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Targeting Apoptosis Pathway with Natural Terpenoids: Implications for Treatment of Breast and Prostate Cancer

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Abstract

Terpenoids represent a large and diverse class of naturally occurring compounds found in a variety of fruits, vegetables and medicinal plants. Structurally some of the terpenoids are similar to human hormones. A diet rich in terpenoids is inversely related with the risk of chronic diseases including cancers. Breast and prostate cancers are hormone-related diseases and the second leading cause of female and male cancer mortality. Diterpenoid paclitaxel, and its semi-synthetic analogue docetaxel, have entered clinical use against established breast and prostate cancers. Here we reviewed potential molecular targets and biological properties of natural terpenoids, including monoterpenoids, diterpenoids, triterpenoids and tetraterpenoids, and their applications in treatment of human breast and prostate cancers. These terpenoids are able to inhibit tumor cell proliferation and induce tumor cell death by inhibiting multiple cancer-specific targets including the proteasome, NF- κ B, and antiapoptotic protein Bcl-2. The efficacy of these terpenoids against breast or prostate cancer cells, as demonstrated in pre-clinical studies supports clinical application of these naturally occurring terpenoids in treatment of hormone-related human cancers.

Keywords

Terpenoids; breast cancer; prostate cancer; proteasome inhibitors; apoptosis

1. INTRODUCTION

Terpenoids represent a large class of natural compounds. They are classified according to the number of containing cyclic structures such as hemiterpenoids (with 1 isoprene unit), monoterpenoids (with 2 isoprene units), sesquiterpenoids (with 3 isoprene units), diterpenoids (with 4 isoprene units), sesterterpenoids (with 5 isoprene units), triterpenoids (with 6 isoprene units), tetraterpenoids (with 8 isoprene units) and polyterpenoids (with a larger number of isoprene units). Terpenoids are extensively found in fruits, vegetables and medicinal plants. This category of compounds exhibits multiple properties including antioxidation, anti-inflammation and anticancer. *In vitro* and *in vivo* studies indicate that terpenoids caused inhibition on cell proliferation and tumor growth in a variety of human cancers. However, because of the space limitation of this review article, we only focus on human breast and prostate cancers. In addition, multiple molecular pathways were found to be involved in the anticancer actions of terpenoids, including activation of apoptosis triggered by inhibition of the ubiquitin-proteasome and NF- κ B pathways. It has been well

documented that suppression of the ubiquitin-proteasome and NF- κ B pathways is essential for induction of tumor cell apoptosis. Therefore in this review, we first summarize the research on the effect of terpenoids on apoptosis, proteasome and NF- κ B pathways and then discuss the potential application of terpenoids for treatment of human breast and prostate cancers.

1.1. Breast and Prostate Cancers

Breast cancer is the most common cancer expected to occur in women, and in 2009 it was the second cause of cancer death in women [1]. Steroid hormone estrogen, especially estradiol plays an important role in the carcinogenesis of breast cancer [2]. Estrogen such as estradiol binds to the estrogen receptor α or β (ER α or ER β) in the nucleus of breast cancer cells and initiates conformational changes in ER structures. The conformational changes result in the dimerization and binding of ER at the appropriate estrogen response elements (EREs) in the promoter region of estrogen-responsive genes, recruiting coactivators and initiating the formation of transcriptional complex. In addition to estradiol binding, ER activity could be enhanced *via* phosphorylation at several specific sites of ER α [3].

Based on gene expression, breast cancer could be divided into three subtypes: (i) the luminal subtype, which expresses the human epidermal growth factor receptor 2 (HER2) and ER, (ii) hormone receptor-negative subtype, and (iii) the basal-like subtype which recently is of particular interest [4, 5]. Basal-like breast cancer is characterized by the absence of ER, progesterone receptor (PR) and HER2 overexpression [4] or referred as ER-, PR- and HER2-negative breast cancer, so often called triple-negative breast cancer [6]. The subtypes vary in prognosis, with worse outcomes observed in hormone receptor-negative patients compared with the luminal subgroups, while triple-negative subtype of breast cancer is a very aggressive tumor with poor prognosis [4, 7].

Prostate cancer is the most common cancer expected to occur in men and the major cause of cancer death, next to lung cancer in men, in 2009 [1]. Steroid hormone androgens contribute to the initiation and promotion of multistage carcinogenesis through binding to hormone receptors [2]. An androgen receptor (AR) is a physiological mediator of the development and function of male reproductive organs [8]. Upon androgen binding, inactive AR is activated through dimerization and nuclear translocation, where it functions as a transcription factor to alter the expression of androgen responsive genes [9]. AR plays an important role in the initiation and progression of prostate cancer by regulating cell proliferation, differentiation and apoptosis [8]. Early stages of prostate cancer can be effectively treated by androgen-ablation therapy through surgical and medical castration. However, most of these prostate cancer patients eventually relapse to a hormone-refractory state that no longer responds to androgen deprivation [10]. AR appears to be a dominant factor in the transition from hormone-sensitive to hormone-refractory disease [11].

There is well-established evidence to show that the AR gene undergoes alterations such as amplification or mutation in hormone-independent cancers. As a result, these hormone-independent cancer cells are very sensitive to low or no androgen environments, and are responsive to a broad range of ligands such as growth factors, other steroid hormones, anti-androgens, *etc* [12, 13]. It has also been reported that wild type AR can be activated by other signaling pathways in a ligand-independent manner [14, 15]. Furthermore, unliganded AR can bind the enhancer elements on the promoters of target genes and mediate their expression even in the absence of androgen, as seen with the prostate specific antigen (PSA) gene in androgen-independent prostate cancer cells [16]. Therefore, finding an effective means to eliminate AR from prostate cancer cells is essential for an effective treatment of prostate cancer.

1.2. Apoptosis

Programmed cell death (apoptosis) is a highly conserved form of cell death that plays crucial roles for embryonic development and tissue homeostasis. Dysregulated apoptosis leads to tumor formation or even development of cancer cell drug resistance [17]. Apoptosis is triggered through two main routes; the extrinsic (depending on triggering of death receptors, transmembrane proteins expressed on the cell surface) and intrinsic (mediated by molecules released from the mitochondria) pathways [18]. A set of cysteine proteases, caspases that cleave substrates at specific aspartyl residues, is regarded as the cascade of both apoptotic pathways. However, caspase-independent apoptosis was also observed [19]. Caspases are expressed in cells as inactive proenzyme forms that require oligomerization and/or cleavage for activation.

The extrinsic apoptosis pathway is initiated through the binding of ligand to death receptors that contain an intracellular death domain. Upon ligand binding, the death receptors trimerize and recruit the adaptor protein Fas-associated death domain (FADD) and procaspase-8 and/or caspase-10 to form a death-inducing signaling complexes (DISC) in the intracellular death domain [20]. In the DISC, caspase-8 or -10 oligomerization drives its activation through auto-proteolytic cleavage [18]. This leads to triggering of the enzymatic activity of downstream effector caspases, such as caspase-3 and -7 [18].

Upon physical or chemical stimulations, such as hypoxia, growth factor deprivation, cell detachment, or stress signals, the intrinsic mitochondrial pathway is activated. The proapoptotic Bcl-2 family members, such as Bax or Bak play an important role in initiating this pathway. They oligomerize and integrate into the mitochondria outer membrane, which is thought to open a pore and cause subsequent mitochondria outer membrane permeabilization (MOMP) [21]. As a result, a component of the mitochondrial respiratory chain, cytochrome-c, present in the intermembrane space, is released from mitochondria into the cytosol [21]. In the cytosol, cytochrome c promotes the oligomerization of apoptotic peptidase activating factor 1 (Apaf-1), leading to recruitment and activation of caspase-9 in a large complex called the apoptosome. Caspase-9 is the initiator caspase associated with the intrinsic or mitochondrial pathway of apoptosis. Once activated, caspase-9 cleaves and activates downstream effector caspases-3 and -7, which then cleave the key regulatory and structural proteins to execute cell death.

1.3. Ubiquitin-Proteasome Pathway and Nuclear Factor *kappa* B (NF κ B)

The ubiquitin-proteasome pathway is responsible for the degradation of most intracellular proteins involved in regulation of cell cycle and apoptosis. A targeted protein is marked with a chain of small polypeptides called ubiquitin. The ubiquitination process is mediated by a series of enzymes, called E1 ubiquitin activating enzyme, E2 ubiquitin-conjugating enzyme, and E3 ubiquitin-ligating enzyme [22, 23]. Polyubiquitinated proteins can then be recognized and degraded by the proteasome.

The proteasome is a large proteolytic complex which is referred as the 26S proteasome (Fig. 1), and expressed in both the nucleus and the cytoplasm in all eukaryotic cells. It consists of one or two 19S regulatory particles and a 20S catalytic core. The 20S proteasome consists of four stacked rings: two inner beta rings and two outer alpha rings, each of which consists of seven subunits [24] (Fig. 1). The 19S particle (700 kDa) consists of both a lid and a base, which is responsible for controlling the access of substrates into the 20S proteasome. Furthermore, the 19S particle is responsible for unfolding and threading the ubiquitinated proteins into the catalytic site of the 20S proteasome [25]. These diverse actions are fulfilled by multiple subcomplexes of the 19S regulatory particle [26].

The 20S proteasome hydrolyzes the target protein through three proteolytic activities, caspase or peptidyl-glutamyl peptide-hydrolyzing (PGPH)-like, trypsin-like and chymotrypsin-like activities. These activities are mediated through three β subunits, β 1 (PGPH-like), β 2 (trypsin-like), and β 5 (chymotrypsin-like), respectively [27]. In accordance with enzymes with similar hydrolytic activities, the β 1 subunit cuts peptides after acidic residues, the β 2 subunit prefers to cleave after basic amino acids, and the β 5 subunit hydrolyzes after hydrophobic residues. All three proteolytic activities depend on the presence of the threonine residue at the amino terminal (Thr1) [28].

Higher levels of proteasome activity were observed in tumor cells compared to normal cells [29, 30], suggesting the importance of the proteasome in the growth of tumor cells. Indeed, several antiapoptotic signaling pathways require proteasomal activity for their function. This is especially true for the pro-oncogenic nuclear factor *kappa* B (NF- κ B) pathway. NF- κ B plays an important role in tumorigenesis *via* transactivation of genes involved in cell proliferation, apoptosis, tumor cell invasion, metastasis, and angiogenesis [31]. Constitutive activation of NF- κ B is commonly observed in many types of cancer [32].

Five members of the NF- κ B family, that are p65 (RelA), RelB, c-Rel, NF- κ B1 or p50, and NF- κ B2 or p52 subunits, have been identified, all of which contain a REL-homology domain that is responsible for DNA binding, dimerization, nuclear translocation and endogenous inhibitor of NF- κ B (I κ B) binding [33]. They form homo- and heterodimers through combinatorial assembly, but p50 and p52 must dimerize with a transactivation domain-containing member such as p65, c-REL or REL-B to activate gene transcription [34] (Fig. 1). The classic form of NF- κ B is p50/p65 heterodimer. I κ B binds to the REL-homology domain of NF- κ B through ankyrin repeats, covering the nuclear localization sequence of NF- κ B [33]. Therefore NF- κ B is normally sequestered in the cytoplasm through association with its endogenous inhibitor I κ B. Upon stimulation, I κ B- α is rapidly phosphorylated by kinase IKK (I κ B kinase) composed of two catalytic subunits, IKK- α and IKK- β , and one regulatory subunit IKK- γ [35]. Phosphorylated I κ B- α is specifically recognized by SCF ^{β -TrCP} ubiquitin ligase at the 19-amino-acid destruction motif in residues 21-41 [36] and transferred to the 26S proteasome for degradation while NF- κ B is allowed to be translocated to the nucleus and mediate transcription of anti-apoptotic genes (Fig. 1). However, accumulation of the I κ B- α protein *via* proteasome inhibition prevents the activation of anti-apoptotic NF- κ B [37], resulting in tumor cell apoptosis. The classical NF- κ B activation is the major pathway through which NF- κ B mediates anti-apoptotic effects. Alternatively, the p100 precursor is activated through limited ubiquitin-dependent proteasome degradation, generating active form p52 [38] (Fig. 1). p52 binds with RelB and transactivates target genes in the nucleus. Therefore, inhibition on the proteasome activity will prevent alternative NF- κ B activation (Fig. 1).

2. MONOTERPENOIDS

A number of monoterpenoids have chemopreventive activity against hormone-related cancers. For example, a *Cinnamomum* monoterpenoid subamone was cytotoxic against prostate cancer cell lines DU-145 and LNCaP [39]. Carveol, uroterpenol, and sobrerol (Fig. 2) were reported to have chemopreventive activity against mammary cancer [40].

D-limonene (Fig. 2) is a major component of citrus peel oil that has been well investigated in both breast and prostate cancer. It demonstrated anti-carcinogenic effects when given during the initiation phase as well as the promotion/progression phase in mammary cancer in rats [41, 42]. Limonene-induced regression was also observed in established mammary carcinomas in rat models [43]. In a phase I clinical trial using d-limonene, partial response

was observed in one breast cancer patient and that was maintained for 11 months before withdraw from the trial because of the progression of cancer in the bone [44].

Several cellular molecules in cancer cells were potentially targeted by d-limonene for mediating its chemopreventive and antitumor activities. Associated with its chemopreventive effect, d-limonene induced phase I and phase II carcinogen-metabolizing enzymes, resulting in carcinogen detoxification [45]. Related to the antitumor activity, d-limonene induced apoptosis, tumor remodeling/re-differentiation to a more benign phenotype, and/or inhibition of the post-translational isoprenylation of cell growth-regulating proteins [40, 45, 46].

Dietary supplementation with limonene can not delay the development of prostate cancer in a rat model [47]. However, it was found that limonene supplement enhanced the antitumor effect of docetaxel against prostate cancer cells without being toxic to normal prostate epithelial cells [48]. Exposure of DU-145 cells to a combined d-limonene and docetaxel resulted in higher reactive oxygen species (ROS) generation and depletion of GSH, accompanied by increased caspase activity than docetaxel alone. The combined beneficial effect could be through the modulation of proteins involved in mitochondrial pathway of apoptosis. It triggered a series of effects involving cytochrome c release, caspase-9 and -3 activation and poly (ADP-ribose) polymerase (PARP) cleavage, as well as a shift in Bad:Bcl-X_L ratio in favor of apoptosis [48].

3. DITERPENOIDS

A well practiced diterpenoid in cancer patients is paclitaxel (Fig. 2). Paclitaxel and second generation docetaxel (Fig. 2) are regarded as first-line or second line therapy as single treatment or in combination with other chemotherapeutics for both early stage or metastatic breast or prostate cancer. Since this area has been extensively reviewed recently [49, 50], we only briefly refer it here.

3.1 Triptolide

Triptolide (PG490) (Fig. 2) is a diterpenoid triepoxide, which is highly oxygenated at the 7,8; 9,11; and 12,13 positions of epoxides. Triptolide is mainly extracted from the tender root of Chinese herb *Tripterygium wilfordii* Hook F (lei gong teng) [51]. The hydrogen-bonded 9,11-epoxy-14beta-hydroxy system was reported to be involved in triptolide's antineoplastic activity [52]. However, recent structure-activity relationship (SAR) studies showed that substitution of the C₁₄ β-hydroxyl group of triptolide, with a chiral epoxy group, resulted in retain of anticancer activity, which challenge the traditional viewpoint on the necessity of C₁₄ β-hydroxyl group of triptolide [53].

Triptolide inhibited cell growth at low concentrations and induced apoptosis at high concentration [54]. *In vivo*, treatment of mice with triptolide inhibited the growth of xenografts formed by different human tumor cell lines including MDA-MB-435 breast cancer line, indicating that triptolide has a broad spectrum of activity against tumors [55]. The antiproliferative activity of triptolide is associated with inhibition of proteasome-mediated NF-κB pathway (Fig. 1). Triptolide inhibited transactivation of NF-κB induced by tumor necrosis factor alpha (TNF-α), and further blocked NF-κB-mediated induction of the inhibitor of apoptosis c-IAP1 and c-IAP2 (IAP, inhibitor of apoptosis protein) [56]. Triptolide inhibited NF-κB transactivation without inhibiting nuclear NF-κB DNA binding activity [56, 57] or even with increased nuclear NF-κB DNA binding activity [58]. Further study demonstrated that triptolide inhibited the binding of p65 to transcriptional coactivator CBP/p300 [59]. Triptolide also suppressed the phorbol myristyl acetate (PMA)-induced activation of NF-κB, in turn, inhibited the overexpression of urokinase-type plasminogen activator receptor (uPAR), which is required for tumor cell invasion [60].

In addition, triptolide inhibited up to 80% mRNA *de novo* synthesis by inhibiting RNA polymerase I and II [61], indicating that it hits a wide spectrum of targets. Indeed, a disintegrin and metalloproteinase 10 (ADAM10) has been identified as a novel molecular target of triptolide in breast cancer MCF 7 cells. ADAM10 is a type 1 transmembrane glycoprotein that cleaves several plasma membrane proteins and involved in malignant cell growth and cancer progression [62]. Triptolide treatment at nM concentrations resulted in a significantly decreased expression of ADAM10 with a concomitant increase in ADAM10 cleaved products in MCF-7 breast cancer cells [62]. This might be correlated with triptolide cellular location. A study using a ³H-labeled triptolide showed that triptolide binding was saturable, reversible, and primarily localized to cell membranes [63]. Triptolide induced suppression of phospholipase D expression, resulting in inhibition of cell in MDA-MB-231 breast cancer cells [64]. Up-regulation of programmed death-1-ligand 1 (PD-L1) in cancer cells is an important mechanism of tumor immune evasion. Triptolide was able to inhibit interferon-gamma-induced PD-L1 surface expression in human breast cancer cells [65]. Therefore, by down-regulating PD-1/PD-L1 pathway, triptolide may also serve as a modulator to promote cancer cell-reactive immune responses [65]. Triptolide could also down-regulate the expression of ER α in MCF-7 cells, which was associated with greater anti-proliferation and apoptotic effects in estrogen-sensitive MCF-7 than in estrogen-insensitive MDA-MB-231 human breast cancer cells [66]. Triptolide was also able to decrease Bcl-2 expression and increase caspase-3 expression, leading to apoptosis induction in prostate carcinoma cells [67].

In brief, triptolide is able to inhibit NF- κ B activation and multiple other targets in human breast and prostate cancer cells, which is responsible for its apoptosis-inducing and anti-tumor activities.

3.2 Oridonin and Ponicidin

Recently, a formula PC SPES (PC for prostate cancer and SPES in Latin for “hope”) composed of one North American plant extract and seven Chinese herbs extracts has been approved as an alternative therapy for advanced prostate cancer. *Rabdosia rubescens* is one of the constituents of PC SPES that contains two diterpenoids, oridonin and poncicidin (Fig. 2). Oridonin exhibited antiproliferative activity toward prostate cancer cells LNCaP, DU145 and PC3 as well as other type of cancer cells [68, 69]. Flow cytometric analysis demonstrated that oridonin induced a G₁ phase arrest in androgen receptor-positive LNCaP cells containing wt p53 through induction of p21 [69], while it blocked the cell cycle at G₂ and M phases in androgen receptor-negative DU-145 cells with mutated p53 [68]. Oridonin inhibits cancer cell growth in a cell cycle-specific manner and shifts the balance between pro- and anti-apoptotic proteins in favor of apoptosis induction in prostate cancer cells. It up-regulated p53 and Bax and down-regulated Bcl-2 expression in a dose-dependent manner [68].

Effects of poncicidin and oridonin on cell cycle distribution and apoptosis were also investigated in human breast cancer MCF 7 and MDA-MB-231 cells along with untransformed MCF-10A cells [70]. Induction of apoptosis by either diterpenoid was observed in MCF-10A cells. MCF 7 is lack of caspase-3 [71]. Oridonin was shown to induce apoptotic nucleosomal DNA fragmentation in MCF-7 cells *via* a postmitochondrial caspase-9-dependent pathway. Moreover, PARP was partially cleaved by calpain rather than by caspase-3 [72]. Oridonin was reported to induce MCF-7 cells and undergo both apoptosis and autophagy simultaneously. Combined treatment with oridonin and 3-MA (the specific inhibitor of autophagy) decreased apoptosis induction compared with treatment of oridonin, indicating that autophagy facilitated apoptosis in oridonin-treated MCF-7 cells [73]. Within the dose range effective for MCF-7 or MCF-10A cells, neither oridonin nor poncicidin

affected the growth of MDA-MB-231 cells, suggesting that they are not effective against highly aggressive breast cancer cells [70].

4. TRITERPENOIDS

Triterpenoid is a class of compounds that are close to steroids in structure. Therefore they are extensively studied in hormone-related cancers although they are also effective against other type of cancers. A number of triterpenoids have demonstrated antitumor properties. For example, betulinic acid was reported to inhibit growth of breast cancer cells [74].

4.1 Celastrol

Celastrol is a quinone methide triterpene (Fig. 3) isolated from lei gong teng (*Tripterygium wilfordii* Hook F). It is also found in *Tripterygium regelii*, a substitute of lei gong teng in northeast of China [75]. Celastrol was predicted as a proteasome inhibitor by molecular modeling, and was confirmed by *in vitro* and *in vivo* experiments [76]. Analysis of atomic orbital energy on celastrol showed that the ketone carbons on B-ring of celastrol had a high susceptibility toward a nucleophilic attack (Fig. 4A). Celastrol could be docked at 92% possibility into the S₁ pocket of $\beta 5$ subunit of the proteasome in a conformation in which it interacts with Thr1, the active site of $\beta 5$ subunit (Fig. 4B). Celastrol potently and preferentially inhibits $\beta 5$ -mediated chymotrypsin-like activity of purified 20S proteasome with an IC₅₀ value 2.5 μ mol/L [76]. In intact human prostate cancer cells, cellular 26S proteasome was inhibited by celastrol at 1–5 μ mol/L. Inhibition of the proteasome activity by celastrol results in accumulation of ubiquitinated proteins and three natural proteasome substrates (I κ B- α , Bax and p27), and induction of apoptosis in androgen receptor-positive or androgen receptor-negative prostate cancer cells (Fig. 1). Treatment of human prostate tumor-bearing nude mice with celastrol (1–3 mg/kg/day, *i.p.*, for 1 to 31 days) resulted in significant inhibition (65–93%) of the tumor growth. Multiple assays using the animal tumor tissue samples from both early and end time-points demonstrated *in vivo* inhibition of the proteasomal activity and induction of apoptosis after celastrol treatment [76].

Antitumor activity of celastrol was also observed in other types of cancer. Celastrol inhibited ~60% tumor growth in breast cancer xenograft through NF- κ B inactivation including inhibition of its DNA-binding activity and inhibition of I κ B- α degradation which was induced by TNF- α or phorbol myristyl acetate (PMA) [77] (Fig. 1). Further investigation showed that celastrol suppressed the NF- κ B activation by targeting cysteine 179 in the I κ B- α kinase (IKK) [77] (Fig. 1). Celastrol was able to potentiate apoptotic effects induced by TNF and chemotherapeutic agents. Celastrol was able to inhibit the TNF-induced activation of I κ B- α kinase, I κ B- α phosphorylation, I κ B- α degradation, p65 nuclear translocation and phosphorylation, and NF- κ B-mediated reporter gene expression [78] (Fig. 1). Finally, celastrol treatment suppressed expression of several TNF-induced genes that are involved in anti-apoptosis, proliferation, invasion, and angiogenesis [78].

Inhibition of NF- κ B was observed in concurrence with of activation of heat shock response, indicating a potential link between the two pathways [79]. Indeed, celastrol induced activation of Hsp70, Hsp40 and Hsp27, which was similar as observed in heat shock treatment [79]. Up-regulation of Hsp70A is a well established cell response to Hsp90 inhibition [80]. Two research groups reported that celastrol is an inhibitor of Hsp90 [81, 82]. Both groups confirmed that celastrol is different from classic Hsp90 inhibitor such as geldanamycin. However, Hieronymus *et al.* reported that celastrol inhibits ATP-binding activity of Hsp90 through mechanisms outside the N-terminal ATP-binding pocket of Hsp90 [82] while Zhang *et al.* reported that celastrol did not interfere with ATP binding to Hsp90 [81]. Celastrol disrupted the interaction between Hsp90 and its cochaperone such as Cdc37 or p23 [81, 82]. Inhibition of Hsp90 by celastrol resulted in suppression of its client proteins

that play an important role in tumor initiation and progression. For example, celastrol depleted AR protein in LNCaP cells [82]. The aryl hydrocarbon receptor (AhR) is another client protein of Hsp 90. It plays a significant role in polycyclic aromatic hydrocarbon (PAH)-induced carcinogenesis. Celastrol suppressed AhR expression, resulting in decreased transactivation of *CYP1A1* and *CYP1B1*, both of which encode proteins that convert PAH to genotoxic metabolites [83].

In conclusion from the literature available, celastrol mainly targets three pathways that link to each other, the ubiquitin-proteasome, the NF- κ B, and the heat shock protein pathways to mediating apoptosis in human breast or prostate cancers.

4.2 Pristimerin

Pristimerin is a natural analog of celastrol (Fig. 3). Not surprisingly, it targets the proteasome [84] (Fig. 4C–D). Nucleophilic susceptibility and *in silico* docking studies show that one ketone carbon of pristimerin is highly susceptible towards a nucleophilic attack by the hydroxyl group of Thr1 of the proteasomal chymotrypsin subunit (Fig. 4C–D). The distance from that carbon to OH group of Thr1 was 3.05 Å, within the range of possible interaction between each other. Consistently, pristimerin potently inhibits the chymotrypsin-like activity of a purified rabbit 20S proteasome (IC₅₀ 2.2 μmol/L) and human prostate cancer 26S proteasome (IC₅₀ 3.0 μmol/L) [84] (Fig. 1). The accumulation of ubiquitinated proteins and three proteasome target proteins, Bax, p27 and I κ B- α , in AR-negative PC-3 prostate cancer cells supports the conclusion that proteasome inhibition by pristimerin is physiologically functional. This observed proteasome inhibition subsequently led to the induction of apoptotic cell death in a dose- and kinetic-dependent manner. Furthermore, in AR-positive, androgen-dependent LNCaP and AR-positive, androgen-independent C4-2B prostate cancer cells, proteasome inhibition by pristimerin results in suppression of AR protein prior to apoptosis [84]. The data demonstrates that the proteasome is a target of pristimerin in prostate cancer cells and inhibition of the proteasomal chymotrypsin-like activity by pristimerin is responsible for its cancer cell death-inducing properties (Fig. 1). Inhibition of the chymotryptic activity of the proteasome was confirmed by another group [85].

Pristimerin-triggered caspase activation was also observed in human breast cancer cells [86]. MDA-MB-231 cells treated with pristimerin showed rapid induction of apoptosis through caspase activation, which could be completely prevented by pretreatment with a pancaspase inhibitor benzyloxycarbonyl-Val-Ala-Asp-fluoromethyl ketone (z-VAD-fmk). Treatment of breast tumor cells with pristimerin resulted in a rapid release of cytochrome c from mitochondria, which preceded caspase activation and the decrease of mitochondrial membrane potential. This process did not depend on Bcl-2 family (Bcl-2, Bcl-X_L and Bax) protein levels and does not require translocation of Bax to the mitochondria. Moreover, the generation of reactive oxygen species (ROS) in MDA-MB-231 cells was also not affected by pristimerin [86].

Therefore, pristimerin can inhibit the proteasome activity, induce cytochrome c release, and activate caspase pathway in human breast or prostate cancer cells.

4.3 Withaferin A

Withaferin A (WA) (Fig. 3) is derived from the medicinal plant *Withania somnifera* Dunal (ashwagandha), which has been used for over centuries in Indian Ayurvedic medicine and as a dietary supplement in the United States recently [87]. WA suppressed human breast cancer growth, which is correlated with apoptosis induction characterized by DNA condensation, cytoplasmic histone-associated DNA fragmentation, and cleavage of PARP. WA-mediated

DNA fragmentation was significantly attenuated by knockdown of protein levels of Bim and its transcriptional regulator forkhead box O3 (FOXO3a) in breast cancer MCF7 and MDA-MB-231 cells, suggesting an important role of FOXO3a and Bim in regulation of WA-mediated apoptosis in human breast cancer cells [88]. Interleukin-6 (IL-6) is involved in angiogenesis and metastasis. WA was found to repress IL-6 gene transcription upon dual inhibition of NF- κ B and activator protein 1 (AP-1) and silencing of IL-6 promoter chromatin accessibility in highly metastatic breast cancer cells [89].

We have reported that WA potently inhibits the chymotrypsin-like activity of a purified rabbit 20S proteasome ($IC_{50}=4.5\text{ }\mu\text{M}$) and 26S proteasome in human prostate cancer cultures (at 5–10 μM) and xenografts (4–8 mg/kg/day) [90] (Fig. 1). Inhibition of prostate tumor cellular proteasome activity in cultures and *in vivo* by WA results in accumulation of ubiquitinated proteins and three proteasome target proteins (Bax, p27, and I κ B- α) accompanied by androgen receptor protein suppression (in androgen-dependent LNCaP cells) and apoptosis induction. Treatment of WA under conditions of the aromatic ketone reduction had significantly decreased the proteasome-inhibitory and apoptosis-inducing activities. Treatment of human prostate PC-3 xenografts with WA for 24 days resulted in 70% inhibition of tumor growth in nude mice, associated with 56% inhibition of the tumor tissue proteasomal chymotrypsinlike activity [90]. WA was also able to induce prostate apoptosis response-4 (Par-4)-dependent apoptosis in androgen-refractory prostate cancer cells and regression of PC-3 xenografts in nude mice [91].

Therefore, at least the proteasome, IL-6, FOX3a and Par-4 are involved in WA-mediated apoptosis process in human breast or prostate cancer cells.

4.4 Ursolic Acid

Ursolic acid (UA) (Fig. 3) is an aprotacyclic triterpene acid found in *Ocimum* leaves and also in berries, leaves, flowers and fruits of *Rosemarinus officinalis*, *Eriobotrya japonica*, *Calluna vulgaris* and *Eugenia jambolana* [92]. Several studies suggest that UA has potent cancer-preventive activity and great therapeutic potential. Breast cancer MCF-7 cells exhibited typical apoptotic features, including chromatin clumps and aggregation and DNA fragmentation after UA treatment, which was in correlation with the down-regulation of Bcl-2 and up-regulation of caspase-3 [93, 94]. An aqueous extract of *Ocimum gratissimum* (OG) leaves containing UA was investigated on tumor progression and angiogenesis. Results showed that the OG leaf extract inhibits proliferation, migration, anchorage-independent growth, 3D growth and morphogenesis and induction of cyclooxygenase-2 (COX-2) protein in breast cancer cells [95]. In addition, the OG extract reduced tumor size and neoangiogenesis in a MCF10 DCIS.com xenograft model [95].

UA inhibited significantly the cell viability and induced apoptosis in PC-3 cells and LNCaP cells associated with down-regulation of Bcl-2 protein [95], suggesting its potential therapeutic use for the treatment of hormone refractory and androgen-sensitive prostate cancer. Bcl-2 down-regulation may be one of the molecular mechanisms by which UA induces apoptosis. This was confirmed in another human hormone-refractory prostate cancer cell line, DU145 [96]. UA caused the activation of c-Jun N-terminal kinase (JNK), resulting in Bcl-2 phosphorylation (Ser70) and degradation and consequent caspase 3 activation in DU145 cells [96]. In conclusion, downregulation of Bcl-2 may be one of the mechanisms responsible for the apoptosis-inducing activity of UA in human prostate cancer cells.

5. TETRATERPENOIDS

One representative of the tetraterpenes family is carotenoids and more than 600 known natural structural variants are present in this family [97]. Higher concentrations of

carotenoids such as lycopene or beta-carotene (Fig. 3) was associated with lower risk of breast cancer [98, 99]. An inverse association has been observed between dietary intake of lycopene and the risk of prostate cancer [100]. On the contrary, it was also reported that β -carotene supplementation had no effect on the incidence of breast, prostate and other types of cancers [101].

5.1 Acyclic Tetraterpenoids

Lycopene is an open chain hydrocarbon containing 11 conjugated and 2 non-conjugated double bonds arranged in a linear array (Fig. 3). It is derived predominantly from tomatoes and tomato products. Lycopene modified breast cancer gene expression involved in various molecular pathways, such as apoptosis, cell communication, mitogen-activated protein kinase (MAPK) pathway and cell cycle [102]. Similarly, it has been reported to trigger G₂/M arrest and suppress Bcl-2 expression in MCF 7 cells [103]. In addition, lycopene inhibited the function of the multidrug resistance (MDR) proteins in MDA-MB-231 cells [104]. It was found that lycopene treatment induced apoptosis in human MDR1-transfected breast cancer MDA-MB-231 cell line [104].

In a phase II clinical trial, the efficacy of lycopene alone or in combination with soy isoflavones on serum PSA levels in men with prostate cancer was investigated. Lycopene and soy isoflavones have activity in prostate cancer patients with PSA relapse disease and may delay progression of both hormone-refractory and hormone-sensitive prostate cancer [105]. However, there may not be an additive effect between these two compounds when taken together [105]. It has also been reported that the combination of tomato and broccoli was more effective at slowing prostate tumor growth than alone in a rat model [106]. Results from a small, randomized clinical trial, showed that lycopene supplementation may increase prostate tissue levels of lycopene, modulate biomarkers of growth and differentiation, and decrease clinical parameters of disease aggressiveness of clinically localized prostate cancer [107].

5.2 Bicyclic Tetraterpenoids

Lutein and zeaxanthin (Fig. 3) are xanthophyll carotenoids found particularly in dark-green leafy vegetables such as kale, spinach, turnip greens, collard, and in egg yolks. Alpha- and beta-carotenes are enriched in dark green-yellow vegetables. Bicyclic tetraterpenoids were shown to modulate drug resistance. For example, lutein, antheraxanthin and violaxanthin (Fig. 3) had moderate effects on the reversal of MDR in the tumor cells. Apoptosis was found in human MDR1-transfected mouse lymphoma cells and human breast cancer MDA-MB-231 (HTB-26) cell lines in the presence of zeaxanthin and capsanthin [104]. Beta-carotene-induced apoptosis has also been investigated in breast cancer model. Peroxisome proliferator-activated receptor gamma (PPAR-gamma) and ROS production have been implicated in β -carotene-regulated apoptosis [108]. Beta-carotene significantly increased PPAR-gamma mRNA and protein levels in MCF 7 cells. Moreover, it induced ROS generation, resulting in mitochondrial dysfunction and cytochrome c release [108].

6. FUTURE DIRECTIONS

Breast and prostate cancers are two major cancers in women and men. It is estimated that 67,970 people died of these two type of cancers in 2009 [1]. It is therefore very urgent to find a powerful strategy to control both breast and prostate cancer progression. Nature has already provided a great source for anticancer drug discovery. Statistics on approved anticancer drugs from 1940 to 2006 reveal that 73% drugs are discovered from nature either directly or semi-synthesized based on the scaffolds of natural products. Paclitaxel and docetaxel are plant-derived or semi-synthetic; both are currently used as first or second line

treatment of breast or prostate cancer. New terpenoid compounds have also been isolated from nature. Currently, most of the research is in preclinical study phase. Although water-soluble analog of triptolide has been developed, many other drugs are not water soluble, which limits their clinical application. Furthermore, the pharmacokinetic characteristics of most of the terpenoids need to be determined. Future investigation should focus on how to translate the bench work into clinical application, including treatment and prevention of human breast and prostate cancers.

7. CONCLUSION

Plants provide a broad spectrum of sources for modern anticancer drug discovery. Plant-derived diterpenoid paclitaxel and its semi-synthetic analog docetaxel are used as standard therapy in breast or prostate cancer patients. Other terpenoids, for example triterpenoids celastrol and withaferin A, have been isolated. Terpenoids evoke apoptosis in cultured breast or prostate cancer cells or xenografts, resulting in inhibition on tumor cell proliferation and growth. Multiple mechanisms are involved in the action of apoptosis induction by various terpenoids. Inhibition of ubiquitin-proteasome and NF- κ B pathways are often observed in terpenoids-treated breast or prostate cancer cells, which is linked with up-regulation of proapoptotic protein Bax and down-regulation of anti-apoptotic protein Bcl-2, resulting in cytochrome c release, caspase activation and apoptotic cell death.

The efficacy of terpenoids as a single or combination strategy against breast or prostate cancers as demonstrated in pre-clinical studies provides strong support for their clinical use in treatment and even prevention of human hormone-related cancers.

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Fig. 1. Inhibition of the proteasome and NF-κB pathways by selected terpenoids

The proteasome contains a 20S multicatalytic core and one or two 19S regulatory caps. IκB-α, a proteasome substrate traps the active form of NF-κB (a dimer of RelA and p50, or of RelB and p52) in the cytosol. NFκB p50 or p52 are generated from the inactive precursor p105 or p100, respectively, through proteasome partial cleavage. After phosphorylation by IKK, a polyubiquitin chain (red square, ubiquitin) is attached to IκB-α with the help of β-TrCP E3 ligase. Then IκB-α is recognized and degraded by the proteasome, setting NF-κB free. The free, active NF-κB complex then enters into nucleus to transactivate target genes required for tumor proliferation. The selected terpenoids could target the ubiquitin-proteasome and NF-κB pathways at indicated specific steps, resulting in apoptosis induction and tumor growth inhibition.

Fig. 2. Chemical structure of selected terpenoids I
Structures of monoterpenoids and diterpenoids are shown.

Fig. 3. Chemical structure of selected terpenoids II
Structures of triterpenoids and tetraterpenoids are shown.

Fig. 4. Molecular docking of celastrol and pristimerin

A, High susceptibility towards nucleophilic attack of celastrol molecule was indicated by red center. **B**, Celastrol was docked the S_1 pocket of β_5 subunit of the proteasome as shown by blue mesh. The distances from ketone carbons to OH group of Thr1 were 2.96 or 4.16 Å. **C**, Similarly, high electronic density was obtained at the ketone carbons surface of the pristimerin molecule. **D**, Pristimerin was shown in a conformation that is suitable to interact with the OH group of Thr 1 of β_5 subunit as shown by rainbow lines. The distances from ketone carbons to OH group of Thr1 were 3.15 or 4.05 Å. The celastrol and pristimerin molecules were shown in pink sticks.