Oleanane triterpenoids in the prevention and therapy of breast cancer: current evidence and future perspectives

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Abstract Breast cancer is one of the most frequently diagnosed cancers and major cause of death in women in the world. Emerging evidence underscores the value of dietary and non-dietary phytochemicals, including triterpenoids, in the prevention and treatment of breast cancer. Oleanolic acid, an oleanane-type pentacyclic triterpenoid, is present in a large number of dietary and medicinal plants. Oleanolic acid and its derivatives exhibit several promising pharmacological activities,

including antioxidant, anti-inflammatory, hepatoprotective, cardioprotective, antipruritic, spasmolytic, antiallergic, antimicrobial and antiviral effects. Numerous studies indicate that oleanolic acid and other oleanane triterpenoids modulate multiple intracellular signaling pathways and exert chemopreventive and antitumor activities in various in vitro and in vivo model systems. A series of novel synthetic oleanane triterpenoids have been prepared by chemical modifications of oleanolic acid and some of these compounds are considered to be the most potent anti-inflammatory and anticarcinogenic triterpenoids. Accumulating studies provide extensive evidence that synthetic oleanane derivatives inhibit proliferation and induce apoptosis of various cancer cells in vitro and demonstrate cancer preventive or antitumor efficacy in animal models of blood, breast, colon, connective tissue, liver, lung, pancreas, prostate and skin cancer. This review critically examines the potential role of oleanolic acid, oleanane triterpenoids and related synthetic compounds in the chemoprevention and treatment of mammary neoplasia. Both in vitro and in vivo studies on these agents and related molecular mechanisms are presented. Several challenges and future directions of research to translate already available impressive preclinical knowledge to clinical practice of breast cancer prevention and therapy are also presented.

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cancer · Oleanolic acid · Synthetic oleananes ·
Treatment

Abbreviations

AML	Acute myelogenous leukemia
AMR	Amooranin
AMR-Me	Amooranin-methyl
CDDO	2-Cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid
CDDO-EA	CDDO-ethyl amide
CDDO-Me	CDDO-methyl
CDDO-Im	CDDO-imidazolide
CDDO-TFEA	CDDO-trifluoroethyl amide
CKD	Chronic kidney disease
COX-2	Cyclooxygenase 2
DMBA	Dimethylbenz(<i>a</i>)anthracene
DR	Death receptor
eGFR	Estimated glomerular filtration rate
EGFR	Epidermal growth factor receptor
ER	Estrogen receptor
FLIP _L	FLICE-like inhibitory protein
HER2	Human epidermal growth factor receptor 2
HSP90	Heat shock protein 90
I κ B- α	Inhibitory kappaB-alpha
i.p.	Intraperitoneal
i.v.	Intravenous
JAK1	Janus-activated kinase-1
JNK	c-Jun N-terminal kinase
MAPK	Mitogen-activated protein kinase
M-CSF	Macrophage-colony stimulating factor
MTD	Maximum tolerated dose
MMP-9	Matrix metalloproteinase-9
MMTV	Mouse mammary tumor virus
mTORC1	Mammalian target of rapamycin complex 1
NF- κ B	Nuclear factor-kappaB
NMU	<i>N</i> -nitrosomethyl urea
PARP	Poly(ADP-ribose) polymerase
PPAR γ	Peroxisome proliferator-activated receptor-gamma
PI3K	Phosphatidylinositide 3-kinase
PyMT	Polyoma middle T
ROS	Reactive oxygen species
SAHA	Suberoylanilide hydroxamic acid
STAT3	Signal transducer and activator of transcription 3
TAM	Tumor-associated macrophages
TRAIL	Tumor necrosis factor-related apoptosis-inducing ligand

Introduction

Breast cancer is one of the most frequently diagnosed cancers and major causes of death in women in the world. High prevalence of this cancer in developed and industrialized countries remains a serious concern. According to the American Cancer Society, 232,340 new cases of invasive mammary cancer will be diagnosed in women in the year 2013 alone, and 39,620 women will die from this disease in the same year (Siegel et al. 2013). In the developed countries, a woman has 1 in 8 chance of developing breast cancer in her lifetime (Lillie et al. 2007). Although breast carcinoma is most common in women, men are also susceptible to developing this malignancy. In the year 2013, 2,240 men will be diagnosed with breast cancer, and 410 men will die of this disease (Siegel et al. 2013). Men and women with similar stages of breast cancer also have similar prognosis. Although the cure for this devastating cancer has yet to be found, in the United States there are more than 2.9 million breast cancer survivors (American Cancer Society 2013).

Both hormonal and non-hormonal risk factors are known to increase the risk of developing breast cancer. Hormonal risk factors include cumulative exposure to endogenous and exogenous estrogens, such as early age of menarche, late onset of menopause, high total numbers of menstrual cycles, nulliparity and use of estrogen-progestin hormones (Bozovic-Spasojevic et al. 2012; Files et al. 2012). Non-hormonal risk factors include alcohol consumption, obesity, diabetes and various life style-related factors (Park et al. 2013; Crujeiras et al. 2013; Pierobon and Frankenfeld 2013; Jia et al. 2013). Genetic factors, including mutations in tumor suppressor genes *BRCA 1* and *BRCA 2*, account for approximately 5–10 % of all breast cancer cases (Lillie et al. 2007; Campeau et al. 2008).

Despite advances in our understanding of the etiology of breast cancer, little progress has been made during the last two decades in the treatment of advanced metastatic disease of the breast. Currently, breast cancer treatment includes surgery and radiotherapy with or without chemotherapy, hormone therapy and monoclonal antibody immunotherapy depending on the characteristics of the tumor, including the stage, grade, molecular profile as well as the patient's age, health, menopausal status and family history. Adverse events

from systemic therapy with cytotoxic drugs can be severe enough to cause damage to major organ systems. Similarly, localized radiotherapy can lead to fibrosis, pain, edema, and irreversible changes in mobility. These problems underscore the need for safer alternatives to conventional cancer therapy. Additionally, there is an urgent need to develop drugs that can be useful for the treatment of estrogen receptor (ER)-negative breast cancer. Especially, triple-negative breast cancer, which lacks receptors for estrogen and progesterone and does not overexpress human epidermal growth factor receptor 2 (HER2), has a poor prognosis (Curigliano and Goldhirsch 2011) and is a good candidate for new therapies. Chemoprevention, an alternate preventive approach through dietary means and/or use of pharmacological and natural agents, could be a winning strategy in reducing breast cancer burden, at least in high-risk population (Jordan 2007; Bozovic-Spasojevic et al. 2012; Cazzaniga and Bonanni 2012; Files et al. 2012; Eccles et al. 2013). According to a recent consensus, preventive therapy needs to be integrated into broader strategies of breast cancer risk reduction, including avoidance of obesity and increase of physical activity (Cuzick et al. 2011). Currently, two selective ER modulators, namely tamoxifen and raloxifen, are only drugs approved by the United States Food and Drug Administration for the prevention of breast cancer in high-risk women. Nevertheless, several adverse effects, such as increased risk of endometrial cancer, thromboembolic events and stroke, preclude the widespread use of these drugs. In view of these limitations, discovery of new breast cancer chemopreventive drugs with acceptable efficacy and toxicity is urgently needed.

Emerging data generated during the last several decades have shown cancer preventive and therapeutic potential of a large number of phytochemicals from dietary and non-dietary origin (Bishayee 2009; Corea et al. 2009; Gullett et al. 2010; Bishayee et al. 2012; Harlev et al. 2013; González-Vallinas et al. 2013; Shanmugam et al. 2013). Various bioactive phytochemicals and compounds based on natural products kill breast tumor cells *in vitro* and prevent the development of breast tumors or suppress the growth of existing tumors *in vivo* through modulation of cellular proliferation, differentiation, apoptosis, oxidative stress, inflammation, angiogenesis and several key signaling pathways implicated in the initiation, promotion and progression of breast cancer (Corea et al. 2005; Barile et al. 2007; Khan et al. 2011;

Reuben et al. 2012; Sinha et al. 2012; Aiyer et al. 2012; Venugopal and Liu 2012; Biersack and Schobert 2012; Vadodkar et al. 2012; Yiannakopoulou 2014). Several clinical intervention trials evaluated the potential effectiveness of various natural products and dietary supplements in breast cancer prevention (Kado et al. 2012).

Terpenoids, also known as terpenes or isoprenoids, represent the largest group of phytochemicals found in a variety of vegetables, fruits and medicinal plants. Based on the number of 5-carbon (C5) building blocks, terpenoids are classified into several groups, including monoterpenes (C10), sesquiterpenes (C15), diterpenes (C20), sesterterpenes (C25), triterpenes (C30), tetraterpenes (C40) and polyterpenes. There are over 40,000 different terpenoids, which have been isolated from plants, animals and microbial species (Withers and Keasling 2007). One of the most important biological effects of natural terpenoids is the ability to prevent and treat several cancers, including breast cancer (Crowell 1997; Gould 1997; Barile et al. 2008; Rabi and Gupta 2008; Rabi and Bishayee 2009; Yang and Dou 2010; Miller et al. 2011; Kuttan et al. 2011; Thoppil and Bishayee 2011).

Within the group of terpenoids, the largest group is represented by triterpenoids. These phytochemicals are most commonly found in various plants, including sea weeds and the wax coating on fruits, such as olives, apples, figs, and cranberries. Triterpenoids are closely related in structure to steroids, and are metabolites of isopentenyl pyrophosphate oligomers. It is estimated that there are over 20,000 triterpenoids in nature (Liby et al. 2007) and various triterpenoids are widely used in Asian traditional medicines (Council of Scientific and Industrial Research 1972; Tang and Eisenbrand 1992). Triterpenoids exhibit a wide spectrum of biological and pharmacological effects, including anti-inflammatory, antibacterial, antiviral, hepatoprotective, gastro-protective, anti-ulcer, cardiovascular, hypolipidemic, antiatherosclerotic, immunoregulatory, anticancer and cancer preventive activities (Ovesná et al. 2004; Laszczyk 2009; Petronelli et al. 2009; Yadav et al. 2010; Bishayee et al. 2011; Lanzotti et al. 2012; Patlolla and Rao 2012; Safe et al. 2012; Shanmugam et al. 2012). Triterpenoids can be sub-classified into several groups including cucurbitanes, cycloartanes, dammaranes, euphanes, friedelanones, holostanes, hopanes, isomalabaricanes, lanostanes, limonoids, lupanes, oleananes, protostanes, squalenes, tirucallanes,

ursanes, and miscellaneous compounds (Setzer and Setzer 2003; Petronelli et al. 2009).

Oleanolic acid (3 β -hydroxyolean-12-en-28-oic acid, Fig. 1), an oleanane-type pentacyclic triterpenoid, has been isolated from more than 1,600 plant species, including many dietary and medicinal plants (Pollier and Goossens 2012). The plants belonging to the Oleaceae family, including olive (*Olea europaea*), are rich sources of oleanolic acid. This compound exists in nature either as a free acid or as an aglycone precursor for triterpenoid saponins, in which it can be linked to one or more sugar chains (Liu 2005; Szakiel et al. 2005). Oleanolic acid can be commonly found in high quantities from olive pulp after the oil is pressed out from the olive fruit and in the leaves which are considered as waste when the trees are pruned. Oleanolic acid and its derivatives possess several promising pharmacological effects, such as antioxidant, anti-inflammatory, hepatoprotective, cardioprotective, antipruritic, spasmolytic, antiallergic, antimicrobial and antiviral activities (Somova et al. 2003; Dzubak et al. 2006; Sultana and Ata 2008; Wang et al. 2010). Emerging studies indicate that oleanolic acid and other oleanane triterpenoids influence multiple intracellular signaling pathways and exert chemopreventive and antitumor activities in various in vitro and in vivo model systems (Ovesná et al. 2004; Sultana and Ata 2008; Sogno et al. 2009; Zhang and Popovich 2009; Kuttan et al. 2011; Shanmugam et al. 2012). Several animal and human studies have indicated systemic absorption, bioavailability and tissue distribution of oleanolic acid following oral administration (Song et al. 2006; Jeong et al. 2007; Yin et al. 2012). It is likely that oleanolic acid is absorbed and deposited in vivo in its intact form, which may be responsible for the biological activity of this molecule (Yin et al. 2012).

Due to its inherent bioactivities, availability and low cost, oleanolic acid has been used as a starter molecule for development of synthetic oleanane triterpenoids. A series of new synthetic oleanane triterpenoids have been prepared by chemical modification of oleanolic acid on its three active sites, such as C-3 hydroxyl, C-12-C-13 double bond and C-28 carboxylic acid (Sporn et al. 2011). Some of these compounds, namely 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid (CDDO), CDDO-methyl ester (CDDO-Me, also known as bardoxolone methyl), CDDO-imidazolide (CDDO-Im), CDDO-ethyl amide (CDDO-EA) and CDDO-trifluoroethyl amide (CDDO-TFEA) (Fig. 1), are considered to be the most potent anti-inflammatory and anticarcinogenic

triterpenoids (Liby et al. 2007). Several aforementioned compounds have the potential to control inflammation and oxidative stress in almost every part of the body and hence are being developed as multifunctional drugs for diseases in which inflammation and oxidative stress contribute to disease pathogenesis (Liby and Sporn 2012). Accumulating studies provide extensive evidence that synthetic oleanane derivatives inhibit proliferation and induce apoptosis of various cancer cells in vitro and demonstrate cancer preventive or antitumor efficacy in animal models of blood, breast, colon, connective tissue, liver, lung, pancreas, prostate, and skin cancers (Liby et al. 2007; Liby and Sporn 2012). The aim of this review is to examine current body of evidence to understand the full potential of natural and synthetic oleanane triterpenoids in breast cancer prevention and therapy. Current limitations and future directions of research on these promising compounds in breast cancer have also been presented.

Oleanane triterpenoids and breast cancer

Research articles presented in this review include preclinical in vitro and in vivo studies conducted to explore chemotherapeutic as well as chemopreventive potential of oleanane triterpenoids and related synthetic analogs in breast cancer. Clinical studies on synthetic oleanane triterpenoid are also described. PubMed, EBOSCOhost, and Google Scholar were used to find all primary literature. There were no date restraints on the articles. Only English language publications were considered for this work. Abstracts of articles were first reviewed and then useful full articles were retrieved. Major keywords used in various combinations included: oleanolic acid, oleananes, synthetic oleanane compound, breast, mammary, cancer, chemoprevention, prevention, treatment, in vivo, in vitro, and clinical studies. The references of the primary articles were also studied to collect additional relevant articles. For obtaining information regarding clinical trials, clinicaltrials.gov was used in addition to aforementioned databases.

In vitro studies

There are numerous in vitro studies that demonstrate the inhibitory effects of oleanolic acid and other

Fig. 1 Chemical structures of oleanolic acid and selected natural and synthetic oleanane triterpenoids studied to explore breast cancer chemopreventive and therapeutic potential

oleanane triterpenoids against proliferation, growth and invasion of a large variety of breast cancer cell lines (Table 1). Interestingly, oleananes exhibited wide variations of antiproliferative activity based on these studies. An ethyl acetate fraction of *Glossogyne tenuifolia* (Asteraceae) (a native traditional anti-inflammatory herb in Taiwan) plant extract containing oleanolic acid has been found to exhibit cytotoxicity against MCF-7 and MDA-MB-231 breast cancer cells (Hsu et al. 2005). Nevertheless, the isolated compound oleanolic acid has been shown to possess weak cytotoxicity against both breast tumors cells (Hsu et al. 2005). An ethyl acetate extract of *Calendulae officinalis flos* (Asteraceae) has been found to contain oleanolic acid. High concentration (more than 75 $\mu\text{g/ml}$) of this extract exhibited antitumor efficacy in T47D

human breast cancer cells (Matysik et al. 2005). BN107, an aqueous preparation of grounded fruit of Chinese traditional medicinal plant *Gleditsia sinensis* Lam. (Fabaceae), is known to contain oleanolic acid. BN107 has been shown to selectively induce apoptosis in ER-negative breast cancer cells, such as MDA-MB-231 and Hs578T, primarily mediated by the mitochondrial pathway as evidenced by mitochondrial transmembrane potential dissipation, caspase-3 and -9 activation, and cytochrome *c* release into cytosol. BN107 also caused rapid alterations in cholesterol homeostasis. BN107 or oleanolic acid treatment resulted in rapid and specific inhibition of lipid raft-mediated survival signaling, such as mammalian target of rapamycin complex 1 (mTORC1) and mTORC2 (Chu et al. 2010). Mencherini et al. (2011)

isolated two terpenes of the oleanane series, namely oleanolic acid and hederagenin, from the crude extract of the roots of *Paenonia rockii* ssp. *rockii*, a plant distributed mainly in the northwest of China. This extract showed a slight inhibitory effect on the proliferation of MCF-7 cells (Mencherini et al. 2011). Oleanolic acid showed significant inhibition of the proliferation of MCF-7 and MCF-7/ADR cells in a time- and concentration-dependent manner (Shan et al. 2011). According to a study conducted by Allouche et al. (2011), oleanolic acid displayed a significant cytotoxic effect against MCF-7 cells which involved cell cycle arrest, reduction of reactive oxygen species (ROS), and protection against oxidative DNA damage. *Oldenlandia diffusa* Roxb. (Rubiaceae), an herb prevalent in East Asia and Southern China, is used to prevent and treat various disorders, especially cancers. An ethanolic extract of *O. diffusa* as well as its bioactive compound oleanolic acid exerted antiproliferative and apoptotic effects selectively in ER- α -positive breast cancer cells. The same study also showed that oleanolic acid upregulated p53 and p21WAF1/Cip1 9 (Gu et al. 2012). Chakravarti et al. (2012) isolated oleanolic acid and ursolic acid from an ethanolic extract of *Wrightia tomentosa* Roem. & Schult. (Apocynaceae), which is used in Indian traditional medicine. Oleanolic acid together with ursolic acid inhibited the proliferation of MCF-7 and MDA-MB-231 cells and induced cell cycle arrest and apoptosis as indicated by significant increase in Annexin-V-positive apoptotic cell counts.

Deng et al. (1999) isolated three new 24,28-dinorolean-3-one derivatives, namely remangilonones A, B and C, from the dried leaves of *Physena madagascariensis* Noronha ex Thouars (Capparaceae), a tree endemic to Madagascar. Remangilonones A and C have been found to be cytotoxic against MBA-MD-231 and MDA-MB-435 cells possibly due to apoptosis induction (Deng et al. 1999). Two new triterpene caffeates, such as 3 β ,23,28-trihydroxy-12-oleanene 23-caffeate and 3 β ,23,28-trihydroxy-12-oleanene 3 β -caffeate, have been isolated from the root bark of *Hibiscus syriacus* (Malvaceae), which is distributed in eastern and southern Asia. Both compounds have exhibited cytotoxicity against MCF-7 cells possibly due to antioxidant mechanism (Yun et al. 1999). Hederacolchisid A₁, a new oleanolic acid monodesmoside isolated from *Hedera colchica* K. Koch (Araliaceae), an ivy species endemic in Georgia, has been evaluated for antiproliferative

activity against MCF-7 cells. Although the results demonstrated a considerable cytotoxicity, the underlying mechanisms were not reported (Barthomeuf et al. 2002).

Maslinic acid, an oleanane-type triterpenoid isolated from apple peels, exhibited antiproliferative and cytotoxic activities against MCF-7 cells although the mechanism of action was not studied (He and Liu 2007). In contrast, Allouche et al. (2011) reported an increased proliferation of MCF-7 cells following maslinic acid treatment. The same investigators observed antiproliferative activity of another oleanane triterpenoid erythrodiol using the same breast cancer cells (Allouche et al. 2011). Investigation of an ethanolic fraction of the aerial parts on *Moringa peregrina* (Forssk.), a wild plant that grows in the eastern desert mountains in Egypt, yielded another oleanane-type triterpenoid β -amyrin. Accompanying study showed in vitro cytotoxicity of β -amyrin against MCF-7 cells (El-Alfy et al. 2011). Achyranthoside H methyl ester, an oleanolic acid saponin derivative isolated from the roots of *Achyranthes fauriei*, a medicinal plant used in East Asia, exhibited significant cytotoxicity against MCF-7 and MDA-MB-453 cells through apoptosis induction (Fukumura et al. 2009).

Since the antitumor activities of naturally occurring oleanolic acid are relatively weak, attempts have been made to synthesize new analogs with greater potency. Several investigators synthesized analogs of oleanolic acid and compared the cytotoxic effect of individual analogs to that of the parent compound. Kang et al. (2012) observed that oleanolic acid azaheterocyclic compounds were more effective in inhibiting the growth of MDA-MB-231 cells compared to oleanolic acid. Similar observations were made by Hao et al. (2013) in which at least five derivatives of oleanolic acid showed superior cytotoxicity against MCF-7 cells to that of oleanolic acid.

In 1999, Suh et al. (1999) developed synthetic oleanane triterpenoid CDDO, which was found to be 100–500 fold more potent than any previous triterpenoid in suppressing inflammatory enzymes with important roles in the development of malignancy, such as inducible nitric oxide synthase and cyclooxygenase 2 (COX-2). CDDO also inhibited the proliferation of ER-positive and ER-negative breast cancer cells (Suh et al. 1999). In a subsequent study, CDDO (at 1 μ M) completely abolished the growth of ER-negative, p53-mutated and HER2-expressing breast cancer

Table 1 In vitro antitumor effects and related mechanisms of action of natural and synthetic oleanane triterpenoids in various breast cancer cells

Compounds	Effects	Mechanisms	Concentrations	References
Oleanolic acid	Displayed weak cytotoxic effects against MCF-7 and MDA-MB-231 cells			Hsu et al. (2005)
	Exhibited antiproliferative activity against T47D cells			Matysik et al. (2005)
	Exhibited cytotoxic effects against Hs578T and MDA-MB-231 cells	↑Apoptosis; cholesterol homeostasis; ↓mTORC1; ↓mTORC2		Chu et al. (2010)
	Elicited modest inhibitory effects on the growth of MCF-7 cells			Mencherini et al. (2011)
	Induced proliferation-inhibiting effects in MCF-7 and MCF-7/ADR cells		28–44 μM	Shan et al. (2011)
	Inhibited the survival and proliferation of MCF-7 cells	Cell cycle arrest; ↓ROS; ↓oxidative DNA damage		Allouche et al. (2011)
	Inhibited the survival of MCF-7 cells	↑p53; ↑p21WAF1/Cip1	6.4 μg/ml	Gu et al. (2012)
	Exerted antiproliferative effects in MCF-7 and MDA-MB-231 cells	Cell cycle arrest; ↑apoptosis	7.0–7.5 μM	Chakravarti et al. (2012)
Remangilonos A Remangilonos C	Exhibited cytotoxic effects in MDA-MB-231 and MDA-MB-435 cells	↑Apoptosis	1.6–8.5 μM	Deng et al. (1999)
3β,23,28-Trihydroxy-12-oleanene 23-cafeate; 3β,23,28-Trihydroxy-12-oleanene 3β-cafeate	Shown cytotoxicity against MCF-7 cells	Antioxidant mechanism	1.8–2.2 μg/ml	Yun et al. (1999)
Hederacolchisid A ₁	Displayed cytotoxicity against MCF-7 cells		~ 10 μM	Barthomeuf et al. (2002)
Maslinic acid	Inhibited the proliferation of MCF-7 cells		136 μM	He and Liu (2007)
	Increased the proliferation of MCF-7 cells		12.5–100 μM	Allouche et al. (2011)
Erythrodiol	Exerted complete inhibition of the proliferation of MCF-7 cells		12.5–100 μM	Allouche et al. (2011)
β-Amyrin	Exhibited cytotoxicity against in MCF-7 cells			El-Alfy et al. (2011)
Achyranthoside H methyl ester	Displayed cytotoxic effects against MCF-7 and MDA-MB-453 cells	↑Sub-G1; PARP cleavage; caspase activation; ↑apoptosis	2–100 μM	Fukumura et al. (2009)
Oleanolic acid derivatives	Exerted antiproliferative effects against MDA-MB-231 cells		44.8–67.9 μM	Kang et al. (2012)
	Exerted growth inhibitory activity against MCF-7 cells		1.98–35.4 μM	Hao et al. (2013)

Table 1 continued

Compounds	Effects	Mechanisms	Concentrations	References
CDDO	Suppressed the proliferation of MCF-7, MDA-MB-231, 21-MT-1, 21-MT-2, 21-NT and 21-PT cells		3×10^{-2} – 1 μ M	Suh et al. (1999)
	Induced the growth inhibitory effects in MCF-7, MDA-MB-231 and MDA-MB-435 cells	$\perp$ G ₁ -S; $\perp$ G ₂ -M; $\uparrow$ apoptosis; $\downarrow$ cyclin D1; $\uparrow$ p21 ^{Waf1/CIP1} ; $\downarrow$ Bcl-2	0.05–10 μ M	Lapillonne et al. (2003)
	Retarded the growth of MCF-7 and MDA-MB-435 cells	$\downarrow$ HER2 phosphorylation; $\downarrow$ HER2 kinase; $\downarrow$ caveolin-1	3.5–7.5 μ M	Konopleva et al. (2006)
CDDO; CDDO-Im	Suppressed the proliferation of MCF-7 cells		10–30 nM	Place et al. (2003)
CDDO; CDDO-Im	Sensitized T47D and MDA-MB-468 cells to TRAIL	$\uparrow$ Apoptosis; $\uparrow$ DR4; $\uparrow$ DR5; $\downarrow$ FLIP _L		Hyer et al. (2005)
CDDO; CDDO-Me	Inhibited the proliferation of MCF-7 cells		0.06–0.16 μ M	Honda et al. (2004)
CDDO-Me	Blocked the proliferation and invasion of 4T1 cells	$\perp$ G ₂ -M; $\downarrow$ STAT3; $\downarrow$ Src; $\downarrow$ Akt; $\downarrow$ c-Myc		Ling et al. (2007)
CDDO-Im; CDDO-Me; CDDO-TFEA	Demonstrated cytotoxic activities against W780 and W0069 cells	$\uparrow$ Apoptosis; $\perp$ G ₂ -M; $\uparrow$ ROS; DNA damage		Kim et al. (2011)
CDDO-Me	Exerted antiproliferative effect in W780 cells	$\perp$ G ₀ -G ₁ ; $\downarrow$ pErbB2	0.1–1 μ M	Kim et al. (2012)
CDDO-Me	Inhibited the proliferation of PyMT cells	$\downarrow$ CCL2; $\downarrow$ CXCL2; $\downarrow$ MMP-9; $\downarrow$ cyclin D1; $\downarrow$ EGFR; $\downarrow$ STAT3	0.1–1 μ M	Tran et al. (2012)
CDDO-Me; CDDO-EA	Reduced the viability of PyMT cells	$\downarrow$ M-CSF; $\downarrow$ MMP-9	0.1–0.3 μ M	Tran et al. (2013)
AMR	Exhibited the growth inhibitory effect in MCF-7 cells		2.5 μ g/ml	Rabi et al. (2002)
	Exerted cytotoxicity against MCF-7 and MCF-7/TH cells	$\uparrow$ Apoptosis; $\perp$ G ₂ -M; $\uparrow$ total caspase; $\uparrow$ caspase-8	3.8–6.8 μ g/ml	Rabi et al. (2003)
	Diminished the survival of MCF-7 and MDA-MB-468 cells	$\uparrow$ Apoptosis; $\perp$ G ₂ -M; $\uparrow$ caspase-3/7; $\uparrow$ p53; $\uparrow$ Bax; $\downarrow$ Bcl2	1.8–7.0 μ M	Rabi et al. (2007)
AMR-Me	Displayed cytotoxic effects against MCF-7 cells		1.8 μ g/ml	Rabi et al. (2002)
	Curbed the proliferation of MCF-7 and MCF-7/p53siRNA cells	$\uparrow$ Apoptosis; $\perp$ G ₂ -M; $\uparrow$ caspase-3/7; $\uparrow$ Bax; $\downarrow$ Bcl2; $\downarrow$ cyclin A; $\downarrow$ cyclin B1; $\uparrow$ JNK; $\uparrow$ MAPK	0.5–0.6 μ M	Rabi and Banerjee (2008)
	Inhibited the proliferation of MCF-7 and MDA-MB-231 cells	$\uparrow$ Apoptosis; $\downarrow$ ER- α ; $\downarrow$ PI3K p85; $\downarrow$ Akt1; $\downarrow$ pAkt; $\downarrow$ NF- κ B-DNA binding	0.15–0.45 μ M	Rabi et al. (2013)

cells. Additionally, CDDO transactivated peroxisome proliferator-activated receptor- γ (PPAR γ), induced cell cycle arrest in G₁-S and G₂-M and triggered apoptotic cell death through regulation of the expression of *cyclin D1*, *p21^{Waf1/CIP1}*, and *Bcl-2* (Lapillonne et al. 2003). Konopleva et al. (2006) investigated the effect of HER2 overexpression on the sensitivity of breast cancer cells to the growth-inhibitory activities of CDDO. While both tumor cell growth and colony formation were preferentially inhibited in HER2-overexpressing cell lines at low concentrations, the growth-inhibitory effects at high concentrations did not corroborate with the expression level of HER2. CDDO also dose-dependently inhibited the phosphorylation of HER2 in HER2-overexpressing cells, diminished HER2 kinase activity, and upregulated breast tumor suppressor gene caveolin-1.

In order to increase the antitumor efficacy of CDDO, several C-28 derivatives have been synthesized. CDDO-Im represents one such synthetic triterpenoid which has been found to be more potent than CDDO in suppressing the proliferation of MCF-7 cells (Place et al. 2003). In a follow-up study (Hyer et al. 2005), both CDDO and CDDO-Im have been found to sensitize tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-resistant T47D and MDA-MB-468 cells to TRAIL-mediated apoptosis with upregulation of cell surface death receptor 4 (DR4) and DR5 and downregulation of FLICE-like inhibitory protein (FLIP_L), an antiapoptotic protein.

Another synthetic oleanane triterpenoid, C-28 methyl ester of CDDO (CDDO-Me), suppressed the proliferation of MCF-7 cells through unknown mechanisms (Honda et al. 2004). Ling et al. (2007) utilized chemotherapy-resistant murine 4T1 breast cancer cells to investigate the effect of CDDO-Me on constitutively activated signal transducer and activator of transcription 3 (STAT3) signaling. The results indicated that CDDO-Me diminished the invasive growth of 4T1 cells with a concomitant accumulation of cells in G₂-M phase, inactivation of STAT3, Src and Akt as well as inhibition of c-Myc. In separate studies, CDDO-Me has also been shown to block the janus-activated kinase-1 (JAK1) $\rightarrow$ STAT3 pathway by directly inhibiting both JAK1 and STAT3 in MDA-MB-468 cells (Ahmad et al. 2008). Treatment with CDDO-Me significantly decreased the levels of Stat3, Jak2 and Src phosphorylation in MDA-MB-468 cells with constitutively activated Stat3 (Duan et al. 2009). To determine the cytotoxic activity of synthetic oleanane

triterpenoids against cultured BRCA1-mutated breast cancer cells, W780 and W0069 cells were exposed to CDDO-Me, CDDO-Im and CDDO-TFEA. CDDO-Me was found to be the most potent inhibitor of cellular proliferation. Mechanistic study revealed that CDDO-Me induced ROS and subsequent DNA damage, thereby facilitating the activation of the DNA damage checkpoint, G₂-M arrest, and finally apoptosis in BRCA1-mutated breast cancer cells (Kim et al. 2011). In BRCA1-deficient mammary tumor cells, CDDO-Me directly interacted with ErbB2, decreased constitutive phosphorylation of ErbB2, and induced G₀-G₁ arrest to inhibit tumor cell proliferation (Kim et al. 2012). Treatment of primary polyoma middle T (PyMT) cells, derived from mammary tumors of female PyMT+/- mice, with CDDO-Me elicited a dose-dependent inhibition of cell viability and this effect was accompanied by decreased levels of chemokines CCL2 and CXCL12, reduced secretion of matrix metalloproteinase-9 (MMP-9), inhibition of cyclin D1, and decreased phosphorylation of epidermal growth factor receptor (EGFR) and STAT3 (Tran et al. 2012). Recently, the same investigators confirmed the antitumor effect of CDDO-Me in PyMT cells and showed similar effect with another CDDO derivative, CDDO-EA. Additionally, it has been demonstrated that the histone deacetylase inhibitor vorinostat (suberoylanilide hydroxamic acid, SAHA) potentiated the antineoplastic effects of synthetic oleanane triterpenoids in breast cancer (Tran et al. 2013).

Amoora rohituka Wright & Arn. (Meliaceae), an evergreen tree that grows wild in India and Bangladesh, has been traditionally used for the treatment of inflammation, liver damage and cancer (Kirtikar and Basu 1980; Prasad 1987). Amooranin (AMR), a triterpenic acid (25-hydroxy-3-oxoolean-12-en-28-oic acid) with a novel structure isolated from the stem bark of *A. rohituka* (Rabi 1996), has been shown to confer cytotoxic effect in MCF-7 cells (Rabi et al. 2002). Subsequently, Rabi and coinvestigators (Rabi et al. 2003, 2007) confirmed the anti-breast cancer effects of AMR and reported the underlying mechanisms of action. AMR has been found to induce oligonucleosome-sized DNA ladder formation (characteristic of apoptosis) in MCF-7 and MCF-7/TH cells with simultaneous elevation of total caspase and caspase-8 activities (Rabi et al. 2003). In a separate study, AMR exhibited a strong inhibitory effect on the survival of MCF-7 and MDA-MB-468 cells compared to MCF-10A normal breast epithelial cells. Additional

studies suggest that AMR induced apoptosis via caspase activation pathway independent of p53 involvement (Rabi et al. 2007).

Since anti-breast tumor efficacy of the natural triterpenoid AMR is weak, new analogues of this compound have been prepared by chemical modification in an attempt to generate more potent agents. The methyl ester of AMR (AMR-Me) has been found to suppress the proliferation of MCF-7 cancer cells with superior potency to the parent compound AMR (Rabi et al. 2002). Subsequent studies have confirmed the striking potency of AMR-Me against MCF-7 cells even at nanomolar range. Mechanistically, AMR-Me has been shown to induce apoptosis in MCF-7 cells through mitochondrial apoptotic pathway associated with DNA fragmentation, poly(ADP-ribose) polymerase (PARP) degradation, altered Bax:Bcl-2 ratios, cytochrome *c* release, and subsequent induction of caspases. AMR-Me also activated two different mitogen-activated protein kinase (MAPK) signaling pathways of p38 MAPK and c-jun N-terminal kinase (JNK) for amplifying the apoptosis cascade in breast cancer cells (Rabi and Banerjee 2008). A follow-up study showed that AMR-Me inhibited phosphatidylinositol 3-kinase (PI3K)/Akt signaling in hormone-dependent MCF-7 cells and diminished the activation of nuclear factor-kappaB (NF- κ B) in hormone-independent MDA-MB-231 cells (Rabi et al. 2013).

In vivo studies

Several laboratories have investigated in vivo breast cancer preventive or therapeutic effects of natural as well as synthetic oleanane triterpenoids (Table 2). Most of these studies have used tumor growth in immunocompromised mouse model whereas a few investigators have utilized carcinogen-induced mammary tumor development in rodents.

Lapillonne et al. (2003) have investigated the effects of CDDO on the growth of implanted MDA-MB-435 cells in female immunodeficient nude mice. 10 days following tumor cell inoculation, the animals were injected intravenously with CDDO for 3 weeks. The results showed a significant reduction in tumor growth in CDDO-treated mice compared to their control counterparts. Konopleva et al. (2006) confirmed the antitumor activity of CDDO using a xenograft model of breast cancer in which parenteral administration of

liposomally encapsulated CDDO retarded tumor growth and reduced tumor size in female nude mice injected with highly tumorigenic MCF-7/HER2 cells. CDDO also significantly decreased HER2 phosphorylation and nuclear cyclin D1 expression in tumors as well as induced tumor cell apoptosis which supported the in vitro results reported from the same laboratory (Konopleva et al. 2006). These results suggest that CDDO could be beneficial for the treatment of patients with HER2-overexpressing breast cancer.

CDDO-Im, a novel CDDO derivative, has been investigated for in vivo antitumor efficacy against a xenograft model of mammary cancer. Though no inhibition of MDA-MB-468 xenograft growth was observed in female BALB/C *nu/nu* mice treated with CDDO-Im, a combination of CDDO-Im and TRAIL was effective in reducing the tumor burden with simultaneous inhibition of tumor cell proliferation (Hyer et al. 2005). Ancillary toxicity studies revealed that the combination was well tolerated by the tumor-bearing animals (Hyer et al. 2005). This study also emphasizes an apparent synergetic effect of CDDO-Im and TRAIL in achieving breast tumor growth suppression.

CDDO-Me inhibited breast cancer growth and lung metastases induced by 4T1 mouse breast cancer cells when the treatment initiated 1 day following tumor implantation and significantly suppressed tumor growth when started 5 days post tumor cell inoculation. CDDO-Me also maintained the mature spleen dendritic cells which possibly contributed to the significant antitumor and antimetastatic effects of this promising agent (Ling et al. 2007). Liby et al. (2008) reported that dietary CDDO-Me significantly delayed the development of ER-negative mammary tumors in female mouse mammary tumor virus (MMTV)-neu mice. It is interesting that a combination of CDDO-Me and the rexinoid LG100268 has been found to be more effective than the individual agents for the prevention of mammary tumorigenesis. Dietary CDDO-Me has also been found to induce tumor regression or arrest the growth of established tumors in this model. The combination of CDDO-Me and LG100268 has been found to be more effective in reducing the tumor volume than CDDO-Me alone. CDDO-Me alone or in combination with LG100268 has also been shown to suppress cell proliferation and induce apoptosis in mammary tumors (Liby et al. 2008). The efficacy of CDDO-Me has been tested in a clinically relevant mouse model of *BRCA1*-mutated breast cancer. A diet

Table 2 Breast cancer chemopreventive and chemotherapeutic effects of natural and synthetic oleanane triterpenoids in preclinical animal models

Compounds	Effects	Mechanisms	Dose/duration	Route	References
CDDO	Reduced the growth of xenografted MDA-MB-435 tumor cells in female nude mice		40 mg/kg/day; twice a week for 3 weeks	i.v.	Lapillonne et al. (2003)
	Blocked the growth and size of transplanted MCF7/HER2 tumors in female nude mice	↓Cell proliferation; ↓cyclin D1; ↑apoptosis;	20 mg/kg/day; 3 times a week for 3 weeks	i.v.	Konopleva et al. (2006)
CDDO-Im	Reduced tumor burden in MDA-MB-468 xenograft model in female BALB/c <i>nu/nu</i> mice	↓Cell proliferation	5 mg/kg/day; 14 days	i.p.	Hyer et al. (2005)
CDDO-Me	Abrogated the growth and metastases of 4T1 tumor cells transplanted in female BALB/c mice	↑Mature spleen dendritic cells	200 µg/mouse; 5 times at 2-day intervals	i.v.	Ling et al. (2007)
	Prolonged the development or arrested the growth of mammary tumors in female MMTV-neu mice	↓Cell proliferation; ↑apoptosis;	60–100 mg/kg diet; 4–45 weeks	diet	Liby et al. (2008)
	Delayed the development of tumors in female <i>Brac1</i> -mutated mice	↓p-ErbB2; ↓p-ErbB2; ↓cyclin D1; ↓γ-H2AX	50 mg/kg diet; 26 weeks	diet	Kim et al. (2012)
	Prolonged the development of tumor and survival of tumor-bearing female PyMT mice	↓Infiltration of TAM	50 mg/kg diet; 30–42 weeks	diet	Tran et al. (2012)
	Delayed development and burden of tumor in female PyMT mice	↓Infiltration of TAM	50–400 mg/kg diet; 26 weeks	diet	Tran et al. (2013)
CDDO-EA	Marginally delayed the development of mammary tumors in female PyMT mice		400 mg/kg diet; 20 weeks	diet	Tran et al. (2013)
AMR	Inhibited the growth of implanted tumor induced by NMU in female Sprague–Dawley rats		10–20 mg/kg/day; 3 weeks	i.p.	Rabi (1996)
AMR-Me	Exhibited lack of antitumor activity against Ehrlich ascites tumor in Swiss mice		50–100 mg/kg/day; 7 days	i.p.	Rabi et al. (2002)
	Inhibited the incidence and burden of mammary tumors induced by DMBA in female Sprague–Dawley rats	↓Cell proliferation; ↑apoptosis; ↑Bax; ↓Bcl-2; ↓ER-α; ↓ER-β; ↓cyclin D1; ↓β-catenin; ↓COX-2; ↓HSP90; ↓NF-κB; ↑IκB-α	0.8–1.6 mg/kg; 3 times per week for 18 weeks	p.o.	Bishayee et al. (2013); Mandal et al. (2013a); Mandal et al. (2013b)

containing CDDO-Me significantly delayed tumor development with simultaneous inhibition in the constitutive phosphorylation of ErbB2 and reduction of the level of cyclin D1 and γH2AX (Kim et al. 2012).

Tran et al. (2012) used PyMT mice, an elegant model of ER-negative breast cancer, to investigate the chemopreventive effect of CDDO-Me. Mice fed with CDDO-Me significantly increased the age of animals

at onset of first tumor and overall survival. This compound also inhibited the infiltration of tumor-associated macrophages (TAM) into the mammary glands of PyMT mice. Recently, the same group showed that the histone deacetylase inhibitor SAHA enhanced the ability of CDDO-Me to delay the formation of mammary tumors in PyMT mice (Tran et al. 2013).

An ethyl amide derivative of CDDO (CDDO-EA) did not delay tumor development in the PyMT breast tumor model. Nevertheless, the combination of CDDO-EA with SAHA was significantly more potent than the individual agents for delaying mammary tumor development (Tran et al. 2013).

Using *N*-nitrosomethyl urea (NMU)-induced mammary adenocarcinomas in rats, Rabi (1996) reported the first in vivo antitumor study of natural oleanane-type triterpenoid AMR. In this study, NMU-induced primary breast carcinomas were subcutaneously transplanted beneath the lower thoracic mammary fat pads of the animals. 50 days after tumor transplantation, the animals were intraperitoneally (i.p.) injected with AMR once every day for 3 weeks. AMR at a dose of 10 or 20 mg/kg/day prolonged the mean survival time of tumor-bearing rats and significantly reduced tumor size.

Administration of AMR-Me at 50 or 100 mg/kg/day for 7 days was found to be inactive in the Ehrlich ascites tumor model in Swiss mice (Rabi et al. 2002). Since AMR-Me exhibited substantial cytotoxic effects against various human breast cancer cells (Rabi et al. 2002; Rabi and Banerjee 2008; Rabi et al. 2013), this compound should be tested against preclinical animal models of breast cancer relevant to human disease. Recently, we have developed a novel method for synthesis of AMR-Me using oleanolic acid as the starting compound (Bishayee et al. 2013). Our custom synthetic scheme eliminates the need for using plant material AMR and hence the dependency to Mother Nature to obtain sufficient quantity of AMR-Me to advance its development as anti-breast cancer drug. Accordingly, we have initiated an extensive research program to investigate mechanism-based chemopreventive effects of AMR-Me using dimethylbenz(*a*)anthracene (DMBA)-induced rat mammary tumorigenesis, a classical animal model that mimics human breast cancer (Malejka-Giganti et al. 2000; Costa et al. 2002). Our results demonstrated that AMR-Me afforded a striking inhibition of DMBA-induced

mammary tumor incidence, total tumor burden, and average tumor weight through suppression of abnormal cell proliferation and induction of apoptosis mediated through regulation of proapoptotic protein Bax and antiapoptotic protein Bcl-2 (Bishayee et al. 2013). We have further reported that AMR-Me downregulated the expression of ER- α , ER- β , and cyclin D1 and disrupted Wnt/ β -catenin signaling during mammary tumorigenesis in rats without any hepatotoxicity and renotoxicity (Mandal et al. 2013a). Very recently, we have provided evidence that AMR-Me downregulated the expression of intratumor COX-2 and heat shock protein 90 (HSP90), suppressed the degradation of inhibitory κ B- α (I κ B- α), and reduced the translocation of NF- κ B from cytosol to nucleus (Mandal et al. 2013b). All these encouraging preclinical results in conjunction with a safety profile should facilitate the clinical development of AMR-Me as breast cancer chemopreventive drug.

Clinical studies

The evidence from preclinical studies set the stage for a few of the novel synthetic oleanane triterpenoids to be moved into clinical trials. As presented in Table 3, several clinical studies have been performed that evaluated anticancer activity of CDDO and CDDO-Me.

A first-in-man phase 1 clinical trial of CDDO was performed by Tsao et al. (2010) with the objective of investigating the in vivo differentiation and proapoptotic effects of CDDO in acute myelogenous leukemia (AML) patients. After 6 days of continuous infusion, PPAR γ mRNA was induced greater than twofold in four patient samples. In the three patients with detected baseline amounts of PPAR γ and DRIP 205 protein, p21 mRNA was also induced by twofold. In one patient, apoptosis induction was observed on day 6 bone marrow cells. All patients did not reach protocol response criteria, differential counts did not significantly change and maximum tolerated dose (MTD) was not reached at the low dose levels in this study. In conclusion, a correlation between PPAR γ levels and apoptosis was not observed in the clinical portion of the study due to very low levels of CDDO. Another phase 1 dose escalation study was conducted to determine the toxicity, maximum tolerated dose,

Table 3 Clinical studies on synthetic oleanane triterpenoids

Human Subjects	Objectives	Findings	Dose	Route	References
Patients with refractory/relapsed AML (9) ^a	To find the correlation of CDDO and PPAR γ on the effects of apoptosis and cell differentiation	No correlation	0.6 - 75.0 mg/m ² /h	Continuous infusion	Tsao et al. (2010)
Patients with advanced solid tumor refractory to standard treatments (7) ^a	To determine MTD for CDDO To determine pharmacokinetics and pharmacodynamics of CDDO	MTD: 1 μ M No antitumor activity	0.6 - 38.4 mg/m ² /h	Continuous infusion	Speranza et al. (2012)
Patients with advanced solid tumors refractory to standard treatments (47) ^a	To determine MTD and dose limiting toxicities for CDDO-Me To characterize pharmacokinetics and pharmacodynamics activity To assess antitumor activity	MTD: 900 mg/d Complete tumor response	5, 50 or 100 mg	Oral	Hong et al. (2012)
Patients with moderate to severe CKD and type 2 diabetes (20) ^a	To assess the effects of CDDO-Me on eGFR To assess safety	Increase in eGFR No serious adverse events	25 or 75 mg	Oral	Pergola et al. (2011a)
Patients with moderate to severe CKD and type 2 diabetes (227) ^a	To estimate the change in eGFR from baseline from CDDO-Me To assess safety	Increase in eGFR maintained throughout 52 weeks Mild adverse events	25, 75 or 150 mg	Oral	Pergola et al. (2011b)

^a The number of human subjects is indicated in the parenthesis

pharmacokinetics, and pharmacodynamics of CDDO in colorectal, bladder, uterine and ovarian cancer patients (Speranza et al. 2012). The observed drug-related adverse effects included thromboembolism, mucositis, nausea, vomiting and anorexia. All patients did develop disease progression and did not continue to receive further treatment of CDDO. No antitumor activity was observed in this study. Hong et al. (2012) completed a phase 1 first-in-human trial on CDDO-Me, also known as bardoxolone methyl, in patients with melanoma as well as colorectal, renal, anaplastic thyroid and other types of cancer. CDDO-Me was administered orally once a day for 21 days for a 28 day cycle with an accelerated titration design used until a grade 2 adverse event occurred. The results of this study showed the dose limiting toxicities to be grade 3 reversible liver transamine elevations and the MTD was established at 900 mg/d.

Pergola et al. (2011a) conducted a study to assess the activity and safety of CDDO-Me in patients with severe chronic kidney disease (CKD) and type 2 diabetes. The results from this study showed a significant increase from the baseline estimated glomerular filtration rate (eGFR). Based on the study, the investigators concluded

that CDDO-Me can safely increase patients' eGFR, and it can be a potential drug for treatment for patients suffering from kidney dysfunction and diabetes. The same investigators (Pergola et al. 2011b) conducted a phase 2 double-blind, randomized, placebo-controlled trial in patients with advanced CKD and type 2 diabetes to assess the long-term effects of CDDO-Me. There were some mild adverse events, with muscle spasms being the most frequent. These results mirrored the results of the previous study showing that the eGFR improved, and added further detailed evidence that CDDO-Me can be a safe and promising future treatment for CKD and diabetes. The bardoxolone methyl evaluation in patients with chronic kidney disease and type 2 diabetes: the occurrence of renal events (BEACON), a multinational, multicenter, double-blind, randomized, placebo-controlled phase 3 trial, was initiated to determine whether long-term administration of CDDO-Me on a background of standard therapy safely reduces renal and cardiac morbidity and mortality. Nevertheless, this trial has been terminated prematurely following a recommendation from the Independent Data Monitoring Committee of the BEACON trial (de Zeeuw et al. 2013).

Conclusion and future directions

Emerging evidence underscores the value of phytochemicals, especially triterpenoids, in the prevention and treatment of various oncologic diseases, including breast cancer. Oleanolic acid, an oleanane-type pentacyclic triterpenoid, has been found to modulate various intracellular targets and signaling pathways and exert anti-breast cancer activities. In order to enhance pharmacological potency, various oleanane triterpenoids have been synthesized using oleanolic acid as a starter compound. Numerous reports published during the last several decades indicate that natural and synthetic oleanane triterpenoids induce cell cycle arrest, apoptosis and immunomodulation, inhibit cellular proliferation, survival, oxidative stress and inflammation, and modulate various signaling pathways in various breast cancer models. All these biological, biochemical and molecular mechanisms could explain why the natural and synthetic oleanane-type compounds diminish the growth of breast cancer cells and prevent the occurrence of mammary tumors.

Overwhelming evidence strongly indicates that plant phytochemicals exert antitumor and cancer preventive effects when they are used in combination rather than individually (Liu 2004; de Kok et al. 2008; Bode and Dong 2009; Ulrich-Merzenich et al. 2009). This is primarily due to the fact that phytotherapy follows a holistic rather than a reductionistic approach (Efferth and Koch 2011). Accordingly, it is very likely that selective combination of oleanane phytochemicals may be effective for multitargeted therapy and even prevention of oncological diseases of the breast. Natural and synthetic oleanane triterpenoids may also be used in combination with currently available anticancer drugs to improve the efficacy and reduce toxicity of standard therapy.

One potential reason of weak efficacy of oleanolic acid and related natural compounds could be limited water solubility affecting bioavailability. Chemical modification of oleanolic acid moiety to synthesize more polar oleanolic acid derivatives may represent one approach to overcome this challenge. Several other techniques to improve the hydrophilicity of oleanolic acid-type compounds may include non-covalent complexes with hydrophilic cyclodextrins and preparation of formulation with liposomes, colloids, micelles as well as nanoparticles.

One of the major challenges in the development of natural molecule-based drugs is the availability of sufficient amount of the bioactive phytochemical. Although oleanolic acid can be extracted from the by-products of the olive industry, obtaining sufficient amount of other oleanane triterpenoids may represent a serious limitation due to low level of these compounds in plant species or presence of lead compounds only in rare plant species (Pollier and Goossens 2012). Innovative biotechnological approaches of biosynthesis of oleanane triterpenoids or their precursors in plant cell cultures could be the most promising and environment friendly option. In particular, these compounds could be obtained in sufficient quantities by expressing the plant biosynthesis genes in heterologous hosts, including yeast (*Saccharomyces cerevisiae*) and *Escherichia coli* (Pollier et al. 2011; Zhang et al. 2011).

Based upon an impressive body of evidence presented here, oleanolic acid, other oleanane triterpenoids and related synthetic compounds show significant promise for the prevention and treatment of breast cancer. Nevertheless, well-designed clinical trials are urgently warranted to evaluate the full potential of these compounds to effectively treat or reduce the risk of human breast cancer.

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