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Unifying Mechanisms of Action of the Anticancer Activities of Triterpenoids and Synthetic Analogs

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Abstract

Triterpenoids such as betulinic acid (BA) and synthetic analogs of oleanolic acid [2-cyano-3,12-dioxooleana-1,9-dien-28-oic acid (CDDO)] and glycyrrhetic acid [2-cyano-3,11-dioxo-18 β -oleana-1,12-dien-30-oic acid (CDODA)] are potent anticancer agents that exhibit antiproliferative, antiangiogenic, anti-inflammatory and pro-apoptotic activities. Although their effects on multiple pathways have been reported, unifying mechanisms of action have not been reported. Studies in this laboratory have now demonstrated that several triterpenoids including BA and some derivatives, celastrol, methyl ursolate, β -boswellic acid derivatives, and the synthetic analogs CDDO, CDODA and their esters decreased expression of specificity protein (Sp) transcription factors and several pro-oncogenic Sp-regulated genes in multiple cancer cell lines. The mechanisms of this response are both compound- and cell context-dependent and include activation of both proteasome-dependent and -independent pathways. Triterpenoid-mediated induction of reactive oxygen species (ROS) has now been characterized as an important proteasome-independent pathway for downregulation of Sp transcription factors. ROS decreases expression of microRNA-27a (miR-27a) and miR-20a/miR-17-5p and this results in the induction of the transcriptional “Sp-repressors” ZBTB10 and ZBTB4, respectively, which in turn downregulate Sp and Sp-regulated genes. Triterpenoids also activate or deactivate nuclear receptors and G-protein coupled receptors, and these pathways contribute to their antitumorigenic activity and may also play a role in targeting Sp1, Sp3 and Sp4 which are highly overexpressed in multiple cancers and appear to be important for maintaining the cancer phenotype.

Keywords

Sp transcription factors; downregulation; reactive oxygen species

INTRODUCTION

Triterpenoids are important components of traditional medicines from plant extracts, and individual triterpenoids and their synthetic analogs are extensively used for biomedical applications [1]. Some of the biological effects of triterpenoids include their bactericidal, anti-inflammatory, antiviral, cytotoxic, growth inhibitory and anticancer activities. For example, betulin and its analogs are used in cosmetics, shampoos and conditioners and as plasticizers, and betulinic acid and its derivatives exhibit antitumor, antiviral, antibacterial, anti-inflammatory and antimalarial activities [2, 3].

The anticancer activities of individual triterpenoids have recently been reviewed in a book entitled “*Pentacyclic Triterpenes as Promising Agents in Cancer*” [1]. Triterpenoids are derived from the intramolecular condensation of the linear 2,3-oxidosqualene to give different classes of triterpenoids including tetracyclic sterols such as lanosterol, the precursor of cholesterol, and the pentacyclic triterpenes which are subsequently converted into a vast array of nearly 200 triterpenoid ring skeletons. The oxidosqualene cyclases β -amyrin synthase, β -amyrin synthase and lupeol synthase catalyze the conversion of 2,3-oxidosqualene into β -amyrin and β -amyrin which contain five 6-membered rings and lupeol which contains four 6-membered and one 5-membered ring, respectively. Some of the most important and active triterpenoid acids include betulinic acid (BA) (from lupeol), ursolic acid (UA) (from β -amyrin), and oleanolic acid (OA) and glycyrrhetic acid (GA) (from β -amyrin) [1]. This report will not extensively review the seemingly diverse antitumorigenic activities of triterpenoids but will primarily focus on some unifying concepts in their overall mechanisms of action.

THE ANTICANCER ACTIVITIES OF TRITERPENOID

Research on the anticancer activity of pentacyclic triterpenoids is extensive [1] but detailed studies are primarily available on BA, UA and OA and synthetic analogs 2-cyano-3,12-dioxooleana-1,9-dien-28-oic acid (CDDO) and 2-cyano-3,11-dioxo-18 β -oleana-1,12-dien-30-oic acid (CDODA) (Fig. 1) [4–7]. Based on the extensive summaries of these compounds and other active triterpenoids [4–7], it is evident that many structurally diverse triterpenoids and their synthetic analogs give overlapping activities; however, direct comparisons of the effects of different triterpenoids are limited and incomplete since most studies have examined diverse responses in different cell lines. Similarities and differences in the effects of various triterpenoids is also complicated by cell context-dependent differences. For example, almost all pentacyclic triterpenoids induce apoptosis and inhibit growth of cancer cells derived from solid and non-solid tumors. The pentacyclic triterpenoid compounds also affect cell cycle progression, angiogenic, metastatic and anti-inflammatory responses; however, these responses have been determined only in a limited number of cell lines [4–7]. Moreover, most reports have focused only on one or two pathways and with the exception of some receptor studies (see next section), the specific sub-cellular receptors or acceptors that are bound or activated by pentacyclic triterpenoids have not been extensively investigated or identified.

Although a systematic study of genes that are affected by treatment of cancer cells with pentacyclic triterpenoids has not been made, several reports show that one or more of these compounds decrease expression of cyclin D1, bcl-2, survivin and angiogenic genes such as vascular endothelial growth factor (VEGF) and its receptors (VEGFR) [8–15]. Research in this laboratory initially focused on the effects of BA in prostate cancer cells, and treatment of androgen-responsive LNCaP cells with this compound decreased expression of cyclin D1, the androgen receptor, VEGF and survivin and this was accompanied by caspase-dependent PARP cleavage [16]. In bladder cancer cells, BA decreased some of the same responses and also decreased expression of the epidermal growth factor receptor (EGFR) and this was accompanied by increased autophagy which is normally suppressed by EGFR in KU7 and 253UB-V bladder cancer cells [17]. Treatment of RKO and SW480 colon cancer cells with BA also decreased expression of cyclin D1, survivin, VEGF and EGFR and both pituitary tumor transforming gene-1 (PTTG-1) and the p65 subunit of NF κ B were also decreased [18].

The results obtained for BA were similar to those observed for CDDO-Me and CDODA-Me in several cancer lines which show that these synthetic triterpenoid esters induce apoptosis and inhibit expression of genes associated with cell proliferation (cyclin D1, EGFR),

survival (bcl-2, survivin), angiogenesis (VEGF, VEGFR) and inflammation (p65) [19, 20]. Previous studies in this laboratory showed that hormone-induced activation of estrogen receptor β (ER β) involved interactions of ER β with transcription factors Sp1, Sp3 and Sp4 bound to GC-rich promoter sites, and knockdown of Sp1, Sp3 and Sp4 by RNA interference (RNAi) decreased hormone-induced and basal expression of multiple genes [21, 22]. Since many of the genes downregulated by BA, CDODA-Me and CDDO-Me also contain GC-rich Sp-binding promoter elements, we used Sp knockdown by RNA interference (RNAi) and identified several genes regulated by Sp1, Sp3 and Sp4 in different cancer cell lines [17, 20, 23–30]. Sp-regulated genes in these cells include cyclin D1, bcl-2, survivin, PTTG-1, EGFR, hepatocyte growth factor receptor (c-MET), VEGF, VEGFR1 and VEGFR2. Moreover, several other pro-oncogenic Sp-regulated genes including YY1, β -catenin, CXCR4 and platelet-derived growth factor receptor have also been identified [unpublished results]. These results suggest that the anticancer activities of BA, CDODA-Me and CDDO-Me may be due, in part, to downregulation of Sp1, Sp3, Sp4 and pro-oncogenic Sp-regulated genes, many of which are individual drug targets of neutralizing antibodies and kinase inhibitors.

Sp1, Sp3 AND Sp4 TRANSCRIPTION FACTORS ARE TARGETED BY TRITERPENOIDs

Sp Transcription Factors and Cancer

Sp1 was the first transcription factor identified and it is a member of the Sp/Krüppel/like factor (KLF) family of zinc finger proteins that modulate expression of multiple genes [31]. Sp1, Sp3 and Sp4 are critical transcription factors required for embryonic and early-life development; however, studies with Sp1 show that expression of this transcription factor decreases with increasing age in humans and rodents [32–34]. Studies in this laboratory show that Sp1, Sp3 and Sp4 are highly expressed in cancer cells and tumors whereas in mice bearing cancer cell xenografts, only minimal to non-detectable levels of Sp1, Sp3 and Sp4 are observed [16–19, 24–27, 29, 30]. The differential expression of Sp1, Sp3 and Sp4 in tumor vs. non-tumor tissue coupled with the pro-oncogenic activity of Sp-regulated genes suggests that these transcription factors are ideal targets for mechanism-based drugs. It has been reported that anticancer drugs such as COX-2 inhibitors alter DNA binding of Sp1 due to altered phosphorylation [35]; however, it has subsequently been shown that the COX-2 inhibitors (celecoxib, nimesulfide and NS-398 [NS; N-[2-(cyclohexyloxy)-4-nitrophenyl]methanesulfonamide]) decreased expression of Sp1 and Sp4 but not Sp3 in colon cancer cells [36]. The COX-2 inhibitors also decreased VEGF expression, and RNAi confirmed that knockdown of Sp1, Sp3 or Sp4 decreased VEGF protein. Subsequent studies on a series of non-steroidal anti-inflammatory drugs (NSAIDs) have identified tolifenamic acid (TA) and related biphenylamine carboxylic acids as a new class of anticancer agents that downregulate Sp1, Sp3 and Sp4 in pancreatic and other cancer cell lines [37–41].

BA, CDODA-Me, CDDO-Me and Other Triterpenoids Downregulate Sp1, Sp3, Sp4 and Sp-regulated Genes

We have shown that BA decreases Sp1, Sp3 and Sp4 proteins in LNCaP prostate [16], KU7 and 253JB-V bladder [17], and RKO and SW480 colon [18] cancer cell lines. Figure 2 shows that treatment of 253JB-V bladder and Panc28 pancreatic cancer cells for 24 hr with 10–25 μ M BA decreases expression of Sp1, Sp3 and Sp4 and the concentration of BA required for this response decreases at longer time points [17]. Since knockdown of Sp transcription factors (individually or combined) results in the induction of both caspase-dependent apoptosis and induction of EGFR-regulated autophagic cell death and growth inhibition [16–18], then the anticancer activity of BA must be due, in part, to downregulation of Sp1, Sp3 and Sp4.

CDDO and related compounds are among the most widely investigated synthetic triterpenoids as anticancer agents in multiple cancer cell lines [7]. Although several mechanisms of action have been proposed for this class of triterpenoid acid derivatives, treatment of Panc1, Panc28 and L3.6pL pancreatic cancer cells with CDDO-Me decreases Sp1, Sp3, Sp4 and Sp-regulated VEGF, cyclin D1, VEGFR2 and survivin [20]. Figure 3 illustrates that CDDO-Me also inhibits growth of KU7 and 253JB-V bladder cancer cells and this is accompanied by downregulation of Sp1, Sp3 and Sp4 proteins. Moreover, knockdown of Sp proteins by RNAi induces apoptosis and inhibits cell growth in these cell lines, and similar results are observed after treatment with CDDO-Me confirming a role for CDDO-Me-induced downregulation of Sp transcription factors in the anticarcinogenic activity of CDDO compounds [20].

CDODA-Me (Fig 1) is synthesized from glycyrrhetic acid and is a structural analog of CDDO-Me. Both compounds contain identical A ring substitution (2-cyano-3-oxo-1-ene) but differ in the position of enone structures (ring C) and the carboxyl group (ring E). Although only a limited amount of work has been published for CDODA-Me, this compound exhibits potent anticarcinogenic activity, and in colon cancer cells, CDODA-Me also downregulates expression of Sp1, Sp3, Sp4 and several Sp-regulated genes [19]. Figure 2C also illustrates that CDODA-Me decreases Sp1, Sp3 and Sp4 proteins in L3.6pL pancreatic cancer cells and similar results have been observed in prostate and breast cancer cells (unpublished results).

Mechanisms of Drug-induced Downregulation Sp Transcription Factors

Proteasome-dependent—Initial studies with TA and BA in Panc1 pancreatic and LNCaP prostate cancer cells showed that downregulation of Sp1, Sp3 and Sp4 proteins is reversed with proteasome inhibitors and activation of the proteasome is a major pathway for targeting Sp transcription factors [18, 37]. However, it should be noted that proteasome activation is both compound- and cell context-specific. For example, BA induces proteasome-dependent degradation of Sp1, Sp3 and Sp4 in SW480 but not RKO colon cancer cells [18]. Similar cell context-dependent differences have been observed for downregulation of Sp1, Sp3 and Sp4 by other anticancer agents [28, 30, 37–41]. The mechanisms for triterpenoid-induced activation of proteasomes and the specific targeting of Sp transcription factors have not yet been delineated.

Proteasome-independent—BA and many of the triterpenoid anticancer agents perturb mitochondria, decrease mitochondrial membrane potential (MMP), and induce reactive oxygen species (ROS) in many cancer cell lines [18, 20, 26–29, 42]. Drug-induced ROS is an important mechanism of action for many anticancer drugs including arsenic trioxide, pro-oxidants and multiple drugs that act through mitochondrial disruption and decreased MMP [42–45]. Treatment of SW480 and RKO colon cancer cells with BA downregulates Sp1, Sp3 and Sp4 in RKO cells and this response is blocked in cells cotreated with BA plus the antioxidants glutathione (GSH) or dithiothreitol (DTT) [18]. Comparable results are observed for nitro-NSAID (GT-094) where GT-094 induces proteasome-dependent downregulation of Sp proteins in SW480 cells, whereas the same response in RKO cells is ROS-dependent [28], and similar results are observed for CDODA-Me [19]. Downregulation of Sp1, Sp3 and Sp4 in Panc1 and L3.6pL pancreatic cancer cells treated with CDDO-Me is reversed after cotreatment with antioxidants [20], and Figure 4 shows that CDODA-Me- and CDDO-Me-induced downregulation of Sp1, Sp3 and Sp4 in pancreatic cancer cells is significantly inhibited after cotreatment with the antioxidants DTT or GSH. In parallel studies, drugs such as arsenic trioxide, curcumin and ascorbate that induce ROS also downregulate Sp1, Sp3 and Sp4 in bladder and colon cancer cells [26, 27, 46]. Moreover, treatment with hydrogen peroxide or *t*-butylhydroperoxide also decreases Sp

proteins and these effects are reversed after cotreatment with antioxidants [46]. These results demonstrate for the first time that ROS downregulates Sp1, Sp3 and Sp4 in cancer cell lines and this response plays a role in the anticancer activities associated with drug-induced ROS.

Lou and coworkers demonstrated that Sp1 expression which is low in human fibroblasts is increased 8- to 18-fold by carcinogen-induced transformation [47]. The resulting transformed cells form tumors in mouse xenograft experiments; however, knockdown of Sp1 in these cells results in their lack of tumorigenicity. These results are consistent with the higher expression of Sp1, Sp3, Sp4 and pro-oncogenic Sp-regulated genes in cancer cells and tumors, but do not provide a rationale for this cancer cell genotype. Benz and coworkers first reported that after treatment of breast cancer cells with HDAC inhibitors, there is a decrease in microRNA-27a (miR-27a) and increased expression of the transcriptional repressor ZBTB10 [48]. Subsequent studies in this laboratory showed that ZBTB10 and ZBTB4 are “Sp-repressors” that competitively bind GC-rich promoters to repress gene expression [49, 50]. High levels of Sp1, Sp3 and Sp4 are related to suppression of ZBTB10 and ZBTB4 by miR-27a and miR-20a/miR-17-5p, respectively, and both miRs are highly expressed in cancer cells [49, 50]. Treatment of colon and pancreatic cancer cells with BA or CDDO-Me, respectively, decreased MMP, induced ROS, decreased miR-27a and induced ZBTB10 expression and this was accompanied by downregulation of Sp1, Sp3 and Sp4 [18, 20]. Cotreatment with BA or CDDO-Me plus antioxidants reverses all responses, demonstrating that ROS targets miR-27a:ZBTB10 and, in ongoing studies, similar effects have been observed for miR-20a/17-5p:ZBTB4 interactions. The mechanisms associated with ROS-dependent downregulation of these miRs have not yet been determined. Moreover, there is evidence for ROS-independent downregulation of miRs and this is currently being investigated. Our results strongly suggest that direct targeting of mitochondria by triterpenoids and the subsequent induction of ROS in some cancer cells is an important mechanism for downregulation of Sp transcription factors (Fig. 5). This pathway is also consistent with the specificity of triterpenoids and other anticancer drugs that decrease MMP and induce ROS in cancer cells. In non-cancer cells/tissues, MMP is relatively low, and minimal induction of ROS and downstream cytotoxic effects are observed, whereas in cancer cells, ROS-induced downregulation of Sp1, Sp3, Sp4 and Sp-regulated genes results in inhibition of cell growth and angiogenesis, induction of apoptosis, and decreased inflammation [18, 20, 28].

TRITERPENOID MODULATE NUCLEAR RECEPTORS AND THEIR DOWNSTREAM GENES AND RESPONSES

PPAR γ

CDDO and related compounds were originally characterized among a series of oleanolic and ursolic acid derivatives as potent anti-inflammatory agents [51–53] which also exhibit diverse anticancer activities [7]. These anticancer responses are highly cell context-dependent and as indicated above include induction of ROS and ROS-mediated perturbation of miR-27a:ZBTB10 and downregulation of Sp1, Sp3, Sp4 and Sp4-regulated genes [20]. Initial screening studies for determining specific targets for CDDO and related compounds showed that both CDDO and CDDO-Me bind peroxisome-activated receptor γ (PPAR γ) [54] and exhibit partial agonist and antagonist activities, respectively. PPAR γ is overexpressed in multiple types of tumors [55], and results obtained for CDDO suggest that PPAR γ is an important intracellular target since this receptor is considered a potential target for cancer chemotherapy [56–60]. Studies in this laboratory showed that CDDO and its derivatives activate PPAR γ -dependent transactivation and inhibit colon cancer cell growth and induction of tumor suppressor caveolin-1 by CDDO-Me is PPAR γ -dependent [61]. However, induction of apoptosis by these compounds is PPAR γ -independent [61] and

results of several studies with CDDO and related compounds suggest that their anticancer activities are primarily PPAR γ -independent [61–64]. However, this does not exclude a possible role for PPAR γ in studies showing that CDDO in combination with retinoids enhanced apoptosis and differentiation in leukemia cells [65, 66].

CDODA and CDODA-Me are glycyrrhetic acid derivatives and are potent inhibitors of pancreatic and bladder cancer cell growth [67]. They have identical A-ring structures with their corresponding CDDO and CDDO-Me counterparts and they also induce PPAR γ -dependent transactivation in colon and prostate cancer cells [68, 69]. In colon cancer cells treated with CDODA-Me, induction of the tumor suppressors caveolin-1 and Krüppel-like factor-4 (KLF4) is PPAR γ -dependent in some cells, demonstrating that PPAR γ plays some role in the antitumor activity of CDODA-Me [68], even though subsequent studies showed that targeting downregulation of Sp transcription factors is a major pathway for the anticancer activity of this compound [19]. CDODA-Me also induces apoptosis and inhibits prostate cancer cell growth [69]; however, in LNCaP prostate cancer cells, the activity is largely PPAR γ -independent and also due to downregulation of Sp transcription factors [unpublished results]. Based on results obtained for CDDO and CDODA compounds, we synthesized the 2-cyano-1-en-3-oxo derivative of betulinic acid (CN-BA), and this compound and its methyl ester (CN-BA-Me) were highly cytotoxic to colon and pancreatic cancer cells [70]. CN-BA and CN-BA-Me induced PPAR γ -dependent caveolin-1 in colon cancer cells and p21 in pancreatic cancers, whereas induction of KLF4 in colon cancer cells was PPAR γ -independent. These results demonstrate that A-ring modified triterpenoids are PPAR γ agonists; however, the role of the receptor in mediating their anticancer activity may not be significant since they also target downregulation of Sp transcription factors [16–20].

FXR

The farnesoid X receptor (FXR, NR1H4) is another nuclear receptor that has multiple endogenous functions in several tissues, particularly in liver where FXR is a “sensor” for bile acids [71, 72]. Excess bile acid levels can lead to significant liver damage, and FXR is activated by bile acids to reprogram expression of genes that play a role in hepatoprotection from these toxicants [71, 72]. FXR is overexpressed in multiple tumors and there is evidence that FXR plays a role in cancer cell growth, survival and metastasis [73–77]. However, it should also be noted that in FXR-deficient mice, there is an increase in liver and intestinal tumor development [78, 79]. Although FXR exhibits a high binding affinity for bile acids, this receptor also binds phytosterols such as lanosterol [80], stigmasterol and stigmasterol acetate (but not β -sitosterol) which decrease FXR-mediated transactivation in HepG2 liver cancer cells [81]. These results suggests that interactions of triterpenoids with FXR (i.e. deactivation) may serve as a novel approach for treating tumors such as pancreatic cancer in which FXR is overexpressed and exhibits pro-oncogenic activity [77].

Estrogen Receptor (ER)

The estrogen receptor (ER) is differentially expressed as ER α and ER β in various tumors. ER β is highly expressed in tumors from a high percentage of early stage breast cancer patients and these individuals can be treated with endocrine therapies. In contrast, ER α expression in tumors is highly variable and is associated with growth inhibition [82]. Several cucurbitane-derived triterpenoids isolated from *Momordica charantia* (bitter lemon) extracts exhibit ER α and ER β agonist/antagonist activities [83]; however, it is unknown if these responses contribute to the anticancer activity of these extracts. 27-Hydroxycholesterol has been characterized as a selective ER modulator (SERM) and there is evidence suggesting that other triterpenoid-derived compounds may also be SERMs with potential for treating estrogen-responsive tumors [84–86].

MEMBRANE RECEPTORS

G-protein-coupled receptors and other membrane receptors are critical sensors of extracellular signals and, although this large family of receptors has not been well characterized, many of the receptors such as the cannabinoid receptors bind lipophilic molecules similar to triterpenoids. It has previously been reported that triterpenoids affect expression of membrane receptors such as the death receptors DR5 and DR [87] and Sp-regulated receptors [17, 25, 30]. However, only a limited number of studies show direct interaction between triterpenoids and membrane receptors and these are briefly summarized below.

Melanocortin Receptors

Betulin is a precursor of BA and exhibits anticancer activity. Binding studies show that betulin binds the melanocortin receptors with variable K_i values and potencies ($MC1 > MC3 > MC5 > MC4$), and the K_i value for MC1 is 1.022 μM [88]. Although betulin does not induce cAMP expression in mouse melanoma cells, betulin inhibits induction of this second messenger by β -melanocyte-stimulating hormone. The importance of melanocortin receptors in mediating anticancer activities of betulin and other triterpenoids has not been further investigated.

TGR5

Like FXR, TGR5 binds diverse bile acids; however, this receptor is a GPCR and a member of the rhodopsin-like superfamily [89, 90]. TGR5 has multiple functions, primarily related to bile acid-regulated responses including lipid and glucose metabolism and energy homeostasis and these effects can be coordinately regulated with parallel FXR activation by bile acids [91]. TGR5 plays a role in bile acid-induced activation of kinases and the epidermal growth factor receptor in gastric carcinoma cells [92] and bile acid-induced proliferation in development of esophageal adenocarcinoma [93]. Recent structure-activity studies show that betulinic acid, oleanolic acid, and ursolic acid all exhibit TGR5 agonist activities in the low μM concentrations in transfected Chinese hamster ovary cells and several other synthetic analogs are also active [94]. Since many of these compounds including BA exhibit anticancer activity, it is likely that TGR5 may also be involved in this type of activity in some tumor types and future studies will be required to investigate these possibilities.

CCR5

A recent study investigated the effects of several lupane-derived compounds for their anti-HIV and anti-chemotactic activity by their interactions with chemokine receptors [95]. 30-Oxo-calenduladiol exhibited high CCR antagonist activity, suggesting a potential role for lupane-derived molecules as antiviral agents. It is possible that the anticancer activity of these compounds may also be related to their effects on chemokine receptors.

Cannabinoid receptors

Cannabinoids (CBs) have been extensively used as anticancer agents, and CB receptors (CB1 and CB2) bind structurally diverse lipophilic compounds [96–102]. Recent studies on the anti-inflammatory and anti-nociceptive activity in a mouse model showed that β - and β -amyryn are highly effective and bind the CB1 ($K_i = 0.133 \text{ nM}$) and CB2 (1.989 μM) receptors [103]. These results are consistent with several reports on the anti-inflammatory activity of β - and β -amyryn in other animal models and cells, and their cytotoxicity in cancer cells [104–107] parallels the anticancer activity of cannabinoids. Ongoing studies in this laboratory have also linked cannabinoid- and specific triterpenoid-mediated activation of

CB1 and CB2 receptors to downregulation of Sp proteins and Sp-regulated genes, and this may be a major pathway for targeting Sp transcription factors.

SUMMARY

The pentacyclic triterpenoids exhibit a broad spectrum of antitumor activities which make them an important class of natural products that can be used as models for anticancer drug development. Many of these compounds activate or deactivate nuclear or membrane receptors and these interactions undoubtedly contribute to their activity. However, based on more extensive studies with BA, CDODA-Me and CDOD-Me, we feel that their effects on downregulation of Sp1, Sp3, Sp4 and Sp-regulated genes may be a highly significant pathway for their anticancer activity. This is particularly true for those cell lines where triterpenoids induce ROS and trigger ROS-dependent disruption of miR-27a:ZBTB10 or miR-20a/17-5p:ZBTB4. Although most of the studies have focused on the effects of BA, CDODA-Me and CDDO-Me on Sp1, Sp3 and Sp4, it should also be noted that celastrol, methyl ursolate and β -boswellic acid derivatives also downregulate Sp proteins [6] and this supports the importance of this pathway in the anticancer activity of triterpenoids.

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Figure 1.
Structures of selected triterpenoid anticancer agents.

Figure 2. Downregulation of Sp1, Sp3 and Sp4 proteins. 253JB-V (A), Panc28 (B) and L3.6pL (C) cells were treated with BA, BA or CDODA-Me, respectively, for 24 hr, and cell lysates were analyzed by western blots [16–20].

Figure 3.
CDDO-Me inhibits bladder cancer cell growth and downregulates Sp1, Sp3 and Sp4. Bladder cancer cells were treated with CDDO-Me for the indicated times, and cell counts were determined (A), and whole cell lysates were analyzed by western blots (B) [16–20].

Figure 4.

Antioxidants reverse CDODA-Me-/CDDO-Me-induced downregulation of Sp1, Sp3 and Sp4 in pancreatic cancer cells. Cells were treated with CDDO-Me or CDODA-Me alone or in combination with antioxidants for 24 hr, and whole cell lysates were analyzed by western blots [16–20].

Figure 5. Proposed mechanisms of triterpenoid-mediated downregulation of Sp proteins and Sp-regulated genes.