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Commentary

Ursolic acid in cancer prevention and treatment: Molecular targets, pharmacokinetics and clinical studies

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ABSTRACT

Discovery of bioactive molecules and elucidation of their molecular mechanisms open up an enormous opportunity for the development of improved therapy for different inflammatory diseases, including cancer. Triterpenoids isolated several decades ago from various medicinal plants now seem to have a prominent role in the prevention and therapy of a variety of ailments and some have already entered Phase I clinical trials. One such important and highly investigated pentacyclic triterpenoid, ursolic acid has attracted great attention of late for its potential as a chemopreventive and chemotherapeutic agent in various types of cancer. Ursolic acid has been shown to target multiple proinflammatory transcription factors, cell cycle proteins, growth factors, kinases, cytokines, chemokines, adhesion molecules, and inflammatory enzymes. These targets can potentially mediate the chemopreventive and therapeutic effects of ursolic acid by inhibiting the initiation, promotion and metastasis of cancer. This review not only summarizes the diverse molecular targets of ursolic acid, but also provides an insight into the various preclinical and clinical studies that have been performed in the last decade with this promising triterpenoid.

1. Introduction

Plants continue to provide a vibrant source for drug discovery [1] since phytochemicals serve as possible starting material for the identification of many novel anticancer drugs [2]. One important class of bioactive phytochemicals is triterpenoids, which represent a large family of compounds classified according to the number of isoprene units [3,4]. Triterpenoids are synthesized in plants by cyclization of squalene and are ubiquitously present in nature [4]. To date, ~20,000 triterpenoids, including dammarane, ergostane, friedelane, lupane, oleanane, taraxastane, and ursane, have been identified from the various parts of medicinal plants. These emphasize the potential of various plant-derived cyclosqualenoid molecules to be used as anti-inflammatory, anticancer and antimicrobial agents [5]. Among various terpenoids, pentacyclic triterpenoids have been shown from various studies to be having wide ranging anti-inflammatory, chemopreventive, and anticancer activities and have been extensively studied previously [6–9].

Ursolic acid, 3β-hydroxy-urs-12-en-28-oic-acid (Fig. 1), an ursane-type pentacyclic triterpenic acid, belongs to the cyclosqualenoid family and is ubiquitous in the leaves and berries of natural medicinal plants, such as *Arctostaphylos uva-ursi* (L.) *Spreng* (bearberry), *Vaccinium macrocarpon Air.* (cranberry), *Rhododendron hymenanthes Makino*, *Rosemarinus officinalis*, *Eriobotrya japonica*, *Calluna vulgaris*, *Ocimum sanctum*, and *Eugenia jambolana* and in the protective wax-like coatings of apples, pears, prunes and other fruits [10]. Ursolic acid may occur as free acid or as aglycone of saponins. Recent evidences have supported the beneficial effects of ursolic acid in a variety of human diseases, including various types of inflammation-driven cancers [11]. Anti-inflammatory and antiproliferative, proapoptotic, antimetastatic and antiangiogenic potential of ursolic acid have been reported in both *in vitro* and *in vivo* models of cancer [7]. Many preclinical efficacy studies using

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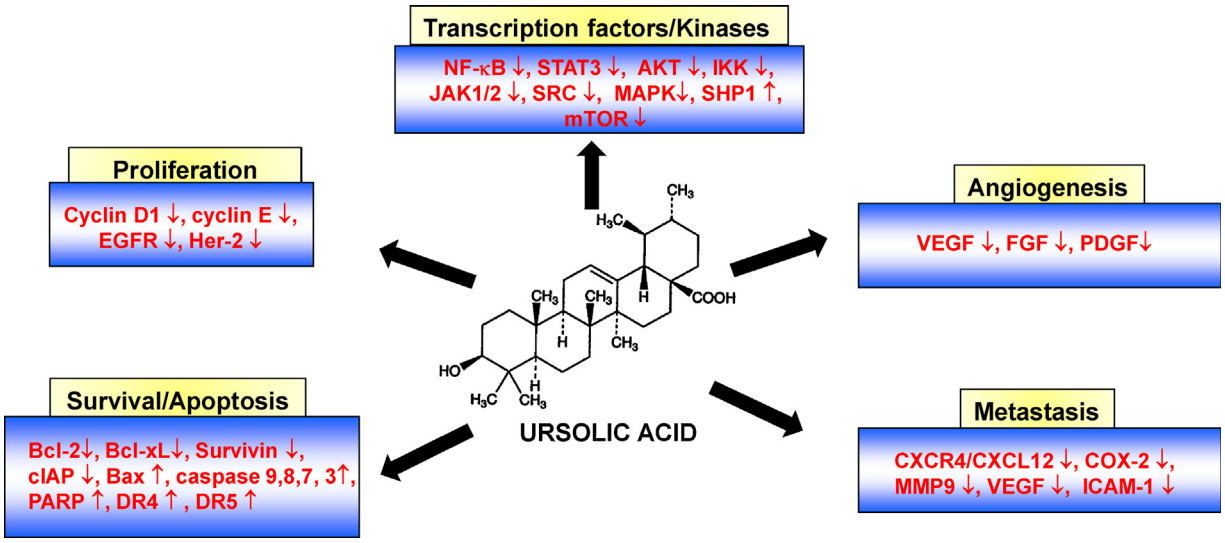

Fig. 1. Oncogenic cascades modulated by ursolic acid in tumor cells. These include wide variety of transcription factors, protein kinases, and molecules involved in tumor cell proliferation, angiogenesis, metastasis, survival and apoptosis.

chemically induced, subcutaneous and orthotopic human xenograft models and recently developed spontaneously developing transgenic tumor growth models have provided ample evidence that naturally occurring and synthetic derivatives of ursolic acid have chemopreventive and therapeutic properties [7]. Here, we discuss in detail the potential of ursolic acid as a chemopreventive and anticancer drug and its reported beneficial effects based on these preclinical and clinical investigations. Also, we highlight in detail various mechanisms by which ursolic acid modulates cellular transcription factors, growth factor receptors, inflammatory cytokines, and other major intracellular molecular targets that regulate cancer cell proliferation, apoptosis, invasion, metastasis and angiogenesis.

2. Isolation and chemical properties of ursolic acid

Isolation of ursolic acid is achieved by a variety of methods [12]. In general, pulverized plant material is extracted with two solvents of increasing polarity, hexane and ethyl acetate, in a Soxhlet apparatus. The ethyl acetate extract is then concentrated in a rotary evaporator and kept in sealed flasks. Since ursolic acid is a ubiquitous compound and is found in many medicinal plants, the most common method of isolation is extraction with an organic solvent using either partition chromatography, column chromatography, high pressure liquid chromatography (HPLC) or high performance thin layer chromatography (TLC) after iodine derivatization [13]. General procedures such as gas chromatography including the essential silylation [14] or methylation step [15], liquid chromatography coupled with UV [16] and MS spectrometry [12] have been used for the isolation and analysis of ursolic acid. Kontogianni et al. [17] demonstrated that the combination of two-dimensional $^{1}H–^{13}C$ HSQC and $^{1}H–^{13}C$ HMBC NMR spectroscopy is a useful and rapid analytical tool for structure elucidation and quantification of the triterpene acids present in complex extracts obtained from plants. Bioassay-guided fractionation is one of the commonly employed methods to detect compounds in new plant extracts that exhibit inhibitory activity against cancer cells. However, this method requires chromatographic fractionation of the crude extract. The fractions are then tested *in vitro* for inhibitory activity against cancer cell cultures. The bioactive fraction is then taken for further fractionation, purification and finally isolation of the bioactive compound. Ursolic acid is isolated as large, lustrous prisms purified from absolute alcohol and forms fine hair-like needles from dilute alcohol, with a melting point of 284 °C, and octanol/water partition coefficient log Kow = 7.92.

3. Anticancer potential of ursolic acid

Decades of research have provided ample evidence that the triterpenoids are multi-faceted in their molecular mechanism(s) as they modulate multiple targets and multiple pathways [7] (Fig. 1). Numerous biochemical and pharmacological effects of ursolic acid, including anti-inflammatory, antioxidant, antiproliferative, anticancer, antimutagenic, antiartherosclerotic, antihypertensive, antileukemic, antiviral, and antidiabetic, have been reported previously [18]. We will discuss the reported anticancer effects of ursolic acid in brief below.

3.1. In vitro *anticancer effects of ursolic acid*

Several inflammatory signaling cascades including nuclear factor-κB (NF-κB), signal transducer and activator of transcription 3 (STAT3), serine/threonine protein kinase B (AKT), and cyclooxygenase-2 (COX-2) have been linked with different stages of cancer progression and are reported to regulate tumor proliferation, survival, invasion, metastasis and angiogenesis [19–21]. The transcription factor, NF-κB is a key regulator of cellular events [22]. NF-κB activation is often associated with chronic inflammation and tumorigenesis [23] and tumor chemoresistance and radioresistance [24]. Numerous publications have provided evidence that up-regulated NF-κB leads to chronic inflammation, which has been causally linked to the development of several human diseases including cancer and that understanding the mechanisms of constitutive NF-κB can lead to the development of novel therapeutics for cancer treatment. Phosphorylation of IκB proteins by IκB kinases is a vital process that leads to NF-κB-DNA binding and transcriptional activation of target genes [25].

Decades of research on natural product agents that inhibit or deregulate this pathway have provided ample evidence that numerous dietary constituents and nutraceuticals may have anti-inflammatory and chemopreventive effects [7,26]. *In vitro* studies using a variety of tumor cell lines have demonstrated the usefulness of ursolic acid in chemoprevention and in the treatment of inflammation-driven disease (Table 1). Although ursolic acid has

Table 1
In vitro anticancer effects of ursolic acid and its derivatives.

Biological effects	References
Multiple myeloma	
Suppressed activation of STAT3	[33]
Suppressed upstream activation of JAK1/2 kinases, and cSRC	[33]
Prostate carcinoma	
Suppressed TNF induced NF-κB activation and IL-6-induced STAT3 activation in LnCaP cells	[34]
Down-regulated CXCR4 expression irrespective of HER2 status in a dose-and time-dependent manner	[30]
CXCR4 down-regulation was mediated by transcriptional regulation of mRNA expression and NF-κB inactivation in DU145 cells	[30]
Induced autophagy in PC3 cells and enhanced the expression of LC3-II	[45]
Induced apoptosis *via* Beclin-1 and Akt/mTOR pathway	[46]
Breast carcinoma	
Inhibited proliferation *via* induction of apoptosis	[48]
Induced apoptosis in MDA-MB-231 *via* Fas receptor, caspase 3 and PARP and mitochondrial pathway	[53]
Suppresses migration and invasion by modulating c-Jun N-terminal kinase, Akt and mammalian target of rapamycin signaling	[92]
Inhibited the expression of FoxM1 in MCF-7 cells	[93]
Hepatocellular carcinoma	
Suppressed angiogenesis by inhibiting HIF-1α, βFGF, VEGF, IL-8, uPA, ROS, and NO	[54,94]
Inhibited hepatoma growth	[58]
Induced apoptosis *via* activation of caspase 3 and induces cell cycle arrest at S phase	[70]
Bladder carcinoma	
Induced IRE1-TRAF2-ASK1 signaling complex and activates apoptotic ASK1-JNK signaling	[36]
Induced AMPK kinase and inhibits cellular proliferation	
Inhibited cell proliferation at G1 phase and induces apoptosis with increase in ROS production	[95]
Colorectal carcinoma	
Sensitized colon cancer cells to TRAIL induced apoptosis	[6]
Induced cell death and modulates autophagy *via* JNK pathway	[38]
Down-regulated NF-κB-regulated gene products cyclin D1, MMP-9, ICAM-1 VEGF, c-FLIP, survivin, Bcl-2, Bcl-xL	[39]
Induced apoptosis by modulating purinergic receptor P2Y(2)/Src/p38/Cox2 pathway	[41]
Reversed multidrug resistance in SW480 and SW620	[48]
Ovarian carcinoma	
Suppressed cell proliferation, upregulated phosphorylation of ERK, and induced caspase 9 and 3 and effectively cleaved PARP	[96]
Down-regulated the expression of survivin, c-Myc and astrocyte elevated gene	[96]
Pancreatic carcinoma	
Inhibited proliferation of MIA-PaCa-2, PANC-1 and Capan-1 *via* PI3 K/AKT/NF-κB and JNK pathways	[40]
Induced cytotoxicity and induced p53, p21waf1, and NOXA in AsPC-1 cells	[71]
Chronic myelogenous leukemia cells and HL60 monocytes	
Induced apoptosis *via* down-regulation of Akt, up-regulation of PTEN and by activating mitochondrial pathway in K562 cells	[42]
Induced HL60 monocyte differentiation and up-regulated C/EBPβ *via* ERK activation	[43]
Reversed multidrug resistance in K562/ADR and HL60/ADR	[48]
Lung carcinoma	
Induced apoptosis in A549, H3255 and Calu-6 in a dose and time dependent manner	[51]
Neuronal glioblastoma (astrocytoma)	
Inhibits proliferation and induces apoptosis in human glioblastoma cell lines U251 by suppressing TGF-beta1/miR-21/PDCD4 pathway	[60]

been shown to prevent a variety of inflammation-associated diseases, the exact molecular mechanism is yet to be elucidated [27–29]. Ursolic acid has been previously reported to suppress the proliferation of a number of tumor cells, induce apoptosis [30] and inhibit tumor promotion, metastasis, and angiogenesis [31]. Shishodia et al. [32] demonstrated that ursolic acid can inhibit NF-κB activation induced by carcinogenic agents, such as tumor necrosis factor (TNF), phorbol ester, okadaic acid, hydrogen peroxide, and cigarette smoke, through the suppression of IκBα kinase and p65/RelA phosphorylation. The inhibition of NF-κB activation correlated with the suppression of NF-κB-dependent cyclin D1, COX-2, and matrix metalloproteinase-9 (MMP-9).

On the other hand, Pathak et al. [33] clearly highlighted the potential of ursolic acid to modulate STAT3 signaling cascade in multiple myeloma. They found that ursolic acid can inhibit both constitutive and interleukin-6-inducible STAT3 activation in a dose- and time-dependent manner that correlated with the suppression of activation of upstream kinases (c-Src, Janus-activated kinase 1, Janus-activated kinase 2, and extracellular signal-regulated kinase 1/2). We recently investigated the effect of ursolic acid on NF-κB and STAT3 signaling pathways in both androgen-independent (DU145) and androgen-dependent (LNCaP) prostate cancer cell lines and also prospectively tested the hypothesis of NF-κB and STAT3 inhibition using a virtual predictive functional proteomics tumor pathway technology platform. Ursolic acid inhibited constitutive and TNF-α-induced activation of NF-κB in DU145 and LNCaP cells in a dose-dependent manner. Ursolic acid also downregulated the expression of various NF-κB and STAT3-regulated gene products involved in proliferation, survival, and angiogenesis and induced apoptosis in both cell lines [34].

Several lines of evidence point to the CXCR4/CXCL12 signaling pathway which plays an important role in distant site metastasis [35]. We recently hypothesized that ursolic acid can modulate CXCR4/CXCL12 pathway in prostate cancer cells and found that this triterpene downregulated the CXCR4 expression irrespective of the HER2 status in a dose- and time- dependent manner and this down-regulation was mediated by transcriptional regulation of mRNA expression and inhibition of NF-κB activation [30]. The antiproliferative effects of ursolic acid have been further confirmed by its ability to induce endoplasmic reticulum stress in human bladder cancer (T24) cell line. Ursolic acid induced the formation of

IRE1-TRAF2-ASK1 signaling complex to activate pro-apoptotic ASK1-JNK signaling. Salubrinal, an ER stress inhibitor, diminished ursolic acid-induced CHOP/Bim expression and anti-T24 cell effects [36]. Ursolic acid suppressed cigarette smoke extract-induced human bronchial epithelial cell injury and prevented the development of lung cancer [37], induced colorectal cancer cell death and modulated autophagy through the JNK pathway in apoptosis-resistant cells [38]. Prasad et al. [39] also reported that ursolic acid can induce apoptosis in colorectal cancer cells by inhibiting constitutive NF-κB activation and down-regulation of cell survival (Bcl-xL, Bcl-2, cFLIP, and survivin), proliferative (cyclin D1), and metastatic (MMP-9, VEGF, and ICAM-1) proteins. Ursolic acid has been shown to suppress growth and induce apoptosis in gemcitabine-resistant human pancreatic cancer cells, (MIA PaCa-2, PANC-1 and Capan-1) *via* modulation of the JNK and PI3K/Akt/NF-κB pathways [40]. Limami et al. [41] demonstrated a novel role involving purinergic receptors and particularly the P2Y(2) receptor in resistance to ursolic acid-induced apoptosis in both colorectal HT-29 and prostate DU145 cancer cells. In a human chronic myelogenous leukemic cell line (K562), ursolic acid induced apoptosis *via* the up-regulation of PTEN gene expression, inhibited Akt kinase activity, altered the mitochondrial transmembrane potential and reduced the release of cytochrome *c*, enhanced the activity of caspases [42] as well as induced HL60 monocytic differentiation and up-regulated C/EBPβ expression *via* activation of the ERK mitogen activated kinase pathway [43]. Ursolic acid is known to chemosensitize tumor cells to a number of chemotherapeutic agents [33,39]. In another study by Koh et al. [44], ursolic acid was shown to radiosensitize DU145, CT26 and B16F10 cells and accelerated cell death, as evident by DNA fragmentation, changes in cellular redox status, mitochondrial dysfunction and modulation of apoptotic marker proteins. Shin et al. [45] demonstrated for the first time that autophagy inhibitors in combination with ursolic acid can enhance autophagy in prostate cancer PC3 cells in association with apoptosis, enhanced the expression of LC3-II (an autophagosome marker in mammals), and also caused monodansyl-cadaverine incorporation into autolysosomes [46].

Drug-resistance is one of the principal causes for chemotherapy failure in clinical practice, and a number of mediators including multi-drug resistance (MDR) proteins and the anti-apoptotic factors play an important role in this process [47]. Shan et al. [48] recently highlighted the promising ability of ursolic acid to revert MDR in human colon cancer cell lines (SW480 SW620), human acute myelocytic leukemia cancer cell lines (HL60 and HL60/ADR), human chronic myelogenous leukemia cell lines (K562 and K562/ADR), human breast cancer cell lines (MCF-7 and MCF-7/ADR), and also in doxorubicin-resistant HepG2 cells [49]. Lung cancer is one of the most common cancers that can affect cigarette smokers [50]. Ursolic acid abolished the invasive and migratory properties of human non-small cell lung cancers, A549, H3255, and Calu-6 cell lines and induced apoptosis, at concentrations as low as 2, 4, 8, or 16 μmol/L [51,52]. In the invasive breast cancer MDA-MB-231 cell line, ursolic acid- induced apoptosis was mediated *via* Fas receptor and cleavage of caspase-8, caspase-3 and PARP, followed by induced Bax up-regulation and Bcl-2 down-regulation and release of cytochrome *c* to the cytosol from mitochondria with concomitant decreased mitochondrial membrane potential [53]. Ursolic acid exhibited antiangiogenic potential in human liver cancer (Hep3B, Huh7 and HA22T) cell lines by inhibiting hypoxia-inducible factor (HIF)-1α, basic fibroblast growth factor (bFGF), VEGF, interleukin (IL)-8, urokinase plasminogen activator (uPA), reactive oxygen species (ROS), nitric oxide (NO) and cell invasion and migration [54]. TRAIL [tumor necrosis factor (TNF)-related apoptosis inducing ligand], a member of the TNF family, is one such apoptosis-inducing cytokine that has shown promise as an anticancer agent [55]. Prasad and coworkers [6] provided the first mechanistic evidence that ursolic acid treatment can indeed result in the sensitization of TRAIL-resistant cells through the ROS and JNK-mediated up-regulation of death receptors (DR4 and DR5) and the down-regulation of anti-apoptotic proteins. Subbaramaiah et al. [56] investigated the effects of ursolic acid on the expression of COX-2 in phorbol 12-myristate 13-acetate (PMA)-treated human mammary and oral epithelial cells. Treatment with ursolic acid suppressed PMA-mediated induction of COX-2 protein and synthesis of prostaglandin E2. Ursolic acid also inhibited PMA-mediated activation of protein kinase C, extracellular signal-regulated kinase 1/2, c-Jun N-terminal kinase, and p38 mitogen-activated protein kinases. Wang et al. [57] tested the ability of ursolic acid and its cis- and *trans*-3-O-p-hydroxycinnamoyl esters to inhibit growth in a panel of tumor cell lines and inhibit MMP activity associated with tumor invasion and metastasis was determined in DU145 prostate tumor cells. Ursolic acid and its esters inhibited tumor cell growth at micromolar concentrations, and inhibited MMP-2 and MMP-9 activity at concentrations below those previously reported for cranberry polyphenolics.

Tian and colleagues [58] reported that ursolic acid could significantly inhibit the proliferation of HepG2 and its drug-resistance strain, R-HepG2 cells, but had minimal inhibitory effect on primarily cultured normal mouse hepatocytes. They also demonstrated that the down-regulation of COX-2 protein and up-regulation of heat shock protein (HSP) 105 mRNA correlated to the apoptosis of HepG2 cells treated with ursolic acid. Also, the apoptotic effect of ursolic acid on human liver cancer HepG2, Hep3B, Huh7 and HA22T cell lines were examined at three different concentration of 2, 4 and 8 μM. Ursolic acid treatment concentration-dependently decreased cell viability and increased DNA fragmentation in HepG2 and Hep3B cell lines. However, it reduced viability and increased DNA fragmentation in Huh7 cell only at 4 and 8 μmol/L. Ursolic acid treatment also concentration-dependently diminished Na(+)-K(+)-ATPase activity and VEGF level in four test cell lines [59]. Wang et al. [60] examined whether ursolic acid could suppress the proliferation of human glioma cell line U251, and if so, through what potential mechanism(s). The results showed that 5–20 μM of ursolic acid suppressed proliferation and induced apoptosis of glioma cells in dose- and time-dependent manners. Ursolic acid increased the activation of caspase-3 and markedly suppressed levels of microRNA-21 (miR-21) in a time-dependent manner. And over-expression of miR-21 in U251 cells abolished the enhancement of PDCD4 protein by ursolic acid. These findings suggest that ursolic acid can inhibit cell growth *via* causing apoptosis in U251 cells by ursolic acid-triggered TGF-β1/miR-21/PDCD4 pathway. Interestingly, the potential effect of 2alpha-hydroxyursolic acid, an ursolic acid analog on cell proliferation and TNF-α-induced NF-κB activation in MCF-7 cells has also been examined [61]. 2α-hydroxyursolic acid significantly inhibited MCF-7 cell proliferation at doses of 20 μM. Pre-incubation with 2α-hydroxyursolic acid suppressed TNF-α-induced NF-κB activation in a dose-dependent manner and significantly inhibited the activation at a dose of 20 μM of 2α-hydroxyursolic acid. Overall, this study suggested that 2α-hydroxyursolic acid has anti-proliferative activities against MCF-7 cells and capabilities inhibiting NF-κB activation induced by TNF-α partially by suppressing proteasome activities.

A series of novel ursolic acid derivatives was synthesized containing an acyl piperazine moiety. These compounds exhibited more potent inhibitory activities against MGC-803 (gastric cancer cell) and Bcap-37 (breast cancer cell) compared with ursolic acid [62]. Likewise in another study by Ma et al. [63] ursolic acid, as well as 2α-hydroxyursolic acid, (with a β-hydroxyl group at C-3 and a carboxyl group at C-17), were found to show cytotoxicity against all the four tumor cell lines tested (HL-60, BGC, Bel-7402 and

HeLa). On the other hand, a series of furoxan-based novel nitric oxide-donating ursolic acid derivatives were synthesized and evaluated for their cytotoxicity using the HepG2 cell line. These compounds showed more significant cytotoxic activities than control, 5-fluorouracil and ursolic acid [64]. Tanaka et al. [65] demonstrated that derivatization of ursolic acid by oxidation with dioxoruthenium (VI) tetraphenylporphyrins produced compounds that exhibited potent cytotoxic activities against C6 rat glioma and A431 human skin carcinoma cell lines at 10–100 μM compared to parent ursolic acid.

Recently, two distinct pathways were reported to produce novel ursolic acid derivatives by metabolism in various Nocardia species. Strains (NRRL 5646, 44822 and 44000) of Nocardia species generated different ursolic acid derivatives namely: ursolic acid methyl ester, ursonic acid, ursonic acid methyl ester, 3-oxoursa-1, 12-dien-28-oic acid and 3-oxoursa-1, 12-dien-28-oic acid methyl ester. These synthetic pathways can presumably be used as strategic routes for the biotechnological production of triterpenoid derivatives [66]. Acetylation of ursolic acid at C-3 alcohol together with coupling an amino acid methyl ester or amino alcohol acetate at C-28 results in derivatives having stronger anti-proliferative ability. Six compounds showed significant antitumor activity against the HeLa, SKOV3 and BGC-823 cell lines by causing cell cycle arrest at the S phase [67]. In another recent study, ursolic acid derivatives with distinct electrical property were synthesized. These compounds were divided into two groups according to their charge at physiological conditions: (1) Group I, negatively charged and (2) Group II, positively charged. These compounds induced cell cycle arrest and apoptosis in a variety of tumor cells including HepG2, AGS, HT-29 and PC-3 cells in the following order of potency: Group I < ursolic acid < Group II. The ursolic acid derivatives in Group II exhibited potent cytotoxicity, with enhancement of the lipophilicity further strengthening cytotoxicity [68]. The same group of researchers also synthesized another ursolic acid derivative, 3β-acetoxy-urs-12-en-28-oyl-1-monoglyceride that was found to trigger the death of BGC-823 cells by inducing apoptosis *via* the mitochondria pathway [69]. Ursolic acid derivative, N-[3β-acetoxy-urs-12-en-28-oyl]-2-aminodiethanol, was tested with increasing concentrations of test compound for 24 h. Dose-dependent induction of apoptosis was observed in various tumor cell lines including, HepG2, BGC-823, SH-SY5Y, HeLa, and HELF [70]. A series of new heterocyclic derivatives of ursolic acid showed potent antiproliferative activity and induction of p53, p21waf1 and NOXA against pancreatic cancer cell lines AsPC-1 [71]. Interestingly, ursolic acid-loaded nanoparticles (UA-NPs) were recently prepared by Zhang et al. [72] using a nano-precipitation method employing amphilic methoxy poly (ethylene glycol)-polycaprolactone (mPEG-PCL) block copolymers as drug carriers. Ursolic acid was effectively transported into gastric cancer SGC7901 cells by nanoparticles and localized around the nuclei in the cytoplasm. The *in vitro* cytotoxicity and apoptosis test indicated that UA-NPs significantly elicited more cell death at almost equivalent dose and corresponding incubation time. Moreover, UA-NPs led to more cell apoptosis through stronger inhibition of COX-2 and activation of caspase-3. Kalani et al. [73] developed a QSAR models for predicting the activities of ursolic acid analogs against human lung (A-549) and CNS (SF-295) cancer cell lines by a forward stepwise multiple linear regression method using a leave-one-out approach. Similarly, the QSAR model for cytotoxic activity against the human CNS cancer cell line (SF-295) also showed a high correlation ($r(2) = 0.99$ and $rCV(2) = 0.96$), and indicated that dipole vector and solvent-accessible surface area were strongly correlated with activity. Overall, these findings indicate that ursolic acid exerts its anti-inflammatory, antiproliferative, and proapoptotic effects in various tumor cells through the modulation of a number of oncogenic signaling cascades. Its effects on NF-κB, STAT3, and TRAIL signaling pathways are summarized in Fig. 2.

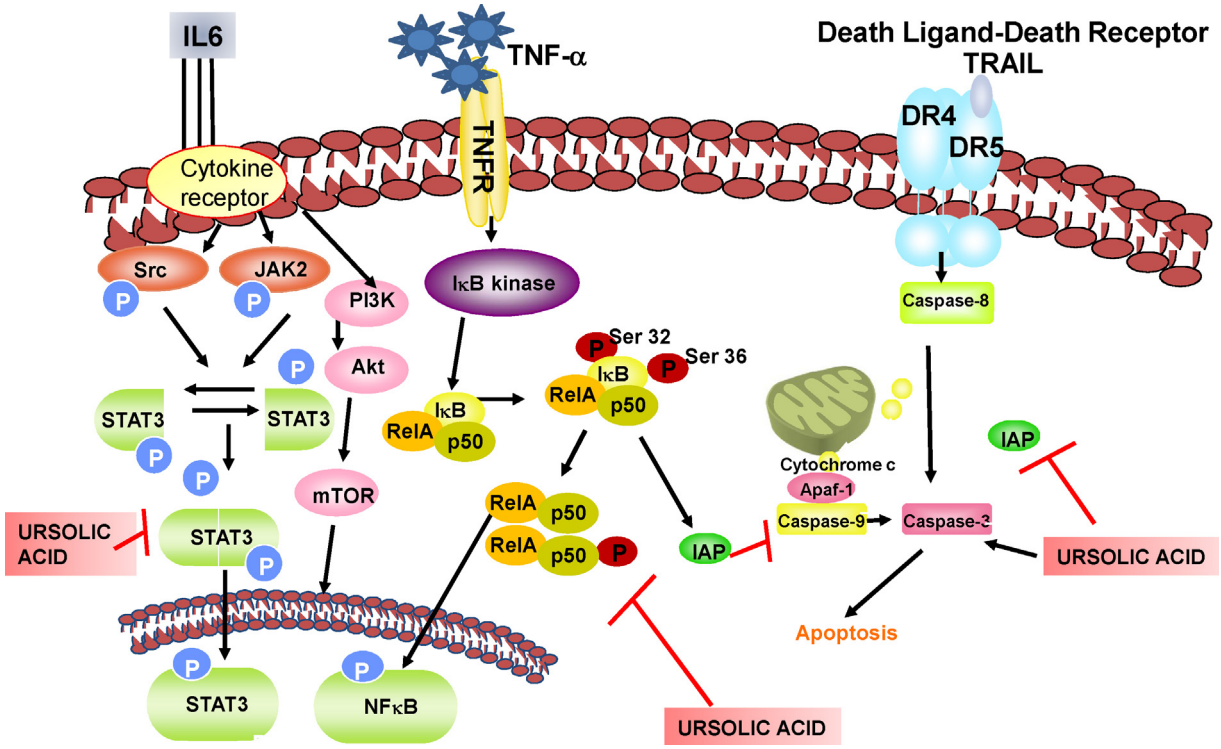

Fig. 2. Key inflammation associated signaling pathways inhibited by ursolic acid. These include nuclear factor-κB (NF-κB), signal transducer and activator of transcription (STAT3), and tumor necrosis factor (TNF)-related apoptosis inducing ligand (TRAIL) signal transduction pathways that have been shown to be modulated by ursolic acid both *in vitro* and *in vivo*.

Table 2
In vivo chemopreventive and anticancer effects of ursolic acid.

Effects	References
Prostate carcinoma (animal model: male Balb/c nude mice) – intraperitoneal injection	
Suppressed subcutaneously implanted neoplasia in male Balb/c nude mice at dose of 200 mg/kg b.w.	[34]
Reduced the expression of VEGF and increased the expression of caspase-3 in tumor tissues	
Transgenic adenocarcinoma of mouse prostate (animal model: TRAMP) – dietary dose (1%, w/w)	
Ursolic acid in TRAMP mice delayed formation of prostate intraepithelial neoplasia, inhibited progression of PIN to adenocarcinoma and demonstrated markedly reduced tumor growth without any significant effects on total body weight and prolonged overall survival	[74]
Down-regulated the expression of COX-2, cyclin D1 and up-regulated the expression of caspase-3	[74]
Reduced CXCR4 expression in tumor tissues of 36 weeks old TRAMP mice	[30]
Hepatocellular carcinoma (animal model: Kunming mice) – intraperitoneal injection	
Inhibited tumor growth in subcutaneously implanted tumor in a dose-dependent manner	[70]
Hepatocellular carcinoma (animal model: Wistar rat) – oral feeding	
Inhibited diethylnitrosamine-induced and phenobarbital-promoted liver cancer	[78]
Reduced oxidative stress and free radicals in liver of Wistar rats	[78]
Colorectal carcinoma (animal model: male nude mice) – intraperitoneal administration	
Inhibited colonic adenocarcinomas in an orthotopic mouse model and this effect was enhanced in the presence of capecitabine	[39]
Inhibited proliferation marker Ki-67 and microvessel density CD-31with concomitant suppression of NF-κB, STAT3 and β-catenin	[39]
Induced death receptors, DR4 and DR5, down-regulated cell survival proteins	[6]
Colonic aberrant crypt foci (animal model: Sprague-Dawley rat) – dietary dose (0.11%, w/w)	
Reduced development of azoxymethane (AOM)-induced colonic aberrant crypt foci	[75]
Leukemia (animal model: NOD/SCID mice) – intraperitoneal injection	
Induced apoptosis in human leukemia cells in a dose- and time-dependent manner	[76]
Down-regulatesed PKB/JNK pathway, caused caspase activation and apoptosis	[76]
Breast carcinoma (animal model: overiectomized female C57BL/6 mice) – dietary dose	
Inhibited syngenic MMTV-Wnt-1 mammary tumors injected in mammary fat pad	[77]
Inhibited AKT/mTOR signaling pathway and induced apoptosis in tumors	[76]
Skin carcinoma (animal model: mice)	
Inhibited skin cancer growth by decreasing epidermal thickness	[80]
Gastric cancer (animal model: male nude mice)	
Inhibited the growth of gastric cancer BGC-803 xenograft and induced apoptosis in tumors	[57]

Abbreviations: Bcl2, B-cell lymphoma 2; COX-2, cyclooxygenase-2; DR, death receptor; EGFR, epidermal growth factor receptor; HER2, human epidermal growth factor receptor 2; IFN-γ, interferon-gamma; IκB, inhibitory subunit of NF-κB; IKK, IκB kinase; IL, interleukin; iNOS, inducible nitric oxide synthase; IAPs, inhibitor of apoptosis; LOX, lipoxygenase; MMP, matrix metalloproteinase; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor-κB; PARP, poly ADP ribose polymerase; ROS, Reactive oxygen species; RNS, Reactive nitrogen species; STAT3, signal transducer and activator of transcription 3; SH-PTP1, protein tyrosine phosphatase-SHP1; TRAMP, transgenic adenocarcinoma of prostate; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; TNF-α, tumor necrosis factor; TGF-β, transforming growth factor beta; TIMP-3, Tissue inhibitor of metalloproteinase 3; VCAM-1, vascular cell adhesion molecule 1; VEGF, vascular endothelial growth factor.

3.2. In vivo *chemopreventive and anticancer activities of ursolic acid*

In vivo, ursolic acid has been reported to inhibit tumor growth in various animal models of cancer (Table 2). Treatment with ursolic acid (200 mg/kg b.w.) for 6 weeks inhibited the growth of DU145 cells in nude mice without any significant effect on body weight [7,34] and produced chemopreventive effects in the transgenic adenocarcinoma of mouse prostate (TRAMP) mouse model [74]. Recently, the effect of diet enriched with 1% w/w ursolic acid was investigated by our group. We found that TRAMP mice fed with ursolic acid in the diet for 8 weeks (weeks 4–12) exhibited delayed formation of prostate intraepithelial neoplasia (PIN) and also inhibition of progression of PIN to adenocarcinoma in the mice fed with ursolic acid diet for 6 weeks (weeks 12–18) was observed. In addition, TRAMP mice fed with this triterpene in the diet for 12 weeks (weeks 24–36) demonstrated markedly reduced tumor growth without showing significant effects on total body weight and prolonged overall survival. It was observed that ursolic acid down-regulated activation of various pro-inflammatory mediators including, NF-κB, STAT3, AKT and IKKα/β phosphorylation in the dorsolateral prostate (DLP) tissues that correlated with the decreased serum levels of TNF-α and IL-6 [74]. *In vivo* studies using H22 xenografts in Kunming mice were conducted by Shao et al. [70] with a novel ursolic acid derivative (compound 14) at doses of 50, 100 and 150 mg/kg body weight. The results revealed that the medium dosage group (100 mg/kg) showed significant anticancer activity ($45.6 \pm 4.3\%$) compared to the control group.

Anti-tumorigenic activity of ursolic acid has also been reported in an orthotopic colorectal nude mouse model. Ursolic acid significantly inhibited tumor volume, ascites formation, as well as distant organ metastasis, and this effect was enhanced with capecitabine [39]. Immuno-histochemistry analysis of tumor tissue indicated that ursolic acid down-regulated biomarkers of proliferation (Ki-67) and microvessel density (CD31). This effect was accompanied by suppression of NF-κB, STAT3, and β-catenin. In addition, ursolic acid suppressed EGF receptor (EGFR) and induced p53 and p21 expression [39]. Ursolic acid (0.11% in diet) reduced the incidence of aberrant crypt foci, one of the earliest precursors of colorectal adenoma development, in particular the tumor initiation phase [75]. Ursolic acid also displayed significant antitumor activity in a leukemic nude mice model. U937 cells (2×10^6 per mouse) were suspended in sterile PBS and injected s.c. into the right flank of NOD/SCID mice. Three days after tumor inoculation, the treatment group received ursolic acid (50 mg/kg, i.p. for 20 days). The control group received an equal volume of solvent control and it was observed that ursolic acid significantly inhibited leukemia growth *in vivo* [76]. Ursolic acid was also found to induce the expression of death receptors, down-regulate cell survival proteins, and activate JNK in orthotopically implanted

human colorectal cancer in a nude mouse model [6]. Likewise, in a transgenic breast cancer model, ovariectomized C57BL/6 mice were randomized to receive control diet (AIN-93G) or diet supplemented with ursolic acid at 1 of 3 doses (wt/wt): 0.05%, 0.10%, or 0.25% (≈54, 106, or 266 mg/kg body weight/day, respectively). After 3 weeks, syngeneic MMTV-Wnt-1 mammary tumor cells were injected in the mammary fat pad, and mice continued on their respective diets for 5 more weeks. Ursolic acid in diet was found to produce significant antitumor activity by modulating Akt/mTOR signaling pathway and by inducing substantial apoptosis [77]. Oral administration of ursolic acid (20 mg/kg) for 6 weeks in diethylnitrosamine (DENA)-induced and phenobarbital-promoted hepatocarcinogenesis in male Wistar rats reduced the oxidative stress-mediated changes in the liver of rats, thereby suggesting that ursolic acid can act as an excellent chemopreventive agent in overcoming free radical- mediated inflammatory diseases like cancer [78].

Ursolic acid was tested for its inhibitory effect on tumor promotion by tetradecanoyl-phorbol-13-acetate (TPA) *in vivo* by Tokuda et al. [79]. They found that ursolic acid can effectively inhibit the tumor promotion in mouse skin and the activities were comparable to that of a known inhibitor of tumor promotion, retinoic acid (RA). Interestingly, ursolic acid was more effective on a single application before initial TPA-treatment than on a continuous application before each TPA-treatment, while RA were ineffective in the same treatment. These data suggest that the role of ursolic acid for inhibitory action on tumor promotion differs slightly from those of RA. Kowalczyk et al. [80] investigated the potential cancer preventive effects of ursolic acid in murine skin carcinogenesis and found that it caused marked decreases of epidermal thickness and (except RES) reduced percentages of mice with mutation in codon 61 of Ha-ras oncogene. Wang et al. [57] explored the effect of ursolic acid on the growth of gastric cancer cell line BGC-803 and hepatoma H22 xenografts and found that the apoptotic rate was significantly increased in tumor cells treated with this triterpenoid. The expression of caspase-3 and -8 was elevated in tumor cells from xenograft treated with ursolic acid. In addition, it was also observed in another study that ursolic acid indeed could inhibit the growth of H22 hepatoma *in vivo* [58]. Interestingly, Lee et al. [81] observed only a moderate retardation of growth in two tumor models *in vivo* upon ursolic acid administration. Singletary and coworkers [82] found that ursolic acid did not significantly affect in 7, 12-dimethyl-benz[a]anthracene-induced mammary tumorigenesis in female rats. Overall, a vast majority of these studies indicate that ursolic acid can inhibit tumor initiation, progression, and metastasis in a wide variety of preclinical cancer models.

4. Pharmacokinetics of ursolic acid

A rapid, sensitive, and accurate liquid chromatography–mass spectrometry (LC–MS) method for the determination of ursolic acid in rat plasma was developed and validated by Liao et al. [83]. In this method, rat plasma was acidified with acetic acid and then extracted with a mixture of hexane-dichloromethane-2-propanol (20:10:1, v/v/v). This LC–MS method has been successfully used for the pharmacokinetic studies after oral administration of Lu-Ying extract containing 80.32 mg/kg ursolic acid to the rats. Furthermore, ursolic acid as an internal standard was established for determination of glycyrrhetic acid and gambogic acid in human plasma, using sensitive liquid chromatography–electrospray ionization-mass spectrometry (LC–ESI-MS) [84,85]. Freeze-dried powder of ursolic acid phospholipid liposomes at low, middle and high doses was used to study for body distribution in mice after i.v. administration. It was found that ursolic acid concentration in the livers of mice was highest in the tested organs at 4 h [86]. Recently an ultra-performance liquid chromatography/tandem mass spectrometry (UPLC/MS/MS) method with high selectivity, sensitivity and throughput was established and validated for quantitation of total ursolic acid in human plasma. This assay exhibited good linearity over the range of 10–5000 ng/mL for ursolic acid in human plasma with a lower limit of quantitation of 10 ng/mL [87]. In another study, Chen et al. [88] developed a method using liquid chromatography and mass spectrometry to determine the concentration of ursolic acid in rat plasma. The concentrations of ursolic acid in rat lung, spleen, liver, heart, and cerebellum tissue were measured. This method was validated in the concentration range 2.5–1470 ng/mL for plasma samples and 20–11760 ng/g for tissue homogenates. Recoveries in plasma and tissues ranged from 83.2% to 106.2% [88]. Ursolic acid was detected in all serum samples 24 h after the last injection. Finally in our recent report, ursolic acid was detected in serum obtained from TRAMP mice fed with ursolic acid (1%, w/w)—enriched diet in nanogram quantity indicating that it is well absorbed in the gastrointestinal tract [74].

5. Human clinical trials with ursolic acid

Liposomal ursolic acid was used to determine the maximum tolerated dose (MTD), dose-limiting toxicity (DLT), and pharmacokinetics of ursolic acid, as a new drug, in healthy adult volunteers and in patients with advanced solid tumors. 63 subjects (4 patients, 35 healthy volunteers, and 24 adults) received a single-dose of ursolic acid liposomes (11, 22, 37, 56, 74, 98, and 130 mg/m^2) administered as a 4-h intravenous infusion. The clinical data reported for the first time that liposomal ursolic acid had manageable toxicities with MTD of 98 mg/m^2. The DLT were primarily hepatotoxicity and diarrhea. Meanwhile, ursolic acid liposomeal formulation had a linear pharmacokinetic profile [89]. Both et al. [90] demonstrated that ursolic acid incorporated into liposomes increased the ceramide content of the skin of human subjects, with increases in hydroxyl-ceramides occurring after only 3 days of treatment. In an another study, Yarosh et al. [91] showed that in clinical tests, ursolic acid incorporated into liposomes increased the ceramide content in human skin over an 11-day period. These studies suggest that ursolic acid has tremendous potential to be developed into a potent anti-inflammatory/anticancer drug.

6. Conclusions and perspectives

This review summarizes the reported chemopreventive and therapeutic potential of ursolic acid in various cancer models. Evidence from both *in vitro* and *in vivo* studies suggests that ursolic acid can indeed suppress multiple molecular targets that play a pivotal role in both chronic inflammation and cancer. However, in future more detailed investigations are needed to completely understand its exact mechanism of action against different cancers. More, importantly, ursolic acid has been found to be bioavailable following oral administration in mice while human pharmacokinetic and pharmacodynamics profiles with the liposomal ursolic acid are also available. Various evidences as discussed above, related to the capability of ursolic acid to suppress various key steps of tumor initiation, progression and promotion, clearly vindicate its traditional use over the past hundreds of years in the treatment of inflammatory diseases, including cancers. Additional clinical trials are required to fully exploit its reported efficacy for the prevention and treatment of various malignancies.

Conflict of interest

None.

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