Guggulsterone inhibits angiogenesis by blocking STAT3 and VEGF expression in colon cancer cells

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Abstract. The plant sterol guggulsterone has been shown to exert anti-tumor effects, making it a candidate chemotherapeutic agent. We investigated the anti-tumor effects of guggulsterone on colon cancer cells and elucidated the underlying molecular mechanisms related to angiogenesis. The apoptotic effects of guggulsterone were examined by cell survival assay. Western blot analysis was used to determine the levels of various down-stream intracellular proteins involved in angiogenesis, including signal transducer and activator of transcription 3 (STAT3), vascular endothelial growth factor (VEGF), hypoxia-inducible factor-1α (HIF-1α) and aryl hydrocarbon receptor nuclear translocator (ARNT). Using chromatin immunoprecipitation assay, we tested whether guggulsterone affects the recruitment of STAT3, ARNT and HIF-1α to the human VEGF promoter. To investigate the effect of guggulsterone on vascular endothelial cell migration and invasion, tube formation and migration assays were conducted using human umbilical vein endothelial cells (HUVECs). Matrix metalloproteinase (MMP)-2 and -9 activities were measured by gelatin zymography. Guggulsterone significantly reduced cell viability in colon cancer cells in a dose-dependent manner and blocked VEGF, ARNT and STAT3 expression prominently in hypoxic conditions. The recruitment of STAT3 and ARNT, but not HIF-1α, to the VEGF promoter was inhibited by guggulsterone treatment. HUVECs produced much foreshortened and severely broken tubes and showed decreased migration activity under guggulsterone effects. In addition, zymography revealed that MMP-2 and -9 enzyme activities were markedly lower in the presence of guggulsterone. The results of this study suggest that guggulsterone not only induces apoptosis, but also inhibits angiogenesis and metastasis in colon cancer cells by blocking STAT3 and VEGF expression, suggesting its therapeutic potential in the treatment of colorectal cancer.

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Introduction

Colorectal cancer is one of the leading causes of cancer mortality worldwide (1,2). Although ~70-80% of patients are eligible for curative surgical resection at the time of diagnosis, ~50% of all newly diagnosed patients ultimately develop metastatic disease (3). These patients eventually receive systemic chemotherapy, but despite significant advances in the development of chemotherapeutic agents, no such drugs for the treatment of colorectal cancer have a non-relapsing cure rate. Current palliative chemotherapy aims only to improve survival and quality of life in patients with advanced colorectal cancer (4). New and specific therapies for unresectable colorectal cancer are urgently needed.

Angiogenesis, the formation of new vessels from existing capillary beds, is a central mechanism in human colorectal cancer development and growth (5,6). In particular, vascular endothelial growth factor (VEGF) is closely associated with the induction of the neovasculature in human colon cancer (7). VEGF is one of the most important endogenous ligands of receptors present on the endothelial cell surface and its binding leads to the initiation of intracellular signal translocation and gene transcription, eventually resulting in endothelial proliferation, active locomotion and the degradation of the extracellular matrix by secretion of proteases, including matrix metalloproteinase (MMP) (8). VEGF is self-produced by neoplastic cells and elevated VEGF levels correlate with a poor clinical outcome (9). A growing body of evidence suggests that modulation of the VEGF pathway could be a primary target for the treatment of advanced colorectal cancer (10). In addition, signal transducer and activator of transcription 3 (STAT3) has been gaining attention as a new molecular target of cancer therapy because of its crucial role in angiogenesis through the modulation of hypoxia-inducible factor-1 (HIF-1)-mediated VEGF expression (11,12).

Plant extracts are a common source for many currently available pharmaceutical products and some phytochemicals

have demonstrable anti-cancer properties both *in vivo* and *in vitro* (13). In Ayurveda, the traditional Indian medicinal system, the gum resin (guggel) of the tree *Commiphora mukul* has been used for thousands of years to treat arthritis, obesity, disorders of lipid metabolism, hypothyroidism and inflammation (14). The active compounds in this extract are the isomers E- and Z-guggulsterone [4,17(20)-pregnadiene-3, 16-dione]. Previously, several investigations have revealed that guggulsterone acts against tumors by inducing apoptosis in various cancer cell types (15,16). While these studies demonstrate the anti-tumor activities of guggulsterone, it remains unknown whether guggulsterone modulates the angiogenesis pathway, especially in colon cancer cells.

We hypothesized that guggulsterone could induce apoptosis and inhibit VEGF in colon cancer cells and that it might represent a possible chemotherapeutic agent for colorectal cancer. Accordingly, we investigated the effect of guggulsterone on tumor apoptosis and angiogenesis in human colon cancer cells and attempted to elucidate the mechanisms behind its actions, paying special attention to its association with STAT3 and HIF-1.

Materials and methods

Cell culture and treatments. The human colon cancer cell line HT-29 [KCLB 30038, Korean Cell Line Bank (KCLB), Seoul, Korea] was used between passages 20 and 40 and maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 1% antibiotics. Human umbilical vein endothelial cells (HUVECs) were obtained from Angiolab Corp. (Daejeon, Korea) and grown in M199 medium (Gibco BRL, Gaithersburg, MD, USA) supplemented with 10% FBS, 50 μg/ml endothelial cell growth supplement (Sigma, St Louis, MO, USA), heparin and antibiotics. HUVECs were used between passages 5 and 8. Both HT-29 and HUVEC cells were cultured at 37˚C in a humidified atmosphere containing 5% CO_2. For hypoxic conditions, cells were incubated at a 5% CO_2 level with 1% O_2 balanced with N_2 in a hypoxic gaspack (BD Biosciences, San Jose, CA, USA). Z-guggulsterone (Sigma-Aldrich, Poole, Dorset, UK) was dissolved in DMSO to create a 50 μM stock solution and stored at -20˚C until use in experiments at a working strength of 5 μM in medium. Cells were treated with various concentrations of guggulsterone (0-200 μM) or with DMSO vehicle.

MTT assay. Cytotoxic studies were carried out using a 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazonium bromide (MTT) assay (Sigma) to determine the impact of increasing guggulsterone concentrations on cell viability. Briefly, cells were harvested from exponential phase cultures growing in DMEM supplemented with 10% FBS, counted, plated in 96-well flat-bottomed microtiter plates (150 μl cell suspensions, $1x10^4$ cells/ml for HT-29 cells) and treated with medium containing various concentrations of guggulsterone. After 48 h, 50 μl of MTT solution (2 mg/ml in PBS) was added to the culture medium, and the reaction mixture was incubated at 37˚C in a 5% CO_2 atmosphere for 4 h. The MTT solution was removed and 200 μl of DMSO was added. The optical density was measured using a spectrophotometer at 550 nm. DMSO-alone controls (0.01%) did not affect cell viability.

Co-immunoprecipitation assay. After treatment with guggulsterone, HT-29 cells were washed in PBS, and cell lysates were prepared by adding 1 ml of RIPA modified buffer (50 mM Tris-Cl, pH 7.5, 150 mM NaCl, 1% Nonidet P-40, 1 mM EDTA, 1 mM PMSF) supplemented with protease inhibitors (Roche Molecular Biochemicals, Mannheim, Germany). After pre-clearance with protein A/G-agarose beads (Santa Cruz Biotechnology, Santa Cruz, CA, USA), lysates were incubated at 4˚C for 1 h with beads and a 1:200 dilution of anti-HIF-1 monoclonal antibody (BD Biosciences). After incubation at 4˚C for 2 h, the beads were washed once with RIPA buffer and twice with PBS and the immune complexes were released from the beads by boiling the samples in sample buffer for 5 min. Following electrophoresis on 10% SDS-polyacrylamide gels, immunoprecipitates were analyzed by Western blotting using rabbit anti-STAT3 polyclonal antibody (Cell Signaling Technology, Danvers, MA, USA) (1:1000), rabbit anti-ARNT polyclonal antibody (Santa Cruz Biotechnology) (1:1000) and rabbit anti-VEGF polyclonal antibody (Santa Cruz Biotechnology) (1:500).

Quantitative real-time PCR analysis. For RT-PCR, cells were treated with 20, 50 or 100 μM of guggulsterone as indicated. Total RNA was extracted using TRIzol Reagent (Invitrogen Life Technologies, Carlsbad, CA, USA) and 4 μg of RNA was reverse-transcribed using the SuperScript First-Strand Synthesis kit (Invitrogen Life Technologies) according to the manufacturer's recommended protocol. RT products were amplified with a 7500 real-time PCR System (Applied Biosystems, Foster City, CA, USA) using the following primer pairs: MMP-2 coding sequence, forward, 5'-AGTCTGAAG AGCGTGAAG-3' and reverse, 5'-CCAGGTAGGAGTGAG AATG-3'; and control GAPDH coding sequence, forward, 5'-TGATGACATCAAGAAGGTGG-3' and reverse, 5'-TTT CTTACTCCTTGGAGGCC-3'. Finally, quantitative analysis was performed using the relative standard curve method. Results are reported as the relative expression or fold change compared to the calibrator after normalization of the transcript level to the endogenous control.

Chromatin immunoprecipitation (ChIP). Cells were treated with guggulsterone for the indicated times and then with the cross-linking reagent formaldehyde (1% final concentration) for 10 min at 37˚C, rinsed twice with cold PBS and swollen on ice in SDS lysis buffer (1% SDS, 10 mM EDTA, 50 mM Tris-HCl, pH 8.1) for 10 min. Nuclei were collected and sonicated on ice. Supernatants were obtained by centrifugation for 10 min and diluted 10-fold in ChIP dilution buffer (0.01% SDS, 1.1% Triton X-100, 1.2 mM EDTA, 16.7 mM Tris-HCl, pH 8.1, 167 mM NaCl). The mixture (i.e., fragmented chromatin) was then incubated with 2 ml of anti-HIF-1α, anti-STAT3, anti-ARNT, or anti-VEGF antibody on a rotator at 4˚C for 4 h. Next, 20 ml of protein A/G PLUS-agarose were added and incubated for 1 h at 4˚C with rotation to collect the antibody/chromatin complex. Cross-linked, precipitated chromatin complexes were recovered and reversed according to Upstate's protocol (Upstate, Chicago, IL, USA). Final DNA

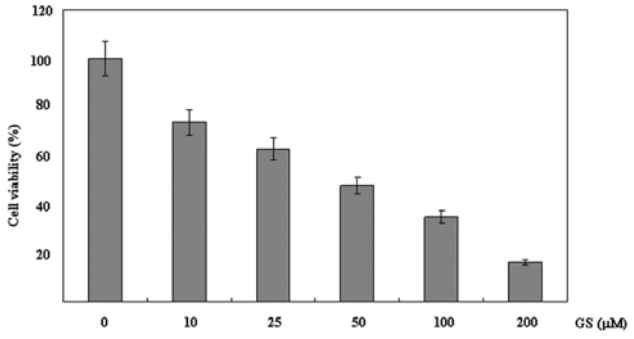

Figure 1. Guggulsterone inhibits proliferation in HT-29 cells. HT-29 cells were cultured in the absence or presence of guggulsterone for two days. Cell growth was monitored by MTT assay, shown by absorbance at 550 nm. GS, guggulsterone.

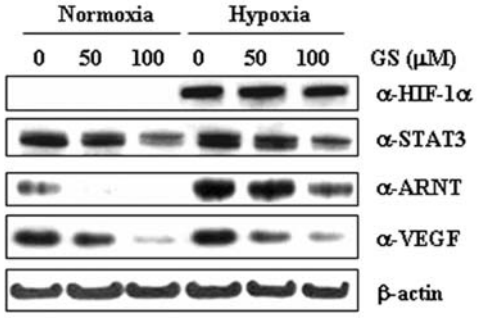

Figure 2. Guggulsterone inhibits the expression of angiogenesis gene products, STAT3, ARNT and VEGF. HT-29 cells were left untreated or incubated with 50 or 100 μM guggulsterone for 16 h. Whole-cell extracts were prepared and 20 μg of the whole-cell lysate were analyzed by Western blotting using antibodies against HIF-1α, STAT3, ARNT and VEGF, as indicated. GS, guggulsterone.

pellets were recovered and analyzed by PCR, using a pair of primers that encompass the VEGF promoter region (235 bp): forward 5'-AGACTCCACAGTGCATACGTG-3' and reverse 5'-AGTGTGTCCCTCTGACAATG-3'.

Determination of MMP-2 and -9 by gelatin zymography. The activities of MMP-2 and -9 in the medium were measured by gelatin zymography protease assays, as previously described (17). Briefly, collected media at appropriate volumes were prepared with SDS sample buffer without boiling or reduction and subjected to SDS-PAGE electrophoresis on a 10% nondenaturing polyacrylamide gel containing 1 mg/ml gelatin (Invitrogen). After electrophoresis, the gels were washed with renaturing buffer (Invitrogen) and then incubated in a reaction buffer (developing buffer, Invitrogen) at 37˚C for 16 h. The gels were then stained with Coomassie brilliant blue R-250.

Endothelial cell tube formation assay. Growth factor-reduced Matrigel (BD Biosciences) was placed in the wells of a pre-chilled 24-well cell culture plate and incubated at 37˚C for 1 h to allow polymerization. HUVECs ($4x10^4$ per well) were plated into the coated wells, incubated at 37˚C in 5% CO_2 for 16 h in the presence of 5% serum from either a medium for zymography experience, or a control. After washing, the cells were viewed under an Olympus fluorescence AX70 microscope (Olympus Optical Co, Tokyo, Japan).

Migration assay. The migration of HUVECs was assessed using the 8.0 μm pore BD Falcon HTS FluoroBlok 24-multi-well insert system (Becton Dickinson, Franklin Lakes, NJ, USA). HUVECs ($4x10^4$ cells/well) were plated on the top of the inserts suspended in 400 μl of media per well. Thereafter, 400 μl of media for zymography experience was added to the bottom of each well as a chemoattractant. The plate was incubated for 8 h at 37˚C, 5% CO_2. After incubation, cells that migrated through the membrane were stained with crystal violet (Dojindo, Kumamoto, Japan) and counted using a microscope.

Results

Guggulsterone reduces the viability of HT-29 cells. First, to determine the biological effect of guggulsterone on tumor cell growth, we measured cell viability rates in response to guggulsterone using an MTT assay (Fig. 1). The viability of HT-29 cells was significantly reduced by treatment with guggulsterone in a concentration-dependent manner. For example, treating HT-29 cells with 50 μM guggulsterone for 48 h caused a 50% reduction in cell viability compared with the DMSO-treated control.

Guggulsterone down-regulates protein levels of STAT3, ARNT, and VEGF, but not HIF-1α. Pharmacological inhibition of the HIF-1 target VEGF has proven to be effective as a cancer therapy (18). VEGF expression is directly regulated by HIF-1α and/or ARNT. Moreover, in recent years, it has been demonstrated that STAT3 is a potential modulator of HIF-1-mediated VEGF activation (12), suggesting that STAT3 may be an effective target for inhibiting tumor VEGF expression and angiogenesis (19). To investigate the effect of guggulsterone on the expression of VEGF, HIF-1, and STAT3, we treated HT-29 cells with guggulsterone for 18 h under normoxic or hypoxic conditions. As shown in Fig. 2, guggulsterone down-regulated the protein levels of STAT3, ARNT, and VEGF stabilized by hypoxia, but did not affect the level of HIF-1α. This finding suggests that the stability of the protein levels of STAT3, ARNT, or VEGF, but not HIF-α, is reduced by guggulsterone.

Guggulsterone inhibits the recruitment of STAT3 and ARNT to the VEGF promoter. Based on the above findings, we hypothesized that VEGF expression is cooperatively regulated by HIF-1α, STAT3 and ARNT. To test this hypothesis, we examined the interaction among HIF-1α, STAT3 and ARNT using co-immunoprecipitation. HT-29 cells were incubated under hypoxic conditions and cell lysates were immuno-precipitated with an anti-HIF-1α antibody, followed by Western blotting with an anti-STAT3 and ARNT antibody. As shown in Fig. 3A, STAT3 and ARNT co-precipitated with HIF-1α in hypoxic cells, demonstrating an association among HIF-1α, STAT3 and ARNT under hypoxic conditions.

To test whether STAT3 and ARNT in association with HIF-1α, recruit to the human VEGF promoter, we performed ChIP assays on chromatin samples from normoxic or hypoxic

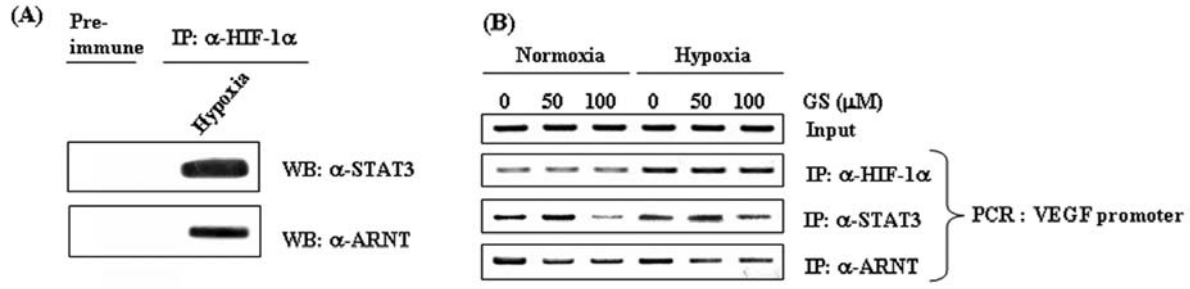

Figure 3. (A) HT-29 cells were incubated under hypoxic conditions and cell lysates were immunoprecipitated with an anti-HIF-1α antibody, followed by Western blotting with an anti-STAT3 and ARNT antibody. STAT3 and ARNT co-precipitated with HIF-1α in hypoxic cells, demonstrating an association among HIF-1α, STAT3 and ARNT under hypoxic conditions. (B) HIF-1α, STAT3 and ARNT are recruited to the VEGF promoter. Cross-linked, sheared chromatin was prepared from HT-29 cells, and grown in the absence or presence of guggulsterone overnight. Chromatin samples were then immunoprecipitated with the antibodies indicated on the right. The precipitates were subjected to PCR analysis using primer pairs spanning the human VEGF promoter. The control was the PCR product of chromatin obtained before immunoprecipitation. The recruitment of HIF-1α, STAT3 and ARNT was greater under hypoxic conditions. Guggulsterone treatment significantly inhibited the recruitment of STAT3 and ARNT, but not HIF-1α, to the VEGF promoter. GS, guggulsterone.

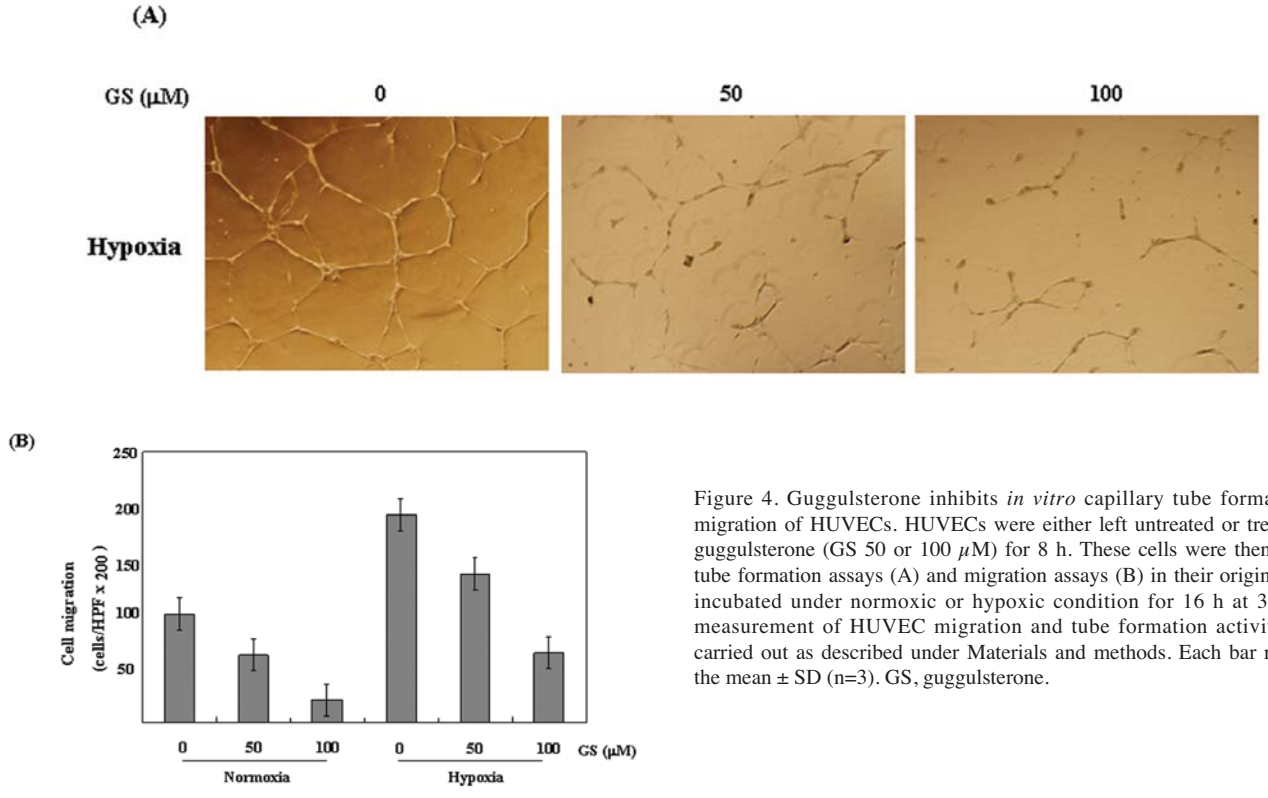

Figure 4. Guggulsterone inhibits *in vitro* capillary tube formation and migration of HUVECs. HUVECs were either left untreated or treated with guggulsterone (GS 50 or 100 μM) for 8 h. These cells were then used for tube formation assays (A) and migration assays (B) in their original media, incubated under normoxic or hypoxic condition for 16 h at 37˚C. The measurement of HUVEC migration and tube formation activities were carried out as described under Materials and methods. Each bar represents the mean ± SD (n=3). GS, guggulsterone.

cells. The occupancy of the promoter was analyzed using specific pairs of primers spanning both the STAT3 binding motif and HIF-1α binding motif of the VEGF promoter. Significantly greater binding of HIF-1α to the promoter was observed under hypoxic conditions (Fig. 3B). The binding of STAT3 and ARNT to the promoter was also significantly enhanced by hypoxic conditions. Guggulsterone treatment significantly inhibited the recruitment of STAT3 and ARNT to the VEGF promoter, but did not inhibit HIF-1α binding.

Guggulsterone directly decreases HUVEC capillary tube formation and migration. To investigate the effect of guggulsterone on vascular endothelial cells under hypoxic conditions, *in vitro* angiogenesis assays were conducted using HUVECs. Guggulsterone was administered to HUVECs seeded on Matrigel beds (10 mg/ml) and incubated for 18 h under normoxic or hypoxic conditions. Guggulsterone strongly inhibited the hypoxia-stimulated network by producing considerably foreshortened and severely broken tubes (Fig. 4A). In angiogenesis, migrating endothelial cells must break and traverse through their basement membrane to form new blood vessels and hypoxia can stimulate endothelial cell invasion (8). Accordingly, the effect of guggulsterone on vascular endothelial cell invasion was studied using a polycarbonate filter in a transwell coated with Matrigel. The HUVECs were placed in the filter and allowed to invade under normoxic or hypoxic conditions. As shown in Fig. 4B, guggulsterone significantly inhibited the hypoxia-stimulated migration of HUVECs.

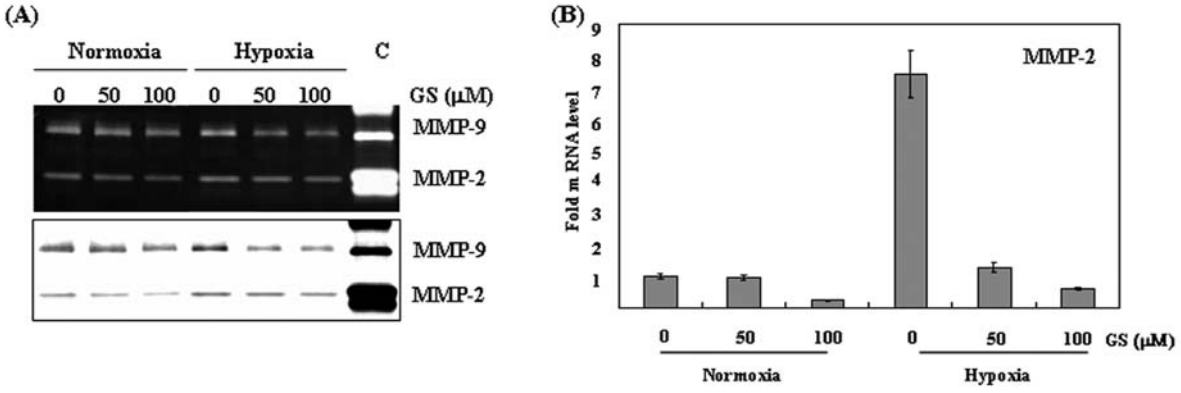

Figure 5. Matrix metalloproteinases determination. (A) The influence of guggulsterone administration on the gelatinolytic activity of HT-29 was investigated by gelatin zymography. This zymogram represents MMP-2 and -9 levels detected in conditioned medium (15 μl) collected from HT-29 cells exposed to 16 h of normoxia or hypoxia followed by reoxygenation for 48 h. Activity is indicated as an achromatic white band. The positions of the active MMP-2 and -9 enzymes, visible as a clear band, were confirmed by comparison to H1080 cells. Hypoxia-induced MMP-2 and -9 enzyme activity markedly decreased in the presence of guggulsterone. (B) Effect of guggulsterone on the expression of endogenous MMP genes. Total RNA was extracted from each cell and subjected to real-time PCR using primer pairs specific for MMP-2 coding sequences. GAPDH was used as an internal control. The total RNA level of MMP-2 decreased in a dose-dependent manner under hypoxic conditions. GS, guggulsterone.

Guggulsterone attenuates hypoxia-induced MMP-2 and -9 in HT-29 cells. Gelatinases (MMP-2 and MMP-9) play a key role during invasion and metastasis of malignant cells (8). To examine whether the anti-angiogenic and anti-migratory activities of guggulsterone correlate with the inhibition of activities of gelatinolytic MMPs, we performed zymography and RT-PCR in HT-29 cells. Zymographic analysis revealed that hypoxia-induced MMP-2 and -9 enzyme activities were markedly lower in the presence of guggulsterone (Fig. 5A). In the RT-PCR analysis, total RNA levels of MMP-2 under hypoxic conditions dropped in a dose-dependent manner (Fig. 5B). These results suggest that guggulsterone could inhibit tumor cell invasion by suppressing the activity of MMP-2 and -9.

Discussion

To our knowledge, this is the first published investigation of the potential anti-angiogenic effects and underlying mechanisms of guggulsterone in human colon cancer cells. Guggulsterone, a phytochemical derived from the gum resin (guggul) of the tree *Commiphora mukul* has recently aroused considerable interest. The anti-arthritic and anti-inflammatory activity of gum guggul was shown as early as 1960 by Gujral *et al* followed by a study of activity in experimental arthritis induced by a mycobacterial adjuvant and another study on the effectiveness of guggul for treating osteoarthritis of the knee (20,21). Guggulsterone also attenuates dextran sulfate sodium-induced colitis by blocking NF-κB signaling in mice (22). In addition to its anti-inflammatory effects, more than a few recent studies have shown that it induces anti-proliferation and cell death in some cancer cells (15,16,23,24). Our finding that guggulsterone inhibited the proliferation of human colon cancer HT-29 cells in a dose-dependent manner confirmed the apoptotic activity of guggulsterone. For guggulsterone to be a potential chemotherapeutic agent for colorectal cancer, however, it should also be able to block angiogenesis, one of the key mechanisms involved in colorectal cancer development and growth. We sought to establish whether this material has an anti-angiogenic activity on colon cancer.

We hypothesized that guggulsterone might modulate the angiogenesis signaling pathway in human colon cancer cells, leading us to evaluate the effect of guggulsterone on hypoxia-induced VEGF expression. Angiogenesis is one of the most important characteristics of cancer cells and VEGF is a potent angiogenic factor with a recognized major role in the promotion of colon cancer (6). Furthermore, transcriptional activation of the VEGF gene is mediated by HIF-1 and when this transcription factor is activated, the expression of its inducible subunit HIF-1α protein increases in kind (25). HIF is a transcription factor found in mammalian cells cultured under reduced oxygen tension and plays a key role in the cellular response to hypoxia. A heterodimer consisting of two subunits [the oxygen-sensitive HIF-1α and the constitutively expressed HIF-1ß, also known as aryl hydrocarbon receptor (ARNT) (26)], HIF regulates the transcription of several genes involved in biological processes including angiogenesis, cell proliferation and survival, glucose metabolism, pH regulation and apoptosis (27). Among them, VEGF is a well-characterized HIF-regulated gene that is involved in endothelial cell proliferation and blood vessel formation in both normal and cancer cells.

Previous studies have demonstrated that STAT3 is a direct transcriptional activator of the VEGF gene, forming a transcriptional complex with HIF-1 (28). Many studies have concentrated on STAT3 as a potential target for cancer therapy and have found that STAT3 inhibition effectively blocks production of VEGF and tumor angiogenesis (19). In the present study, we observed that guggulsterone down-regulated the protein levels of VEGF, STAT3 and ARNT stabilized by hypoxia but did not affect the level of HIF-1α. From a mechanistic perspective, guggulsterone suppressed ARNT and hypoxic induction of VEGF expression without altering HIF-1α. Interestingly, curcumin, another phytochemical that may have chemotherapeutic effects, inhibits HIF-1 by

degrading ARNT, which seems to be similar to our findings (29). Little is known about the mechanism of ARNT regulation. Further studies are needed to evaluate the usefulness of ARNT inhibition as an anti-cancer therapy. In addition, some other studies have emphasized that STAT3 is a critical requirement for HIF-1α expression and that HIF-1α expression is blocked by STAT3 inhibitors (19). In contrast, our data showed that down-regulation of the STAT3 level by guggulsterone did not correlate with suppressed HIF-1α expression. Additional efforts are needed to further explain this discrepancy.

We found that guggulsterone inhibited STAT3 expression. Increasing evidence has indicated that STAT3 activation is necessary for the malignant phenotype (30). Many studies suggest that STAT3 is also involved in tumor progression through VEGF (31). Consequently, STAT3 inhibition and the subsequent reduction in VEGF expression could represent a clinical therapy for patients with solid tumors. Moreover, we identified an interaction between HIF-1 and STAT3 at the VEGF promoter area using co-immunoprecipitation and ChIP assays, suggesting that HIF-1 and STAT3 serve as transcriptional factors binding to the VEGF promoter. These observations are consistent with other studies (28). Taken together, the overall findings of our study indicate that the STAT3/HIF-1 pathway of VEGF expression is likely to be a main target of guggulsterone action. Further preclinical and clinical studies are needed to validate the efficacy of this plant sterol as an anti-angiogenic compound in the treatment of colorectal cancer.

We further revealed that guggulsterone directly suppresses hypoxia-stimulated endothelial migration, invasion and tube formation in guggulsterone-treated vascular endothelial cells. Several studies have shown that STAT3 activation is required for VEGF receptor signaling in endothelial cells (32,33). Blocking STAT3 in endothelial cells inhibits their migration and vessel formation (33). Based on our observations, guggulsterone may have a STAT3/HIF-1-dependent tumor suppressive effect, which is orchestrated by both tumor and endothelial cells. Because the identification of anti-angiogenic factors appears crucial for colorectal cancer therapy, guggulsterone could be an ideal candidate. Finally, metastasis is a definitive characteristic of cancer and is promoted by tumor hypoxia. Recent studies have demonstrated that HIF-1 may also regulate the invasiveness of colon cancer cells by altering the expression of genes encoding intermediate filaments, extracellular matrix components and proteases (34). MMP expression, required for the degradation of extracellular matrix, is critical for tumor angiogenesis and metastasis and VEGF upregulates the expression of MMP (8). Our zymography and RT-PCR results show that guggulsterone inhibits MMP expression in proportion to guggulsterone concentration (Fig. 5). Down-regulation of MMP expression could be due to the suppression of VEGF expression by guggulsterone.

In conclusion, guggulsterone strongly down-regulates STAT3 protein levels and activity under hypoxic conditions, eventually leading to the inhibition of VEGF gene expression in colon cancer cells. Guggulsterone might be a promising therapeutic agent for colorectal cancer by preventing tumor angiogenesis and migration.

Acknowledgements

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