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Review

Ursolic acid: An anti- and pro-inflammatory triterpenoid

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There is growing interest in the elucidation of the biological functions of triterpenoids, ubiquitously distributed throughout the plant kingdom, some of which are used as anticancer and anti-inflammatory agents in Asian countries. Ursolic acid (UA), a natural pentacyclic triterpenoid carboxylic acid, is the major component of some traditional medicine herbs and is well known to possess a wide range of biological functions, such as antioxidative, anti-inflammation, and anticancer activities, that are able to counteract endogenous and exogenous biological stimuli. In contrast to these beneficial properties, some laboratory studies have recently revealed that the effects of UA on normal cells and tissues are occasionally pro-inflammatory. Thus, UA may be designated as a double-edged sword with both positive and negative effects, and further evaluations of the effects of UA on the biological status of target cells or tissues are necessary. This review summarizes previous and current information regarding UA, and provides new insights into the underlying molecular mechanisms of its activities.

Keywords: Anti-inflammation / CD36 / Pro-inflammation / Triterpene / Ursolic acid

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1 Introduction

Plants synthesize an enormous variety of metabolites classified as primary metabolites, that are synthesized for metabolic processes within the plant, as well as secondary metabolites that stimulate ecological interactions between plants and their environment [1]. Isoprenoids, more commonly called terpenoids, are the most functionally and structurally varied group of plant metabolites, and plant-produced terpenoids are essential components of the human diet.

Terpenoids are synthesized in a great number of organisms, and are especially abundant and diverse in plants, with tens of thousands of compounds reported to date [1 – 4]. Further, many are present in all types of plants, where they function as primary metabolites with roles in respiration, photosynthesis, and regulation of growth and development. For example, vitamin A acts as a photoreceptor in animals and is the chromophoric element of a light-driven proton pump in certain bacteria. Carotenoids, such as β-carotene, act as light-protecting pigments in plants and have

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Abbreviations: **B[*a*]P,** benzo[*a*]pyrene; **COX,** cyclooxygenase; **DMAPP,** dimethylallyl diphosphate; **DMBA,** dimethylbenz[*a*]anthracene; **EBV**, Epstein-Barr virus; **EGF**, endothelial growth factor; **ERK**, extracellular signal-regulated kinase; **FGF**, fibroblast growth factor; **FPP**, farnesyl diphosphate; **GA**, glycyrrhetinic acid; **GGPP,** geranylgeranyl diphosphate; **GM-CSF**, granulocyte macrophage colony-stimulating factor; **GPP**, geranyl diphosphate; **H_2O_2**, hydrogen peroxide; **IBD**, inflammatory bowel disease; **IFN**, interferon; **IL**, interleukin; **iNOS**, inducible nitric oxide synthase; **IPP**, isopentenyl diphosphate; **JNK**, c-Jun NH_2-terminal kinase; **LPS**, lipopolysaccharide; **MAPK**, mitogen-activated protein kinase; **MCP**, monocyte chemoattractant protein; **MEP**, methylerythritol 4-phosphate; **MIF**, macrophage migration inhibitory factor; **MIP,** macrophage-inflammatory protein; **MMP**, matrix metalloproteinase; **MVA**, mevalonic acid; **Mϕ**, macrophages; **NF-κB**, nuclear factor-kappa B; **NO**, nitric oxide; **NOX**, NADPH oxidase; **O_2^-**, superoxide anion; **OA**, oleanolic acid; **$^{\bullet}$OH**, hydroxyl radical; **$ONOO^-$**, peroxynitrite; **oxLDL**, oxidized low-density lipoprotein; **PDGF**, platelet-derived growth factor; **PG**, prostaglandin; **pMϕ**, peritoneal M*ϕ*; **RA**, retinoic acid; **RNS**, reactive nitrogen species; **ROS**, reactive oxygen species; **SOD**, superoxide dismutase; **SR**, scavenger receptor; **TBARS**, thiobarbituric acid reactive substances; **TGF**, transforming growth factor; **TNF**, tumor necrosis factor; **TPA,** 12-*O*-tetradecanoylphorbol-13-acetate; **UA**, ursolic acid

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been claimed to have anticancer activities, while steroids, such as cholesterol, are essential components of eukaryotic cell membranes. However, terpenoids with the greatest variety are secondary metabolites that function to protect plants against herbivores and pathogens, as well as to attract pollinators and seed-dispersing animals, while they also act as allelochemicals that influence competition among plant species [1, 2]. In addition, many compounds that are secondary metabolites with a triterpenoid origin have important commercial value and are used as chemotherapeutic agents, such as flavors, pigments, polymers, fibers, glues, waxes, drugs, and agrochemicals [5, 6]. Paclitaxel (Taxol®), a taxane-type diterpene from the yew tree, and the semi-synthetic Taxotere® have rapidly achieved a central position in chemotherapy for malignant tumors, such as ovarian and mammary carcinomas [7]. Genetic engineering appears to be a powerful tool to direct the production of both primary and secondary terpenoids products in plants; however, only limited knowledge of the pathways involved in the biosynthesis of their precursors has been available until very recently.

Triterpenoids are ubiquitously distributed throughout the plant kingdom in the form of free acid or aglycones for saponins, types of triterpenoids [8 – 10], and have long been considered to have a number of biological effects [11]. Presently, more than 80 different triterpenoid structures have been identified as being common in plants, and an exponential increase in the number of reports regarding bioactive triterpenoids in the last decade reflects their growing importance as sources of medications and preventive medicines. In fact, some are increasingly being used for medicinal purposes for a variety of clinical diseases in many Asian countries as antioxidant, anticancer, and anti-inflammatory agents [11, 12].

Ursolic acid (UA; 3β-hydroxy-12-urs-12-en-28-oic acid, Fig. 1), a natural pentacyclic triterpenoid carboxylic acid, was long considered to be biologically inactive [12], whereas in recent years it has attracted considerable interest because of its pharmacological effects combined with low toxicity [13]. Both *in vitro* and *in vivo* studies have demonstrated that UA has many important biological functions [11, 14 – 21], *e. g.*, suppression of the expression of lipopolysaccharide (LPS)-induced pro-inflammatory mediators in RAW264.7 mouse macrophages (Mϕ) [21] and of 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced skin tumor promotion [16]. It has been proposed that these functions are the result of inhibition of nuclear factor-kappa B (NF-κB) activation [21, 22]. Meanwhile, an interesting finding that UA activated NF-κB for releasing pro-inflammatory mediators in non-stimulated mouse Mϕ was recently reported [23]. Thus, it is possible that UA has contrasting anti- and pro-inflammatory activities that are dependent on the biological status of cells and tissues (Fig. 2); however, comprehensive *in vitro* and *in vivo* studies of the action mechanisms of UA are limited. In addition, we recently found that UA has similar effects in non-stimulated mouse Mϕ [24, 25] and skin [26]. This review summarizes previous and current information regarding UA, and provides new insights into its underlying molecular mechanisms.

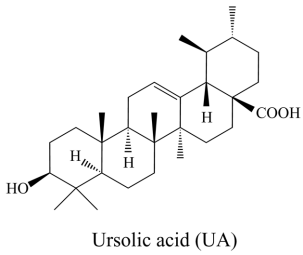

Figure 1. Structure of UA.

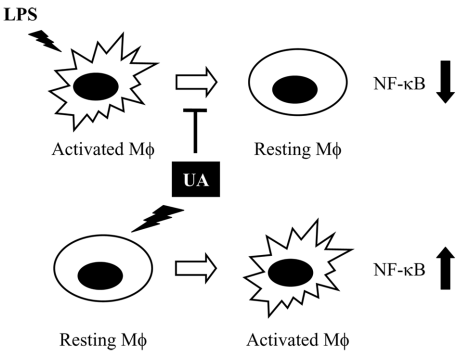

Figure 2. Different responses of RAW264.7 cells to UA. UA attenuates the expression of LPS-induced inflammatory mediators through NF-κB abrogation in activated Mϕ. Further, the compound also induces production of inflammatory mediators *via* NF-κB activation in resting Mϕ.

2 Biosynthesis

Terpenoids are synthesized in all living organisms and recent experimental results have demonstrated two different pathways for them in plants, *via* mevalonic acid (MVA) and methylerythritol 4-phosphate (MEP) (Fig. 3). Biosynthesis of isoprene building units is the first step in the biosynthesis of terpenoids, although this step differs between the two pathways. In the MVA pathway, it has been speculated that isopentenyl diphosphate (IPP) is synthesized from acetyl-CoA and then isomerized to dimethylallyl diphosphate (DMAPP) (Fig. 3) [3, 4]. In contrast, the MEP pathway synthesizes IPP and DMAPP from pyruvate and D-glyceraldehyde 3-phosphate (Fig. 3) [27, 28].

The biosynthesis of triterpenoids and sterol exhibits a common route from IPP and DMAPP to the formation of the C_{30} units squalene and squalene 2,3-oxide (Fig. 3). Interestingly, several experiments using labeled compounds have revealed that cytosolic IPP and DMAPP are preferential precursors for pentacyclic triterpenoids, such as UA,

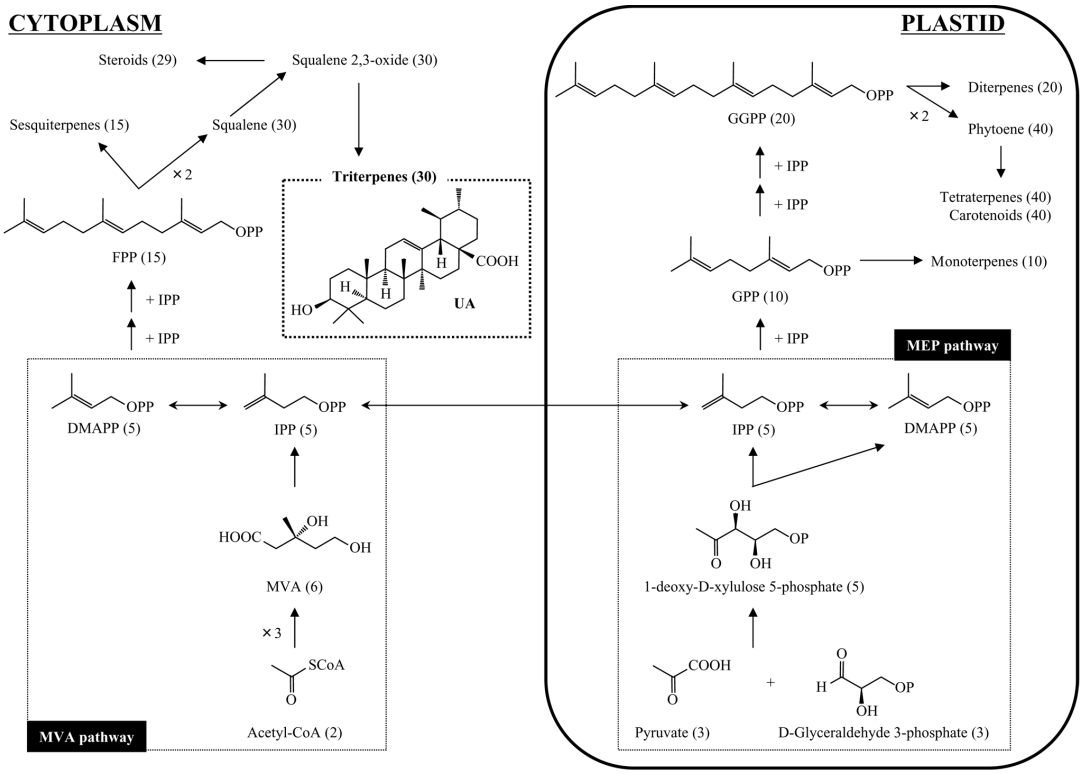

Figure 3. Terpenoid skeleton biosynthesis within plant cells between cytosol (MVA pathway), and plastid (MEP pathway) and UA biosynthesis pathways. Numbers in parentheses indicate the carbon chain number.

and sterol biosynthesis (Fig. 3) [29–32]. In addition, it has been postulated that active biosynthesis of triterpenoids occurs when sterol formation has been satisfied. In contrast, the MEP pathway synthesizes plastid IPP and DMAPP, thus providing the precursors geranyl diphosphate (GPP, C_{10}) and geranylgeranyl diphosphate (GGPP, C_{20}), and ultimately monoterpenes, diterpenes, and tetraterpenes (Fig. 3). Further, crosstalk between these two different IPP biosynthetic pathways has been documented [33–36].

3 Distribution

Interestingly, herbal medicines derived from plant extracts are utilized to treat a wide variety of clinical diseases, although there is relatively little knowledge of their action mechanisms. Of these, triterpenoids are compounds that have attracted considerable interest, based on their remarkable antioxidative, anti-inflammatory, and anticancer biological functions [11, 12]. UA is naturally found in various plants such as fruits and herbs (Table 1) in the form of aglycones or glycosides [8–10], and traditional uses of plants containing UA in folk medicine are abundant. For example, the leaves and twigs of the Labrador tea (*Ledum groenlandicum Retzius*), an Ericaceae widely distributed throughout North America, are used in Native American traditional medicine to treat several inflammatory pathologies such as inflammatory disease [37], asthma [38], rheumatism [39], and diseases of the liver [40] and kidney [38, 41]. Also, thyme (*Thymus*) leaves are utilized as herbal traditional medicine for anti-inflammation in Morocco [42, 43]. Plantain (*Plantago major* L.) leaves have been used as a wound-healing remedy for centuries in nearly all parts of the world as well as for treatment of many other types of diseases, including skin diseases, respiratory organs illness, and cancer [44]. Recently, a number of therapeutic effects of plants containing UA have been confirmed by contemporary scientific research [15, 16, 18, 19, 42–64] and it is thought that some of these effects may contribute to the use of plants that contain it in folk medicine. Table 1 summarizes representative example of plants containing UA and its biological activities.

4 Beneficial effects

4.1 Overview

Increasing evidence suggests that certain phytochemicals have marked anti-inflammatory and cancer chemopreventive properties [65, 66], with UA regarded to be one such compound, as it possesses many biological activities, such as antioxidative, anti-inflammatory, anticancer, and hepato-

Table 1. Representative example of distribution of UA in folk medicines and its pharmacological activities

Plant name	Botanical name	Biological activity	Reference
Apple	*Malus pumila*	Anti-proliferation Anti-cancer	[45]
Basil	*Ocimum basilicum*	Antiviral	[46]
Blueberry	*Vaccinium* spp.	Anti-cancer	[47]
Cranberry	*Vaccinium macrocarpon*	Anti-cancer	[47]
Ground ivy	*Glechoma hederacea* L.	Anti-cancer	[15, 16]
Guava	*Psidium guajava*	Unknown	[48]
Heather flower	*Calluna vulgaris*	Anti-inflammatory	[18]
Japanese cherry	*Prunus serrulata var. spontanea*	Unknown	[49]
Labrador tea	*Ledum groenlandicum Retzius*	Antioxidant Anti-inflammatory Anti-cancer	[50]
Loquat	*Eribotrya japonica* Lindl.	Anti-mutagenic	[51, 52]
Olive	*Olea europaea*	Antioxidant Anti-atherosclerotic Anti-hypertensive	[53, 54]
Oregano	*Origanum vulgare*	Anti-leukemic Antioxidant	[55 – 57]
Persimmon	*Diospyros leucomelas*	Antioxidant Anti-inflammatory	[58, 59]
Plantain	*Plantago major* L.	Antioxidant Anti-inflammatory	[44, 60]
Rosemary	*Rosmarinus officinalis* L.	Anti-inflammatory Anti-cancer	[19, 61, 62]
Sage	*Salvia officinalis* L.	Anti-inflammatory	[63, 64]
Thyme	*Thymus*	Anti-inflammatory	[42, 43]

protective activities, as well as an ability to induce apoptosis. In this section, details of these desirable effects are described.

4.2 Oxidative stress

The predominant sources of reactive oxygen species (ROS) and reactive nitrogen species (RNS), such as superoxide anion (O_2^{-}), hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\bullet}OH$), nitric oxide (NO), and peroxynitrite ($ONOO^{-}$), in sites of inflammation are phagocytes, including neutrophils, monocytes, and Mϕ, with NADPH oxidase (NOX) responsible for O_2^{-} generation [67]. Meanwhile, it is thought that the generation of O_2^{-} from xanthine oxidase, which is mainly expressed in epithelial cells, is lower than that of NOX in inflammatory cells. Although ROS have been recognized to be important signal transduction mediators that regulate gene expression, cell differentiation, immune activation, and apoptosis [68], they are potentially toxic. Thus, inappropriate ROS production *via* NOX damages host tissue [69] and induces pro-inflammatory cytokines, including interleukin (IL)-1β, tumor necrosis factor (TNF)-α, and granulocyte macrophage colony-stimulating factor (GM-CSF) through mechanisms that are dependent on NF-κB [70, 71]. Further, both ROS and RNS are capable of causing oxidative damage to macromolecules, including DNA, protein, RNA, and lipids, as well as others, thereby amplifying the inflammatory response in diseases such as cancer [72, 73], vascular disease [74], inflammatory bowel disease (IBD) [75], asthma [76], and rheumatism [77] (Fig. 4).

In the early 1990s, the antioxidative activities of UA against free radical-induced damage were studied [78, 79]. In these experiments, four standard systems, ascorbic acid, carbon tetrachloride, ADP/iron, and adriamycin, were used to induce lipid peroxidation in isolated rat liver and heart microsomes *in vitro*. Interestingly, UA remarkably reduced the lipid peroxidation by scavenging free radicals. In other experiments, administrations of UA to rats at a dose of 20 mg/kg controlled ethanol-induced oxidative stress in the liver and heart by decreasing lipid peroxidation products [thiobarbituric acid reactive substances (TBARS), lipid hydroperoxides, and conjugated dienes], increasing the activities of antioxidant enzymes [superoxide dismutase (SOD), catalase, glutathione peroxidase, and glutathione *S*-transferase], and increasing the levels of non-enzymic antioxidants (reduced glutathione, ascorbic acid and α-tocopherol) [80, 81]. Additionally, administration of UA (60 mg/kg) significantly reduced the lipid peroxides levels by scavenging free radicals in isoproterenol-induced myocardial ischemia, thereby decreasing TBARS and maintaining the activities of enzymic and non-enzymic antioxidants [82]. UA was also reported to show neuroprotective effects by decreasing oxidative stress [57, 83, 84], and pretreatment with it prevented PC12 nerve cells from ROS toxicity by amyloid β, which increases free radical production and lipid

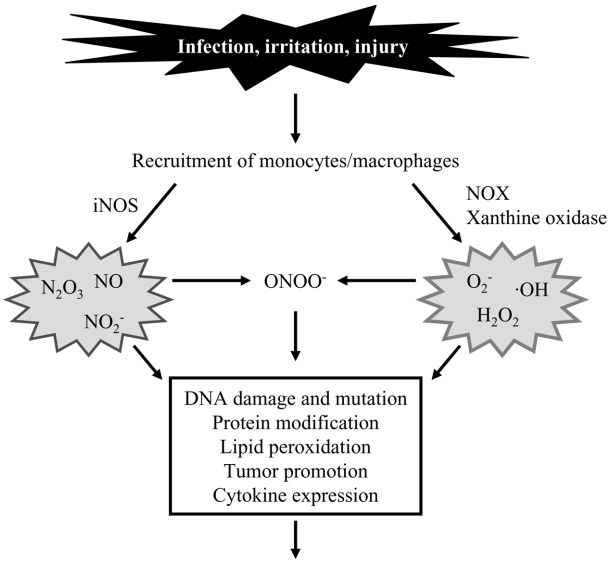

Figure 4. Generation of ROS and RNS from activated monocytes/macrophages is pivotal in the pathogenesis of various diseases.

Table 2. Products released by Mϕ

Enzymes	**Reactive metabolites of oxygen**
Neutral proteases	ROS, RNS
Elastase	
Lysozyme	**Growth factor**
Collagenase	PDGF, EDF, FGF
Plasminogen activator	
Acid hydrolases	**Plasma protein**
Phosphatases	Complement components
Lipases	(*e.g.* C1 to C5, properdin)
	Coagulation factors
Lipid mediators	(*e.g.* factor V, VIII, tissue factor)
Eicosanoids	
	Cytokines and chemokines
	IL-1, IL-6, IL-10, IL-12, IL-15, IL-18, TNF-α, MIF, TGF-β, GM-CSF IL-8, MCP-1, MIP-1

EGF, endothelial growth factor; FGF, fibroblast growth factor; GM-CSF, granulocyte macrophage colony-stimulating factor; IL, interleukin; MCP, monocyte chemoattractant protein; MIF, macrophage migration inhibitory factor; MIP, macrophage-inflammatory protein; Mϕ, macrophages; PDGF, platelet-derived growth factor; RONS, reactive oxygen and nitric species; TGF, transforming growth factor; TNF, tumor necrosis factor.

peroxidation, leading to apoptosis and cell death [57, 85]. The neuroprotective effect of UA (10 mg/kg) against D-galactose-induced neurotoxicity in brain might be caused by the increase in the activity of antioxidant enzymes with a reduction in lipid peroxidation [83]. Further, Shih *et al*. [84] studied the protective effect of UA on the hippocampal neurons against kainate-induced excitotoxicity in rats and proved that free radical scavenging may account, at least partially, for the protective effects of UA. In addition, UA isolated from *Ledum groenlandicum* twigs showed antioxidative activity *in vitro* in ORAC assay results and inhibited *tert*-butylhydroperoxide-induced oxidation of 2′,7′-dichlorofluorescin-diacetate in murine fibrosarcoma L-929 cells [50]. These results suggest that the pharmacological action of UA may offer a novel therapeutic strategy for treatment of oxidative stress-related diseases.

4.3 Inflammation

Inflammation is a complex, highly sequential series of events that is provoked by a variety of stimuli including pathogens, noxious mechanical and chemical agents, and autoimmune responses. The subsequent cascade of events is characterized by such signs and symptoms as redness, swelling, heat, and pain. Inflammation is the primary process through which the body repairs tissue damage and defends itself against stimuli. In physiologic conditions, a regulated response protects against further injury and clears damaged tissue, while in pathologic situations, inflammation can result in tissue destruction and lead to organ dysfunction.

The inflammatory process is usually tightly regulated and involves numerous signals that initiate, maintain, and terminate inflammatory processes. However, when the positive and negative signals become imbalanced, inflammation does not shut down, resulting in cellular and tissue damages. For example, even though inflammatory mediators, such as IL-1β, IL-6, TNF-α, NO, and prostaglandin (PG)E_2, are essential for the host defense system, it is well known that excessive production of these mediators in an inflammatory site may lead to chronic diseases such as cancer [86–88] and neoplasms [89, 90]. Since Mϕ produce a wide range of biologically active molecules, including ROS, RNS, eicosanoids, matrix metalloproteinases (MMPs), inflammatory cytokines and chemokines, and others (Table 2), which participate in both beneficial and detrimental outcomes of inflammation, they are considered to play a key role in chronic inflammatory diseases.

The anti-inflammatory effects of UA on Mϕ have been investigated since the early 1990s, with the earliest report of potential anti-inflammatory effects of UA published in 1992, in which it was isolated from heather flowers (*Calluna vulgaris*) as an inhibitor of arachidonate metabolism [18]. Next, Suh *et al*. [21] showed that pretreatment with UA attenuated the expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX)-2 *via* NF-κB repression in RAW264.7 mouse Mϕ, and Ryu *et al*. [20] demonstrated that UA strongly inhibited NO production in the same cell lines. Similarly, Subbaramaiah *et al*. [91] indicated that treatment with UA suppressed TPA-mediated induction of COX-2 protein and synthesis of PGE_2 in human mammary epithelial cells. In a study conducted to

determine its action mechanism, UA inhibited TPA-mediated activation of protein kinase C, extracellular signal-regulated kinase 1/2 (ERK1/2), c-Jun NH_2-terminal kinase 1/2 (JNK1/2), and p38 mitogen-activated protein kinase (MAPK). In addition, it blocked the binding of activator protein-1 to the COX-2 promoter [91]. These findings are important for understanding the anti-inflammatory properties of UA.

It is evident that carrageenan-induced edema is commonly used as an experimental *in vivo* model for evaluating the anti-inflammatory potentials of plant extracts [92 – 94]. A methanol extract from *Mallotus peltatus* leaves, in which UA is the major compound, exhibited significant anti-inflammatory activity against carrageenan-induced rat paw edema and its potency was comparable to that of the non-steroidal anti-inflammatory drug indomethacin [95]. In addition, UA isolated from *Nepeta sibthorpii Bentham* and *Verbena officinalis* also inhibited carrageenan-induced paw edema in rats by scavenging free radicals [96, 97]. Likewise, UA has been shown to inhibit ear edema production by TPA and croton oil [42, 59, 61, 63, 98, 99]. These anti-inflammatory effects have been attributed to the inhibition of different events of inflammatory reactions, such as histamine release, 5-lypooxygenase, elastase activity, and production of NO and PGE_2 [12, 20, 21, 60, 100].

4.4 Mutagenesis and carcinogenesis

Tumorigenesis is a multi-step process [101] that is induced primarily by various environmental carcinogens (such as cigarette smoke, industrial emissions, and gasoline vapors), inflammatory agents (such as TNF-α and H_2O_2), and tumor promoters (such as TPA and okadaic acid). This multi-step process of carcinogenesis consists of three phases, including tumor initiation, promotion, and progression phages. Extensive research has revealed that regular consumption of certain fruits and vegetables can reduce the risk of several cancers [102].

UA inhibited mutagenicity produced by benzo[*a*]pyrene (B[*a*]P) in bacteria [103], and by aflatoxin B_1 in *Salmonella typhimurium* TA100 and TA98 assay systems [52]. Also, its administration to mice at a dose of 80 mg/kg reduced the number of micro-nucleated polychromatic erythrocytes induced by the mutagen mitomycin C by 76% [104].

Nearly 20 years ago, Koshimizu *et al*. [105] performed screening of antitumor promotion suppressants in edible plants using TPA-induced Epstein-Barr virus (EBV)-associated activation of Raji cells. In this process, they identified UA as an active component of *Glechoma hederacea* L. for inhibiting the tumor-promoting effects of TPA, as well as another tumor promoter, teleocidin B-4 [15, 105, 106], and found that the activity levels were nearly equal to those of retinoic acid (RA) and glycyrrhetinic acid (GA), known inhibitors of tumor promotion [15].

In a two-stage mouse carcinogenesis model, in which dimethylbenz[*a*]anthracene (DMBA) and TPA were used as a tumor initiator and promoter, respectively, topical application of UA (41 nmol) before TPA treatment (4.1 nmol) delayed the formation of papillomas in skin as compared with TPA alone [16]. Further, UA effectively reduced both the percentage of papilloma-bearing mice and the average number of papillomas *per* mouse and the activity was comparable to that of RA [16]. Interestingly, a single application of UA before initial TPA treatment enhanced the activity much more as compared with the case of multiple applications, while RA was ineffective with the same treatment. This fact suggests that the role of UA in inhibiting tumor promotion differs slightly from that of other triterpenoids, and continuous applications of UA might be harmful to mouse skin.

Although the action mechanisms underlying the anti-cancer activities of UA in the skin remain to be fully elucidated, several plausible explanations have been reported. Shishodia *et al*. [22] and Rhew *et al*. [107] indicated that UA inhibits the activation of NF-κB through suppression of the inhibitor of NF-κB and p65 phosphorylation, thereby causing down-regulation of the expression of certain oncogenes, such as COX-2, MMP-9, cyclin D1, c-Jun, and c-Fos. In addition, UA may inhibit skin tumor formation by inhibiting the binding of B[*a*]P and DMBA to epidermal DNA [19], and TPA to the epidermal membrane [108]. Further, it is well known that UA suppresses tumor promoter-induced inflammation [19, 109] and ornithine decarboxylase [5, 19, 110], while it induces differentiation of F9 teratocarcinoma stem cells into endoderm cells [111] and M1 cells into Mϕ-like cells [112]. Also, UA-induced apoptosis of M4Beu melanoma cells may have some correlation to the suppression of tumorigenesis [113].

It should be noted that the use of UA is recommended for skin cancer therapy, and topical cosmetic preparations containing UA have been patented in Japan for the prevention of skin cancer [114, 115]; however, to the best of our knowledge, there are no results from a human study regarding the effect of UA. In addition, a series of novel derivatives of UA, which have significant antioxidative and anti-inflammatory effects [116, 117], are now being utilized for the development of a useful new chemopreventive agent that may be useful for malignancy therapy. However, we have concerns about the risks of UA in humans, since its effects on normal cells and tissues have not been fully addressed, which will be discussed later in this review.

4.5 Apoptosis

Establishment of a natural balance between cell death and cell renewal in mature animals by destruction of excess, damaged, or abnormal cells is important, thus apoptosis is necessary for the maintenance of normal tissue homeostasis. In fact, impaired apoptosis is associated with hyperpro-

liferation and tumorigenesis. UA has been demonstrated to induce apoptosis in many cell types, including HL-60 and K562 human leukemia [118, 119], HT-29 human colon cancer [120], MCF-7 human breast cancer [121, 122], A549 and H460 human lung cancer [123, 124], PC-3 human prostate cancer [125], M4Beu melanoma [113], and HepG2 human hepatoblastoma [126] cell lines. It is believed that the action mechanisms of UA-induced apoptosis are dependent on inhibition of the initiation of DNA replication [126], up-regulation of MMP family gene expression [124], induction of p53 [122], activation of JNK1/2 [119], caspase-3, -8, and -9 [113, 120, 121], and decrease of the expression of bcl-2 family proteins [119, 123, 125].

4.6 Hepatoprotection

UA is well known for its hepatoprotective effects on acute chemically induced liver injury, as well as chronic liver fibrosis and cirrhosis [12, 127 – 129], and it is presently used alone or in combination with other hepatoprotective ingredients as oral medications. The beneficial effects of UA on the liver could be due to its antioxidative and anti-inflammatory effects, as noted above. This triterpenoid is an effective inducer of metallothionein, a small cysteine-rich protein that functions like glutathione in defense against toxic insult in the human body. Induction of the hepatic metallothionein responsible for the detoxification of heavy metals is believed to be one of the hepatoprotective mechanisms of this triterpenoid, although it is not the sole mechanism for protection against chemically induced liver injury [128].

5 Potentially harmful effects

5.1 Overview

As noted in the previous sections, both *in vitro* and *in vivo* experiments have demonstrated that UA has many important biological functions, such as antioxidative, anti-inflammatory, and anticancer activities. Shishodia *et al*. [22] reported that UA inhibited TNF-α-, phorbol ester-, okadaic acid-, H_2O_2-, and cigarette smoke-induced NF-κB activation in various cell lines. In addition, it has been reported that UA attenuated the expression of iNOS and COX-2 [21, 91], and that topical application of UA to mouse skin delayed the formation of TPA-induced papillomas [16]. It has been proposed that these functions, at least in part, are the result of inhibition of NF-κB activation [21, 22]. On the other hand, conflicting experimental results that bring into question these general results were reported in 2001 [23], in which treatment with UA caused enhancement of NO and TNF-α mRNA induction *via* transactivation of NF-κB in non-stimulated RAW264.7 Mϕ. These findings imply that UA has dual effects on NF-κB activation that are dependent upon the biological status of the target Mϕ (Fig. 2), namely, non-stimulated or stimulated. In this section, we discuss the undesirable effects of UA both *in vitro* and *in vivo*, as well as the possible underlying mechanisms.

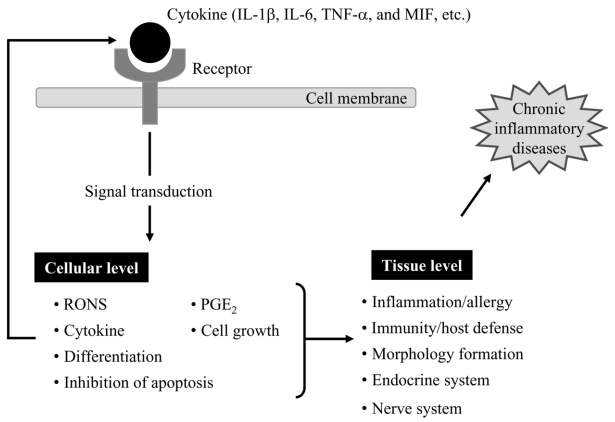

Figure 5. Biological responses to cytokines.

5.2 *In vitro* observations

Prolonged inflammatory responses cause excessive production of cytokines, which leads to activation of not only inflammatory cells but also transcriptional factors. Thus, it is thought that chronic inflammatory diseases might be caused in part by the uncontrolled production of cytokines. Indeed, the release of IL-1β, IL-6, TNF-α, and macrophage migration inhibitory factor (MIF) has been shown to be enhanced in patients with IBD [130, 131]. Further, excessive production of these cytokines has been observed in many cancerous areas, such as skin [132], colon [133], breast [134], gastric [135], and ovarian [136] tissues, indicating that these various disorders are triggered by cytokine release, which is caused by the release of ROS, RNS, and PGs from cytokine-activated inflammatory cells (Fig. 5).

Five years ago, we screened agents that modify the production of MIF, a distinct pro-inflammatory cytokine that functionally inactivates the tumor suppressor protein p53 [137, 138], and has attracted attention as a key molecule involved with linking the processes of inflammation and carcinogenesis. A total of 13 food factors and ten synthetic agents were separately added to interferon (IFN)-γ -treated RAW264.7 Mϕ and then incubated for 12 h, after which MIF protein in media was quantified using an ELISA kit. Although (−)-epigallocatechin gallate (green tea polyphenol), benzylisothiocyanate (cruciferous isothiocyanate), 1400W (iNOS inhibitor), and NS-398 (COX-2 inhibitor) showed significant suppressive effects, IFN-γ-induced MIF protein production was enhanced by pretreatment with UA (Table 3). These findings along with those of a previous report [23] led us to further study the potential pro-inflammatory effects of UA *in vitro* and *in vivo*, as well as the underlying molecular mechanisms.

Table 3. Effects of natural and synthetic compounds on MIF protein releated from IFN-γ-treated RAW264.7 cells

Treatment	MIF (ng/ml)	Inhibition (%)	Cell viability (%)
DMSO	11.6	–	104
IFN-γ	37.1	–	100
Natural compounds			
IFN-γ + (−)-Epigallocatechin-3-gallate (Green tea polyphenol)	18.9	71.2	81.7
+ Capsaicin (Chili peppers capsaicinoid)	27.5	37.7	87.5
+ Benzyl isothiocyanate (Cruciferous isothiocyanate)	27.6	37.2	107
+ Nobiletin (Citrus flavonoid)	31.2	23.3	96.7
+ (−)-Catechin (Green tea polyphenol)	31.3	22.7	87.7
+ Silymarin (Milk Thistle flavonoid)	32.0	19.9	95.0
+ Glutathione (Antioxidant)	36.9	0.91	106
+ Sodium butyrate (histone deacetylase inhibitor)	41.6	−17.5	87.6
+ Rutin (Quercetin-3-rutinoside)	46.0	−34.8	107
+ Ferulic acid (Phenolic compound)	68.9	−125	95.3
+ α-Tocopherol (Vitamin E)	72.0	−137	106
+ 13-HOA (Corn fatty acid)	73.2	−141	91.5
+ Ursolic acid (Pentacyclic triterpenoid)	73.9	−144	84.0
Synthetic compounds			
+ Indomethacin (COX inhibitor)	23.2	54.5	77.0
+ NS-398 (COX-2 inhibitor)	28.5	33.9	86.3
+ 1400W (iNOS inhibitor)	31.5	22.1	85.0
+ SB202474 (Inactive analog of SB203580)	32.9	16.5	97.5
+ Acetylsalicylic acid (COX inhibitor)	35.0	8.1	86.4
+ PD98059 (MEK1 inhibitor)	36.6	2.1	98.4
+ Pentoxifilline (PDE inhibitor)	40.1	−11.9	93.0
+ SB203580 (p38 inhibitor)	44.7	−29.7	101
+ SP600125 (JNK1/2 inhibitor)	45.0	−30.9	102
+ U0126 (MEK1/2 inhibitor)	75.9	−152	94.7

RAW264.7 cells were pretreated with each compound for 30 min before exposure to IFN-γ for 12 h. After then, MIF released into the media was evaluated using ELISA. All compounds were tested at a concentration of 20 μM. COX, cyclooxygenase; IFN, interferon; iNOS, inducible nitric oxide synthase; JNK, c-Jun NH_2-terminal kinase; MEK, mitogen-activated protein kinase/extracellular signal-regulated kinase; PDE, phosphodiesterase; 13-HOA, 13-hydroxy-10-oxo-trans-11-octadecenoic acid.

We initially attempted to investigate the effects of UA on the production of MIF in resting RAW264.7 mouse Mϕ, and found that it decreased intracellular MIF protein levels and promoted the release of MIF into culture media in dose- and time-dependent manners, without affecting mRNA levels [24]. The mechanisms for MIF release by UA appear to be related to the activation of ERK1/2, while the involvement of ERK2 and, to a lesser extent, ERK1 following activation caused by the release of MIF was revealed in experiments using small interfering RNAs. It is conceivable that the UA-triggered activation of ERK2 may also be associated with the activation of NF-κB and the resultant induction of pro-inflammatory mediators [24].

We also considered it worthwhile to examine the effects of this triterpenoid on the production of inflammatory cytokines, such as IL-1β, IL-6, TNF-α, and MIF, in peritoneal Mϕ (pMϕ), which are more appropriate for mimicking *in vivo* situations than established cell lines, because the pro-inflammatory effects of UA have only been demonstrated in resting RAW264.7 cells, an established cell line [23, 24]. UA markedly amplified ATP binding cassette transporter-mediated IL-1β secretion from murine pMϕ at transcriptional, translational, and post-translational levels, presumably through intracellular ROS generation, which resulted in activation of both the ERK1/2 and p38 MAPK pathways and caspase-1 activation [25]. Similarly, this triterpenoid induced IL-6 and MIF, but not TNF-α, protein release, with action mechanisms that may be similar to those seen with IL-1β release, because IL-6 was previously shown to be strongly induced by ROS generation, as well as the ERK1/2, p38 MAPK, and NF-κB signaling pathways [139, 140], and MIF production was induced by ROS-activated ERK1/2 [141]. Interestingly, UA was found to aggregate in culture media and the aggregates were suggested to be responsible for IL-1β production [25]. Meanwhile, members of the scavenger receptor (SR) family, including SR-A, CD36, and CD68, are known to recognize high-molecular weight substances, such as oxidized low-density lipoprotein (oxLDL) and amyloid β [142, 143]. Further, Nishimura *et al*. [144] suggested that oxLDL binding to SRs can generate intracellular ROS production through NOX activation, thereby activating NF-κB. From these results, it is possible that aggregated UA is recognized by SRs. In fact, in experiments using SR neutralization antibodies, a surface plas-

mon resonance assay, and genetically modified mice, we identified CD36 as one of the cell surface receptors [25]. Thus, it is considered that the pro-inflammatory effects of UA are, at least in part, mediated by CD36 signaling. Collectively, the effects of UA shown in experiments with RAW264.7 cells [23, 24] may also be mediated by binding to CD36, because UA binds to the same cell lines [25], in which this SR is expressed in a constitutive manner.

5.3 *In vivo* observations

As noted above, there is ample evidence that UA induces several pro-inflammatory mediators, such as NO, TNF-α, MIF, IL-1β, and IL-6, in resting Mϕ [23–25]. Although these mediators have critical roles in the early stages of skin tumor formation and colon cancer, there is limited information on the effects of UA *in vivo*. Thus, the effects of UA on TPA-induced tumor promotion in mouse skin and mouse colonic mucosa were addressed [25, 26]. Interestingly, topical UA applications to mouse skin at a high dose (1600 nmol) twice a week for 2 wk increased TNF-α, COX-1, and COX-2 mRNA levels, supporting the *in vitro* observations [23–25], although there are also a number of reports showing its antitumor promoting activities [16, 19, 22]. UA-increased pro-inflammatory mediator production is known to contribute greatly to skin tumor promotion. For example, skin-tumor development in TNF-α-deficient mice was delayed, and tumor multiplicity and diameter were dramatically reduced as compared with wild-type mice following its application [132]. The importance of not only COX-2 but also COX-1 in mouse-skin carcinogenesis was extensively investigated by Tiano *et al*. [145], who reported that homozygous COX-1 and COX-2 deficient-mice had tumor numbers reduced by 75% as compared with their wild-type counterparts. Together, increases in TNF-α, COX-1, and COX-2 expression may have a major role in potentiating the development of tumor formation in mouse skin. Surprisingly, although UA-induced pro-inflammatory mediator production is known to contribute greatly to skin tumor promotion, a high dose of UA dramatically reduced DMBA/TPA-induced tumor formation over a period of 20 wk [26]. Although the precise reasons for these contrasting outcomes are not known, they may be due to the following effects. First, repetitive, long-term high-dose applications of UA may lead to accumulation of non-absorbed UA on the skin, thereby preventing TPA from reaching the target tissue, whereas 2 wk of applications may not have been enough to produce such a physical barrier. Second, continuous and high-dose applications of UA may induce apoptosis in DMBA-initiated cells, thereby abolishing papilloma genesis, which is supported by the above-mentioned results of several *in vitro* studies [112, 117–125]. At this moment, although the relationships between the short-term (2 wk) effects of UA and those over the long term (20 wk) are unclear, it should be emphasized that the dose and duration of UA treatment may be conditional determinants of its effects toward mRNA expression of pro-inflammatory mediators and TPA-induced tumor promotion in mouse skin.

On the other hand, intraperitoneal administration of UA at a high dose (200 mg/kg) induced IL-1β production and myeloperoxidase activity in colonic mucosa [25], together with increased IL-1β production in pMϕ [25]. Several studies have shown that IL-1β production was enhanced in human IBD and dextran sulfate sodium-induced colitis murine models [146–149]. In addition, Youngman *et al*. [150] proposed that the major source of IL-1β in the large intestine was chronically activated Mϕ in the mucosal and submucosal layers in the site of colitis, indicating that Mϕ-derived IL-1β may be closely and critically associated with disease pathology. Therefore, it is presumed that UA has the potential to trigger inflammation in the large intestine.

These results suggest that it is necessary to perform more comprehensive evaluations *in vivo*, because the action mechanisms of UA in mouse skin are highly complex and may exhibit undesirable effects on colonic mucosa. Nonetheless, topical cosmetic preparations containing UA are now patented in Japan for the prevention of skin cancer [115].

6 Other functional triterpenoids

6.1 Oleanolic acid (OA, 3β-hydroxy-12-olea-12-en-28-oic acid)

OA (Fig. 6), a UA isomer, widely exists in plants in the form of free acid or aglycones for saponins group of triterpenoids [8–10], and plants that include OA have been used as a drug for longevity as well as a cure-all in East Asian countries since ancient times. Presently, OA has been isolated from more than 120 different plant species [10]. Although we give no details of the biological activities of OA in this review, a pharmaceutical preparation containing OA has been patented for treatment of non-lymphatic leukemia [151] and OA has been shown to have virtually the same biological effects as UA, including antioxidative [78, 79], anti-inflammatory [21, 152, 153], anticancer [15, 16, 103, 105], and antihepatotoxic [12, 127–129] activities.

6.2 Glycyrrhetinic acid (GA, 3β-hydroxy-11-oxoolean-12-en-30-oic acid)

Licorice (*Glycyrrhiza* glabra L.) has been used as an antidote, demulcent, and elixir in folk medicine for generations in China. In both Japan and the United States, it is used as a sweetening and flavoring agent in candy, gums, chocolate candy, cigarettes, liquors, and beer [5, 6]. GA (Fig. 6), the aglycone of glycyrrhizin, a naturally occurring saponin triterpenoid and the major constituent of licorice, is similar in structure to UA and OA, and has been shown to possess sev-

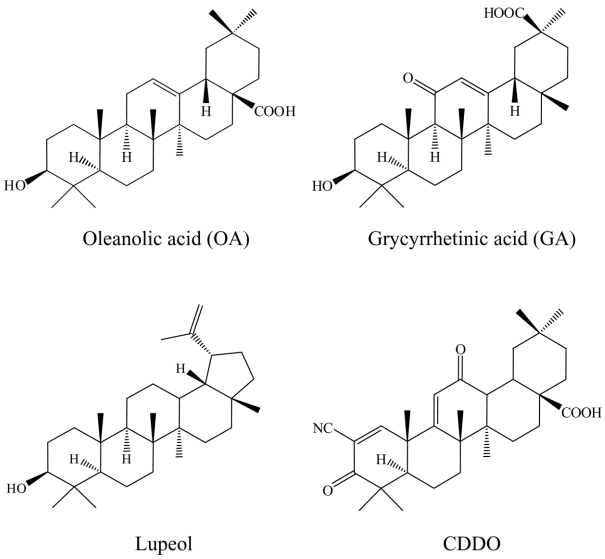

Figure 6. Structures of OA, GA, lupeol, and CDDO.

eral beneficial pharmacological effects, such as anti-inflammatory [154, 155], anticancer [16, 156], and antihepatotoxic [157] activities, as well as inhibition of the growth of melanomas in mice [158]. In fact, GA inhibited paw edema induced by vasoactive agents, such as carrageenan and histamine, in rats [159]. Further, this compound inhibited the mutagenicity of B[*a*]P, 2-aminoflourene and aflatoxin B_1 in *Salmonella typhimurium* TA100 and TA98 assay systems, and was also effective in inhibiting the covalent binding of B[*a*]P to epidermal DNA and tumor initiation by B[*a*]P and DMBA [5].

6.3 Lupeol (Lup-20(29)-en-3β-ol)

Lupeol (Fig. 6) is a naturally occurring triterpene found in various edible plants, such as olive, mango, strawberry, red grape, and fig, as well as medicinal plants, and has been used by native people in North America, Japan, China, Latin America, and the Caribbean islands [160 – 162]. This triterpenoid has exhibited strong antioxidative, anti-inflammatory, anticancer, and antiarthritic activities in both *in vitro* and *in vivo* systems [163], and has also induced Fas-mediated apoptotic death of androgen-sensitive prostate cancer cells and inhibited tumor growth in a xenograft model [164]. Further, lupeol induced apoptotic death of human pancreatic adenocarcinoma cells *via* inhibition of the Ras signaling pathway [165], while it also inhibited paw edema induced by carrageenan in rats [166] and tumor promotion in two-stage mouse skin carcinogenesis by modulating the NF-κB and phosphatidylinositol 3-kinase/AKT pathways [167]. It was also shown that lupeol has a low-density lipoprotein (LDL) protective activity in LDL oxidation studies performed in *in vivo* [168], while another study found that it inhibited lipemic-oxidative stress in experimental hypercholesterolemia [169].

6.4 CDDO (2-cyano-3,12-dioxooleana-1,9-dien-28-oic acid)

Since naturally occurring triterpenoids have relatively weak anti-inflammatory and anticancer activities, a series of novel derivatives of UA, including CDDO (Fig. 6), have been synthesized [116, 170, 171]. CDDO has some important properties, such as anti-inflammatory effects and antiproliferative, differentiating, and apoptosis-inducing activities. In previous studies, CDDO was 400-fold more potent than UA in suppressing the expression of iNOS and COX-2 [117, 171], and it induced apoptosis of human acute myeloid leukemia [172, 173], breast cancer [174], lung cancer [175], osteosarcoma [176], multiple myeloma [177], and chronic lymphocytic leukemia [178] cells. CDDO has also been shown to have several mechanisms of actions including disrupting intracellular redox balance [177], activating p38 MAPK and JNK1/2 [179], up-regulating the expression of cyclin D1 and p21 [174], and inducing apoptosis through both intrinsic and extrinsic pathways [173, 180]. Further, it potently prevented ileitis induced by *Toxoplasma gondii* infection [181] as well as activation of NF-κB by TNF-α and IL-1β [182, 183]. It is noteworthy that several antioxidants that display anti-inflammatory activities also inhibit NF-κB signaling [184 – 186], and a recent report linked the antioxidative activity of CDDO to increased expression of phase II response enzymes, such as heme oxygenase 1 and SOD, *via* activation of nuclear factor E2-related factor 2 signaling, suggesting a plausible mechanism for the anti-

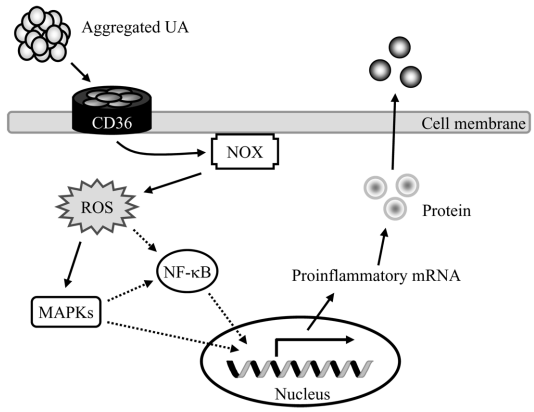

Figure 7. Proposed molecular mechanisms by which UA induces pro-inflammatory cytokines in murine Mϕ. Aggregated UA is recognized by CD36 and then ROS are intracellularly generated, presumably by NOX. This process triggers the activation of MAPK pathways and NF-κB for promoting transcription of pro-inflammatory mediator (such as iNOS, TNF-α, and IL-1β) genes, leading to the expression of pro-inflammatory mediators for intracellular protein production. Then, intracellular proteins are released and exhibit their biological functions.

Table 4. Representative biological effects of UA

Effects	Proposed mechanisms [Ref]	Experimental systems
Antioxidation		
	ROS ↓ [50]	Fibrosarcoma cells
	ROS ↓, Antioxidant enzymes ↑ [57]	Nerve cells
	Lipid peroxidation production ↓ [80 – 82]	Liver & Heart
	Antioxidant enzymes ↑ [80, 82]	
	Non-enzymic antioxidants ↑ [80-82]	
	Antioxidant enzymes ↑ [83,84]	Brain
Anti-inflammation		
	Lipoxygenase and COX ↓ [18]	Leukemic cells
	NO ↓ [20]	Macrophages
	iNOS ↓, COX-2 ↓, NF-κB ↓ [21]	
	COX-2 ↓, PGE2 ↓ [91]	Mammary epithelial cells
	Edema ↓ [42, 59, 61, 63, 95 – 99]	Paw & Ear
Anti-cancer		
	EBV activation ↓ [15, 105, 106]	Burkitt's lymphoma cells
	Apoptosis ↑ [113]	Melanoma cells
	DNA binding of NF-κB ↓ [22, 107]	Skin
	DNA binding of tumor initiator ↓ [19]	
	Epidermal membrane binding of TPA ↓ [108]	
	Tumor promotion by TPA ↓ [16]	
	Inflammation by TPA ↓ [19, 109]	
	Ornithine decarboxylase ↓ [5, 19, 110]	
Pro-inflammation		
	NO ↑, TNF-α ↑, NF-κB ↑ [23]	Marcophages
	MIF ↑, ERK2 ↑ [24]	
	IL-1β ↑, IL-6 ↑, MIF ↑, ROS ↑, ERK1/2 ↑, p38 MAPK ↑ [25]	
	TNF-α ↑, COX-1 ↑, COX-2 ↑ [26]	Skin
	IL-1β ↑, Myeloperoxidase ↑ [25]	Colonic mucosa

↑: enhanced, ↓: suppressed
COX, cyclooxygenase; EBV, Epstein-Barr virus; ERK, extracellular signal-regulated kinase; IL, interleukin; iNOS, inducible nitric oxide synthase; MAPK, mitogen-activated protein kinase; MIF, macrophage migration inhibitory factor; NF-κB, nuclear factor-kappa B; NO, nitric oxide; PG, prostaglandin; ROS, reactive oxygen species; TNF, tumor necrosis factor; TPA, 12-*O*-tetradechanoylphorbol-13-acetate

inflammatory effects of this agent [187]. This compound is now being utilized in the development of a useful new chemopreventive agent that may be used in the future for malignancy therapy.

7 Conclusions and perspectives

Fruits and vegetables are recommended for the prevention of cancer and other disease. However, their action mechanisms are poorly understood. Extensive research during the last quarter century has identified UA from several plants (Table 1) as an antioxidative, anti-inflammatory, and anticancer activity agent (Table 4). Therefore, the use of UA is recommended for skin cancer therapy, and topical cosmetic preparations containing UA have been patented in Japan for the prevention of skin cancer, although, to the best of our knowledge, there are no results from a human study regarding the effect of UA. In addition, CDDO, a derivative of UA, is being utilized for the development of a useful new chemopreventive agent for malignancy. However, undesirable effects of UA, which may be partially mediated by CD36, are now emerging from both *in vitro* and *in vivo* experiments conducted over the past 5 years (Fig. 7, Table 4). Currently, it is considered that the effects of UA on normal cells and tissues are occasionally pro-inflammatory, although UA is able to counteract endogenous and exogenous inflammatory stimuli. Because comprehensive studies of the action mechanisms of UA in resting Mϕ and tissues are limited, additional extensive investigations regarding the desirable or undesirable effect of UA on normal tissue and human are required to determine the risks and benefits of this triterpene. Such approaches will likely show the diversity of the physiological effects and underly-

ing action mechanisms of functional phytochemicals, including UA, and help to develop cosmetics as well as functional foods with additional safety.

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