Gambogic Acid Inhibits STAT3 Phosphorylation through Activation of Protein Tyrosine Phosphatase SHP-1: Potential Role in Proliferation and Apoptosis

Sahdeo Prasad, Manoj K. Pandey, Vivek R. Yadav, and Bharat B. Aggarwal

Abstract

The transcription factor, STAT3, is associated with proliferation, survival, and metastasis of cancer cells. We investigated whether gambogic acid (GA), a xanthone derived from the resin of traditional Chinese medicine, Garcinia hanburyi (mangosteen), can regulate the STAT3 pathway, leading to suppression of growth and sensitization of cancer cells. We found that GA induced apoptosis in human multiple myeloma cells that correlated with the inhibition of both constitutive and inducible STAT3 activation. STAT3 phosphorylation at both tyrosine residue 705 and serine residue 727 was inhibited by GA. STAT3 suppression was mediated through the inhibition of activation of the protein tyrosine kinases Janus-activated kinase 1 (JAK1) and JAK2. Treatment with the protein tyrosine phosphatase (PTP) inhibitor pervanadate reversed the GA-induced downregulation of STAT3, suggesting the involvement of a PTP. We also found that GA induced the expression of the PTP SHP-1. Deletion of the SHP-1 gene by siRNA suppressed the ability of GA to inhibit STAT3 activation and to induce apoptosis, suggesting the critical role of SHP-1 in its action. Moreover, GA downregulated the expression of STAT3-regulated antiapoptotic (Bcl-2, Bcl-xL, and Mcl-1), proliferative (cyclin D1), and angiogenic (VEGF) proteins, and this correlated with suppression of proliferation and induction of apoptosis. Overall, these results suggest that GA blocks STAT3 activation, leading to suppression of tumor cell proliferation and induction of apoptosis.

Cancer Prev Res; 4(7); 1084–94. ©2011 AACR.

Introduction

The successes of several recent clinical trials in preventing cancer in high-risk populations suggest that chemoprevention is a rationale and appealing strategy. Chemoprevention includes the use of natural or synthetic substances to reverse, suppress, or prevent the initiation, promotion, or progression of cancer. In particular, natural compounds, which include fruits and vegetables, are important in the treatment of life-threatening conditions. As many as 70% of all drugs discovered within the past 25 years have their roots in natural products (1). Thus, there is growing interest in the possible therapeutic potential of natural products against a variety of ailments. Moreover, almost 80% of the world population relies on natural products for their medical needs. Because natural compounds are considered to be affordable and safe, many potential compounds are now in different phases of clinical trials. Moreover, because cancer is a complex and multigenic disease, agents that modulate multiple targets are preferred. Natural products, because of their ability to bind multiple targets, thus have an advantage over rationally designed monotargeted agents.

One potentially useful natural compound is gambogic acid (GA; C_{38}H_{44}O_{8}; Fig. 1A), a xanthone derived from the resin of Garcinia hanburyi (also called mangosteen), a plant that mainly grows in South China, Cambodia, Vietnam, and Thailand (2). It has been shown that GA can suppress the growth of various cancer cells such as non–small cell lung cancer cells (3), human hepatocellular carcinoma (4), oral squamous cell carcinoma (4), oral squamous cell carcinoma (5), human breast cancer cells (6), human malignant melanoma (7), human gastric carcinoma (8), and human leukemia cancer cells (9). GA has also been shown in animal models to inhibit the development of tumors (10, 11). A variety of mechanisms have been proposed by which GA inhibits the proliferation of cancer cells and induces apoptosis. These include inhibition of antiapoptotic proteins Bcl-2 (6, 12) and survivin (13); induction of apoptosis-associated proteins p53 (14), bax, and procaspase-3 (7); activation of c-jun-NH₂-kinase, p38 (15), and GSK-3β (16); inhibition of topoisomerase II by binding to its ATPase domain (17), and NF-κB and its regulated gene products (9); downregulation of the MDM2...
oncogene and subsequent induction of p21 (14); and downregulation of human telomerase reverse transcriptase (10). It has also been shown to directly bind to c-myc (18) and transferrin receptors (19), and to block VEGF signaling (20, 21). Recently, a proteomic approach revealed suppression of expression of 14-3-3 protein sigma and stathmin by the GA (8).

Extensive research in the past few decades has revealed that most chronic illnesses, including cancer, exhibit dysregulation of multiple cell signaling pathways. One of the major signaling molecules in this regard is STAT3. The latter is transcription factor involved in cell proliferation, differentiation, and apoptosis (22–24). STAT3 is constitutively active in many human cancer cells, including multiple myeloma, leukemia, lymphoma, and solid tumors (25, 26). STAT3 can also be activated by certain interleukins (e.g., IL-6), growth factors [e.g., epidermal growth factor (EGF)], carcinogens, and tumor promoters (27). The activation of STAT3 is regulated by phosphorylation of tyrosine 705 by receptor and nonreceptor protein tyrosine kinases such as EGF receptor (EGFR) kinase (28), Src (29), Janus-activated kinase (JAK; refs. 30, 31), and extracellular signal-regulated kinase (32). On activation, STAT3 undergoes phosphorylation-induced homodimerization, leading to nuclear translocation, DNA binding, and subsequent gene transcription.
Because of the critical role of STAT3 activation in tumor cell survival, proliferation, and chemoresistance, we hypothesized that GA may mediate its effects through suppression of the STAT3 pathway. We found that GA indeed suppressed both constitutive and inducible STAT3 activation and downregulated the expression of cell survival, proliferative, and angiogenic gene products, leading to suppression of proliferation and induction of apoptosis.

Material and Methods

Cell lines

Human multiple myeloma lines U266, MM.1S (melphalan-sensitive); head and neck squamous cell carcinoma SCC4; prostate cancer PC-3 and DU1145; and breast cancer MCF-7 cells were obtained from the American Type Culture Collection. MCF-10A cells were kindly provided by Dr. Kapil Mehta from our Institute. U266 (ATCC TIB-196) is a plasmacytoma of B-cell origin and is known to produce monoclonal antibodies and IL-6. MM.1S cell line, established from the peripheral blood cells of a patient with IgA myeloma, secretes EL chain, is negative for the presence of the Epstein–Barr virus genome, and expresses leukocyte antigen DR, plasma cell Ag-1, and T9 and T10 antigens. U266, MM.1S, and MM.1R cells were cultured in RPMI 1640 containing 10% FBS. SCC4 cells were cultured in Dulbecco’s modified Eagle’s medium containing 10% FBS, nonessential amino acids, pyruvate, glutamine, and vitamins. All media were also supplemented with 100 units/mL of penicillin and 100 µg/mL of streptomycin. The above-mentioned cell lines were procured more than 6 months ago and have not been tested recently for authentication in our laboratory.

Reagents

GA was obtained from Alexis Laboratories. A 10 mmol/L solution of GA was prepared in dimethyl sulfoxide, stored as small aliquots at −20°C, and then diluted as needed in cell culture medium. Hoechst 33342, MTT, Tris, glycine, NaCl, SDS, and bovine serum albumin were purchased from Sigma-Aldrich.

RPMI 1640, FBS, 0.4% trypan blue vital stain, and antibiotic–antimycotic mixture were obtained from Invitrogen. Rabbit polyclonal antibodies to STAT3 and mouse monoclonal antibodies against phospho-STAT3 (Tyr705), phospho-STAT3 (Ser727), phospho-JAK1 (Tyr1022/1023), JAK1, phospho-JAK2 (Tyr1007/1008), JAK2, Bcl-2, Bcl-xl, Mcl-1, SHP-1, cyclin D1, procaspase-3, procaspase-9, and PARP were obtained from Santa Cruz Biotechnology. Goat anti-mouse horseradish peroxidase was purchased from Transduction Laboratories, and goat anti-rabbit Alexa Fluor 594 was purchased from Invitrogen. Bacteria-derived recombinant human IL-6 was kindly provided by Novartis Pharmaceuticals. The siRNA for SHP-1, and the scrambled control were obtained from Ambion. GST-JAK2 substrate was kindly provided by Dr. Zhizhuang Joe Zhao (Department of Pathology, University of Oklahoma Health Sciences Center, Oklahoma City, OK).

Propidium iodide staining for apoptosis

Cells were pretreated with GA (2.5 µmol/L) for different times. Propidium iodide (PI) staining for cell distribution across the cell cycle was carried out with a FACSCalibur device (Becton Dickinson) as described elsewhere (33). A total of 10,000 events were analyzed by flow cytometry by using an excitation wavelength set at 488 nm and emission set at 610 nm.

Annexin V/PI assay

An early indicator of apoptosis is the rapid translocation and accumulation of the membrane phospholipid phosphatidylserine from the cytoplasmic interface of membrane to the extracellular surface. This loss of membrane asymmetry can be detected by using the binding properties of annexin V. To identify apoptosis, we used an annexin V antibody, which was conjugated with a fluorescein isothiocyanate (FITC) fluorescent dye. Briefly, 2 × 10^6 cells were pretreated with GA for different times at 37°C and subjected to annexin V staining. The cells were washed in PBS, resuspended in 100 µL of binding buffer containing an FITC-conjugated anti–annexin V antibody, and then analyzed with a flow cytometer (FACSCalibur; BD Biosciences).

Live/Dead Assay

To measure apoptosis, we used the Live/Dead Assay (Invitrogen), which assesses intracellular esterase activity and plasma membrane integrity. This assay was carried out as described previously (33).

Cytotoxicity assay

The cytotoxic effects of GA were determined by the MTT uptake method (9).

Western blot analysis

To detect various proteins, cells treated with GA were washed with PBS and protein extracted by incubation for 30 minutes on ice in lysis buffer containing 20 mmol/L HEPES (pH 7.4), 2 mmol/L EDTA, 250 mmol/L NaCl, 0.1% NP-40, 2 µg/mL leupeptin, 2 µg/mL aprotinin, 1 mmol/L phenylmethylsulfonyl fluoride, 0.5 µg/mL benzamidine, 1 mmol/L dithiothreitol, and 1 mmol/L sodium vanadate. The lysate was centrifuged, and the supernatant was collected. Whole-cell extract protein (40 µg) was resolved on 7.5% to 12% SDS-PAGE onto a nitrocellulose membrane, blotted with antibodies, and then detected by electrochemiluminescence (Amer sham Biosciences).

Immunocytochemistry for STAT3 localization

GA-treated cells were plated on a glass slide by centrifugation, using a Cytospin 4 (Thermoshendon), air-dried for 1 hour at room temperature, and fixed in 4% formaldehyde. After a brief washing in PBS, slides were blocked with 5% normal goat serum for 1 hour and then incubated with rabbit polyclonal anti-human STAT3 antibody (dilution, 1:100). After overnight incubation, the slides were washed.
and then incubated with goat anti-rabbit IgG-Alexa 594 (1:100) for 1 hour and counterstained for nuclei with Hoechst (50 ng/mL) for 5 minutes. Stained slides were mounted with mounting medium and analyzed under an epifluorescence microscope (Labophot 2; Nikon). Pictures were captured by a Photometrics Coolsnap CF color camera (Nikon) and MetaMorph version 4.6.5 software (Molecular Devices).

Electrophoretic mobility shift assay for STAT3–DNA binding

STAT3–DNA binding was analyzed by electrophoretic mobility shift assay (EMSA) by using a 32P-labeled high-affinity sis-inducible element (hSIE) probe (forward, 5′-CTTCATTCCCGTAAATCCCTAAGCT-3′ and reverse 5′-AGCTTTAGGGATTTACGGGAAATGA-3′) as previously described (33). Briefly, nuclear extracts were prepared from GA-treated cells and incubated with the hSIE probe. The DNA–protein complex formed was separated from free oligonucleotide on 5% native polyacrylamide gels. The dried gels were visualized, and the radioactive bands were quantitated with a Storm 820 and Imagequant software (Molecular Devices).

Kinase assay

To determine the effect of GA on JAK2 activation, we carried out an immunocomplex kinase assay by using GST-JAK2 as the substrate, as described previously (33). In brief, the JAK complex from whole-cell extracts was precipitated with antibody against JAK2 and treated with protein A/G-agarose beads (Pierce). After 2 hours, the beads were washed with whole-cell extract buffer and then resuspended in a kinase assay mixture containing 50 mmol/L HEPES (pH 7.4), 20 mmol/L MgCl2, 2 mmol/L dithiothreitol, 20 μCi [γ-32P]ATP, 10 μmol/L unlabeled ATP, and 2 μg of substrate GST-JAK2. After incubation at 30°C for 30 minutes, the reaction was terminated by boiling with SDS sample buffer for 5 minutes. Finally, the protein was resolved on 10% SDS-PAGE, the gel was dried, and the radioactive bands were visualized with the Storm 820 imaging system. To determine the total amounts of JAK2 in each sample, 40 μg of whole-cell proteins was resolved on 10% SDS-PAGE, electrotransferred to a nitrocellulose membrane, and then blotted with anti-JAK2 antibody.

Results

The goal of this study was to determine whether GA can inhibit the STAT3 cell signaling pathway, leading to suppression of proliferation and induce apoptosis. We investigated the effect of GA on both constitutive and IL-6–inducible STAT3 activation. Whether GA affects STAT3-regulated gene products involved in cellular proliferation, survival, and apoptosis was also investigated.

GA induces apoptosis in multiple myeloma cells

We first examined the apoptosis-inducing effects of GA by using the annexin V/PI assay, which detects phosphatidylserine externalization. For this, human multiple myeloma U266 cells were exposed to a 2.5 μmol/L concentration of GA for different times. GA significantly induced apoptosis in time-dependent manner (Fig. 1B, left).

To confirm the GA-induced cell death, we also measured apoptosis by PI staining of DNA. We found that GA induced apoptosis from 1% in control cells to 30% in GA-treated cells within 24 hours (Fig. 1B, right).

We also measured apoptosis by intracellular esterase activity and plasma membrane integrity by using the live/dead assay. The results indicated that GA treatment induced apoptosis from 2% in control cells to 65% in GA-treated cells within 24 hours (Fig. 1C).

Next, we examined the effect of GA on the activation of caspase-9, caspase-3, and PARP cleavage. We found that GA cleaved procaspase-9 and procaspase-3, leading to the appearance of caspase-9 and caspase-3, respectively, in a time-dependent manner. We also found that GA induced PARP cleavage in time-dependent manner (Fig. 1D, left). Taken together, all these results suggest that GA can induce apoptosis in human multiple myeloma cells.

To determine whether GA is selectively more cytotoxic to tumor cells than normal cells, we employed human breast cancer MCF-7 and human normal counterpart MCF-10A cells. Under the conditions when GA induced 75% cytotoxicity in MCF-7 cells, only 13% cytotoxicity was observed in MCF-10A cells. These results thus indicate that GA is highly cytotoxic to tumor cells (Fig. 1D, right).

GA inhibits constitutive STAT3 phosphorylation in multiple myeloma cells

We investigated whether GA modulates constitutive STAT3 activation in multiple myeloma cells. We incubated U266 cells with different concentrations of GA for 6 hours and examined them for phosphorylated STAT3 by Western blot analysis by using an antibody that recognizes STAT3 phosphorylated at the tyrosine 705 site. As shown in Figure 2A, GA inhibited constitutive STAT3 activation in the U266 cells, with maximum inhibition occurring at 2.5 μmol/L GA. GA had no effect on STAT3 protein expression.

We also determined the effect of GA incubation time required to suppress STAT3 activation in U266 cells. As shown in Figure 2B, STAT3 inhibition was time-dependent, with maximum inhibition occurring 6 hours after the beginning of GA treatment. Interestingly, GA also inhibited STAT3 phosphorylated at serine 727 site (Fig. 2B). Under these conditions, GA had no significant effects on cell viability.
GA suppresses the nuclear translocation of STAT3

Because tyrosine phosphorylation causes dimerization of STATs and then nuclear translocation, whether GA inhibited nuclear translocation of STAT3 was examined in U266 cells by immunocytochemistry. Our results showed that GA was able to inhibit the nuclear translocation of STAT3 (Fig. 2C).

GA inhibits binding of STAT3 to the DNA

When STAT3 is translocated to the nucleus, it binds to the DNA, an event that in turn regulates gene transcription. Whether GA inhibits DNA binding activity of STAT3 was examined by EMSA. Nuclear extracts prepared from U266 cells showed STAT3 DNA-binding activity and also showed that GA inhibited this binding in a dose-dependent (Fig. 2D, left) and time-dependent (Fig. 2D, right) manner. No loss of cell viability was noted under these conditions.

GA inhibits IL-6–induced STAT3 phosphorylation

Because IL-6 is a growth factor for multiple myeloma cells and induces STAT3 phosphorylation, we determined whether GA could inhibit IL-6–induced STAT3 phosphorylation. Multiple myeloma cells, which lack constitutively active STAT3, were treated with IL-6 for different times and then examined for phosphorylated STAT3. IL-6 induced phosphorylation of STAT3 as early as 15 minutes, but phosphorylation began to decline at 60 minutes (Fig. 3A). IL-6–induced STAT3 phosphorylation was suppressed in multiple myeloma cells pretreated with GA for 6 hours (Fig. 3B).
GA suppresses the constitutive activation of JAK1 and JAK2

STAT3 has been reported to be activated by soluble tyrosine kinases of the JAK family; thus, we sought to determine whether GA affects the constitutive activation of JAK1 in U266 cells. We found that GA suppressed the constitutive phosphorylation of JAK1 (Fig. 4A). Levels of nonphosphorylated JAK1 remained unchanged under the same conditions.

To determine the effect of GA on JAK2 activation, GA-treated cells were used for Western blot with the anti–phospho-JAK2 antibody. As shown in Figure 4B, JAK2 was constitutively active in U266 cells and pretreatment with GA suppressed this phosphorylation in a time-dependent manner.

We further investigated whether GA affects JAK2 activity in U266 cells, using immunocomplex kinase assays with GST-JAK2 acting as the substrate. We found that GA suppressed the constitutive facilitation of JAK2 in a time-dependent manner (Fig. 4C).

GA-induced inhibition of STAT3 activation involves a protein tyrosine phosphatase

Because protein tyrosine phosphatases (PTP) have been implicated in STAT3 activation, we determined whether GA-induced inhibition of STAT3 tyrosine phosphorylation could be due to the activation of a PTP. Treatment of U266 cells with the broad-acting tyrosine phosphatase inhibitor sodium pervanadate prevented the GA-induced inhibition of STAT3 activation (Fig. 5A). This suggests that tyrosine phosphatases are involved in the GA-induced inhibition of STAT3 activation.

GA induces SHP-1 expression in U266 cells

SHP-1 is a nontransmembrane PTP expressed most abundantly in hematopoietic cells (34). PTP have been shown to be involved in the negative regulation of JAK/STAT signaling in leukemia and lymphoma. Therefore, we examined whether GA modulates SHP-1 expression in U266 cells. We incubated cells with GA for various times. As shown in Figure 5B, GA induced SHP-1 protein expression in U266 cells. Our results suggest that the stimulation of SHP-1 expression by GA could be associated with the downregulation of constitutive STAT3 activation in U266 cells.

Gene silencing of SHP-1 reverses the effect of GA on STAT3

We determined whether the suppression of SHP-1 expression by siRNA would abrogate the inhibitory effect of GA on STAT3 activation. Western blotting showed that GA-induced SHP-1 expression was effectively abolished in the cells treated with SHP-1 siRNA; treatment with
scrambled siRNA had no effect (Fig. 5C, left). We also found that GA failed to suppress STAT3 activation in cells treated with SHP-1 siRNA (Fig. 5C, right). These results suggest the critical role of SHP-1 in the suppression of STAT3 phosphorylation by GA.

**Gene silencing of SHP-1 reduces GA-induced apoptosis**

We showed earlier that SHP-1 plays a critical role in the suppression of STAT3 phosphorylation by GA. Whether SHP-1 siRNA also affects GA-induced apoptosis was
determined. We found that knockdown of SHP-1 significantly decreased the apoptotic effects of GA (Fig. 5D). By contrast, treatment with control siRNA had no effect (Fig. 5D).

**GA downregulates the expression of antiapoptotic proteins**

STAT3 has been shown to regulate the expression of various gene products involved in proliferation and cell survival (22, 23); thus, whether downregulation of STAT3 activation by GA leads to downregulation of these gene products was examined. The results showed that GA inhibited the expression of c-IAP, survivin, Mcl-1, Bcl-2, and Bcl-xL in a time-dependent manner. The inhibition was less pronounced for Bcl-2 than for the other gene products. Maximum suppression was observed at around 12 to 24 hours (Fig. 6A).

**GA suppresses the expression of proliferative proteins**

Cyclin D1, which is required for cell proliferation and for transition from the G1 to S-phase of the cell cycle, is also regulated by STAT3. We therefore examined the effect of GA on constitutive expression of cyclin D1 in U266 cells. Our results showed that GA treatment suppressed the expression of cyclin D1 in a time-dependent manner (Fig. 6B).
GA downregulates the expression of angiogenic proteins

VEGF, a major mediator of angiogenesis, is regulated by STAT3 activation. Therefore, we examined the effect of GA on constitutive VEGF expression in U266 cells. Our results show that GA inhibited the expression of this protein in U266 cells in a time-dependent manner (Fig. 6B).

Discussion

Because STAT3 activation has been linked with most chronic diseases, including cancer, our findings that GA modulates the STAT3 cell signaling pathway provide a rationale for its use to treat various types of cancer. We show that GA was effective in blocking the activation of the STAT3 pathway. It suppressed both constitutive and inducible activation of STAT3. This inhibition was linked to the downregulated activation of various kinases linked to STAT3 activation and induction of phosphatases. Downregulation of STAT3 activation led to the suppression of expression of various proteins involved in the survival and proliferation of tumor cells (Fig. 6C).

We investigated in detail how GA induces apoptosis. First, we found that GA inhibited the phosphorylation of STAT3 at both tyrosine residue 705 and serine residue 727. Although the role of tyrosine 705 in STAT3 activation is well known (22), PKC, MAPK, and CDK5 have been implicated in the phosphorylation of STAT3 at serine 727 (22). PKC-ε has been shown to interact with STAT3 directly and phosphorylate serine 727 (35). Whether GA affects any of these kinases is not clear at present. Similarly, a large number of tyrosine kinases have been linked to phosphorylation of STAT3. These include EGFR (36), JAK1 and JAK2 (30, 31), and c-Src (29). We found that GA inhibited c-Src, JAK1, and JAK2 activation. C-Src–mediated STAT3 activation has been linked to the transformation of cells. Various tumors exhibit persistently active STAT3 that is associated with activated Src, including breast cancer (37), and melanoma (38). Inhibition of Src in these tumors by GA should downregulate STAT3 activation and suppress growth.

We also found evidence that inhibition of STAT3 activation is linked to the induction of a PTP by GA. Numerous PTPs have been implicated in STAT3 signaling, including SHP-1 (39), SHIP-2 (40), TC-PTP (41), PTEN (42), PTP-1D (43), CD45 (43), and PTP-ε. We found that GA inhibits the STAT3 activation pathway through the induction of SHP-1. GA was found to stimulate the expression of SHP-1 protein in U266 cells, which correlated with the downregulation of constitutive STAT3 phosphorylation in these cells. Silencing of the SHP-1 gene by siRNA reversed the STAT3 inhibitory effect of GA, thereby further implicating a critical role of this phosphatase in GA-induced downregulation of STAT3 activation. The silencing of SHP-1 also reversed GA-induced apoptosis. Loss of SHP-1 has been shown to enhance JAK3/STAT3 signaling in anaplastic lymphoma kinase–positive anaplastic large-cell lymphoma (39). SHP-1 has been shown to be inactive in various human tumors, including multiple myeloma (44) and lymphoma (39). DNA methylation has been described as one of the mechanisms for inactivation of SHP-1 in different cancers (44).

Previously, we showed that GA can also suppress NF-κB activation (9). Whether the suppression of STAT3 activation by GA is also linked to the inhibition of NF-κB activation is not clear. The p65 subunit of NF-κB has been shown to interact with STAT3 (45). STAT3 and NF-κB, however, are activated in response to different cytokines: IL-6 is a major activator of STAT3 and TNF is a potent activator of NF-κB. Interestingly, erythropoietin has been shown to activate NF-κB through the activation of JAK2 kinase (46). Thus, it is possible that the suppression of JAK2 kinase activation is the critical target for the inhibition of both NF-κB and STAT3 activation by GA.

We also found that GA suppresses the expression of STAT3-regulated proteins, including cell proliferative cyclin D1, COX-2, the angiogenic protein VEGF, and antiapoptotic gene products, including c-IAP, Mcl-1, survivin, Bcl-2, and Bcl-xL. However, no appreciable change was observed in the expression of ICAM-1 by GA treatment. Among the many genes controlled by NF-κB and STAT3, either synergistically or individually, some genes are prominent targets for both NF-κB and STAT3, such as Bcl-xL, Bcl-2, c-IAP, cyclin D1, VEGF, and COX-2, whereas A1 and c-FLIP are mostly NF-κB dependent and Mcl-1 and survivin are STAT3 dependent (22, 45, 47, 48). The downregulation of Bcl-2 and survivin by GA that we found is in agreement with previous reports (12, 13). Expression of Bcl-xL has been reported to be regulated by STAT3 (38), and it is overexpressed in multiple myeloma cells (49). Bcl-xL has also been shown to block cell death induced by a variety of chemotherapeutic agents, in parallel with an increase in chemoresistance. The downregulation of Bcl-xL expression that we found is likely linked to the ability of GA to induce apoptosis in multiple myeloma cells. The downregulation of Bcl-2, Bcl-xL, and survivin expression is likely linked to the ability of GA to induce apoptosis in multiple myeloma cells. We further observed that GA induced the downregulation of Mcl-1 protein. Because VEGF expression is also regulated by STAT3, GA may mediate antiangiogenesis through the downregulation of VEGF. We and others have indeed shown that GA can suppress angiogenesis (9, 20, 21).

Constitutive STAT3 activation is associated with various types of carcinoma, sarcoma, lymphoma, and leukemia (23). Thus, the suppression of constitutively active STAT3 in multiple myeloma cells raises the possibility that GA might also inhibit constitutively active STAT3 in other types of cancer cells (22). We observed that GA inhibited the growth of head and neck cancer, breast carcinoma, and human prostate carcinoma cells.

Perhaps one of the best in vitro models of premalignancy for cancer prevention is STAT3 as suggested by the evidence, first that STAT3 plays a major role in oncogenesis and regarded as an oncogene (50–52); second, STAT3 is activated by an oncogenic Src (29, 50); third, STAT3 regulates transformation, inflammation, survival, proliferation, and angiogenesis of the tumors through expression of several proteins including c-myc, COX2, Bcl-xL, survivin, cyclin D1,
and VEGF (53–56). Because our evidences indicate that GA downregulates STAT3 activation and STAT3-regulated gene expression, it suggests chemopreventive role of GA in an in vitro premalignancy model of cancer prevention. Overall, our results show that GA inhibits growth and induces apoptosis in various tumor cells through suppression of both inducible and constitutive STAT3 activation via the induction of tyrosine kinase phosphatase. Further studies in animals are needed to validate human clinical trials. Moreover, in China, this agent is already in clinical trials (13).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References


Acknowledgment

We thank Virginia M. Mohlere for carefully editing the manuscript. B.B. Aggarwal is the Ransom Horne, Jr., Professor of Cancer Research.

Grant Support

This work was supported by a grant from the Clayton Foundation for Research (B.B. Aggarwal), a core grant from the NIH (CA-106672), a program project grant from NIH (CA-124787-01A2), and a grant from Center for Targeted Therapy of MD Anderson Cancer Center.

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Received November 17, 2010; revised February 7, 2011; accepted March 28, 2011; published OnlineFirst April 13, 2011.

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