistical analysis with ANOVA showed significant difference between either drug alone and combination of both ($p<0.01$). Similarly, *Serenoa repens* enhanced anti-proliferative activity of docetaxel against RPMI 8226 cells (Fig. 5A, right panel). We next studied the interaction of AG490 (JAK inhibitor) and docetaxel (Fig. 5B). For example, RPMI 8226 cells were pre-incubated with AG490 (20 nM) for 3 h and exposed to docetaxel (10^{-9} M, 48 h). Either AG490 or docetaxel alone inhibited the growth of RPMI 8226 cells by 34.8 or 84.6%, respectively. When cells were exposed to the combination of both, the cell growth was inhibited by 23.9% compared to control cells ($p<0.001$) (Fig. 5B, right panel). The enhanced anti-proliferative effect was also observed when U266 cells were treated in combination with AG490 (20 nM) and docetaxel (10^{-9} M) (Fig. 5B, left panel). These data indicate that both *Serenoa repens* and AG490 sensitize MM cells to anti-proliferative effect of docetaxel through inhibition of STAT 3 signaling.

Discussion

Phytotherapy (including herbal) have been used for many centuries for treatment of cancer. For example, Chinese herbal mixture denoted PC-SPES was shown to inhibit the growth of different kinds of cancer cells such as non-small cell lung

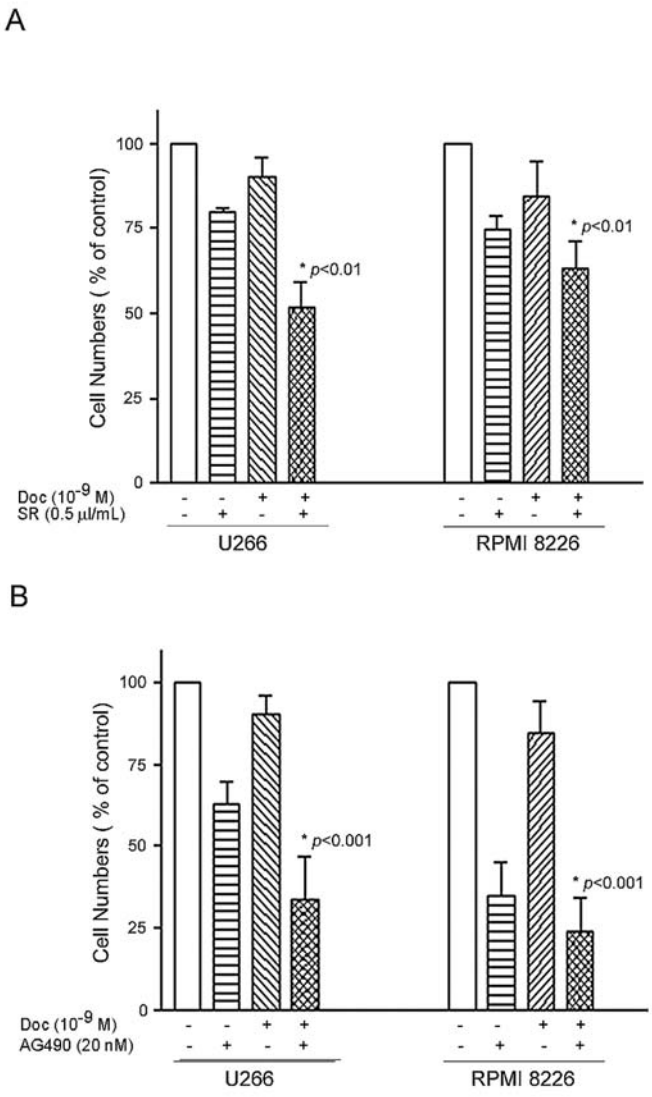

Figure 5. Inhibition of STAT 3 signaling by *Serenoa repens* or AG490-sensitized MM cells to anti-proliferative effect of docetaxel. (A) Trypan blue exclusion test. U266 and RPMI 8226 cells (10^5/ml) were plated in 96-well plates and cultured either with or without *Serenoa repens* (0.5 μl/ml) for 3 h and then cells were exposed to docetaxel (10^{-9} M) for 2 days. Cell numbers and their viability were evaluated at the indicated time point by trypan blue exclusion test. Results represent the mean ± SD of three experiments performed in triplicate. (B) Trypan blue exclusion test. U266 and RPMI 8226 cells (10^5/ml) were plated in 96-well plates and cultured either with or without AG490 (20 nM). After 3 h, cells were exposed to docetaxel (10^{-9} M) for 2 days, cell numbers and their viability were evaluated at the indicated time point by trypan blue exclusion test. Results represent the mean ± SD of three experiments performed in triplicate. The statistical significance was determined by one-way analysis of variance.

cancer cells and prostate cancer cells (27,28). Recently, another herbal *Rabdosia rubescens* was reported to induce apoptosis of lung cancer cells or to inhibit the growth of breast cancer cells (29,30). Oridonin purified from *Rabdosia rubescens* was also to be found to induce growth inhibition and apoptosis of a variety of human cancer cells (31). Baicalin, a major component of PC-SPES, inhibited the proliferation of human cancer cells such as prostate cancer cell lines, breast cancer cell lines, myeloblastic leukemia cell lines and promyelocytic leukemia cell lines (32). In addition, recent data showed that *Serenoa repens* induced growth arrest and apoptosis of androgen-dependent prostate cancer LNCaP cells (25). It is worthwhile to explore the effect of *Serenoa repens* on other type of cancer cells. In this study, we found that *Serenoa repens* effectively inhibited the growth of a variety of human leukemia cells including U266 and RPMI 8226 multiple myeloma cells (Figs. 1A and 2A). Flow cytometry assay and TUNEL assays showed that *Serenoa repens* induced apoptosis of U266 cells in a time- and dose-dependent manner (Fig. 1B and D). Moreover, *Serenoa repens* up-regulated the level of p27 and cleaved PARP in a variety of human leukemia cells (Figs. 1E and 2B). These results indicated for the first time that *Serenoa repens* is one of the effectively phyto-therapeutic agents inducing growth arrest and apoptosis of human leukemia especially multiple myeloma.

Previous studies have demonstrated that STAT 3 signaling has a critical role in tumor formation and this signaling can be activated by IL-6 (33-37). IL-6 is a pleiotropic cytokine which stimulates the proliferation of MM cells in either an autocrine or a paracrine fashion and acts as an anti-apoptotic factor in these cells (38) and MM cells often have enhanced expression of IL-6 resulting in activation of both JAK/STAT and Ras/ERK signal pathways (39,40). In this study, we found for the first time that *Serenoa repens* down-regulated the phosphorylated form of STAT 3 in U266 cells (Fig. 3A). Also, IL-6-induced the level of phosphorylated form of STAT 3 and ERK were reduced after *Serenoa repens* treatment in U266 cells (Fig. 3B). To block the STAT 3 pathway, JAK inhibitor AG490 was used and the proliferation of U266 and RPMI 8226 cells was effectively inhibited in a dose-dependent manner (Fig. 4B). We also found that the interaction of AG490 and *Serenoa repens* effectively inhibited the growth of these MM cell lines (Fig. 4C). These data were similar to a previous study showing that Saw Palmetto induced growth arrest and apoptosis of androgen-dependent prostate cancer LNCaP cells via inactivation of STAT 3 signaling (25), suggesting that STAT 3 signaling may play an important role in survival of MM cells and *Serenoa repens* might inhibit MM cell growth via inhibition of this signaling.

Docetaxel has activity against multiple malignancies including MM (26). A previous study indicated that docetaxel had the ability to synergize the anti-tumor activity of anti-cancer drug R115777 in MM cells. The treatment of docetaxel with R115777 in MM cells from patients with multiple myeloma was much more beneficial than that of single agents treatment (41). Therefore, we explored the combined effect of *Serenoa repens* with docetaxel in MM cells. The combination of *Serenoa repens* or AG490 with docetaxel effectively inhibited the growth of U266 and RPMI 8226 cells (Fig. 5). These data indicated that inhibition of STAT 3 signaling by *Serenoa repens* or AG490 sensitized MM cells to docetaxel-mediated growth inhibition.

In summary, we found that *Serenoa repens* was an important phytotherapeutic drug against multiple myeloma cells through inhibition of STAT 3 signaling. *Serenoa repens* may be useful as an adjunctive therapeutic agent for treatment of individuals with multiple myeloma and other types of cancer in which STAT 3 signaling is activated.

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