

Anticancer effect of ursolic acid via mitochondria-dependent pathways (Review)

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Abstract. Ursolic acid is a plant-derived pentacyclic triterpenoid found in various medicinal herbs and fruits. It has generated clinical interest due to its anti-inflammatory, antioxidative, antiapoptotic and anticarcinogenic effects. An increasing amount of evidence supports the anticancer effect of ursolic acid in various cancer cells. One of the hallmarks of malignant transformation is metabolic reprogramming that sustains macromolecule synthesis, bioenergetic demand and tumor cell survival. Mitochondria are important regulators of tumorigenesis as well as a major site of the metabolic reactions that facilitate this reprogramming and adaptation to cellular and environmental changes. The current review explored the close association between the anticancer effect of ursolic acid and the activation of mitochondrial-dependent signaling pathways.

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1. Introduction

Aerobic glycolysis in cancer cells, also referred to as the 'Warburg effect', was first observed and described by Warburg

90 years ago (1,2). The Warburg effect suggests that tumor cells produce excessive amount of lactate in the presence of oxygen (3). This effect has been studied extensively improving the understanding of various metabolic characteristics of tumor cells (4-6). Elucidating the mechanisms underlying the expression levels and/or activity of key catalytic enzymes involved in tumor cell metabolic pathways may improve cancer diagnosis and treatment.

Normal cells can metabolize glucose to produce pyruvate by the process of glycolysis, which occurs in the cytoplasm. The tricarboxylic acid cycle oxidizes the majority of glycolysis substrates into pyruvate and carbon dioxide. Oxygen and the mitochondrial electron transport chain are required to generate an electrochemical gradient and facilitate adenosine triphosphate (ATP) production (7). The metabolic abnormalities of tumor cells are manifested when aerobic glycolysis is severely altered (6). The tumor-associated changes in glycolysis include weakened oxidative phosphorylation, accelerated pentose phosphate metabolic pathway, activated glutamine catabolism, fatty acid *de novo* synthesis and β -oxidations (8). In order to meet the increased demand of tumor cells for energy and substance anabolism, metabolic reprogramming redefines and directs the flow and flux of nutrients to the metabolic network of tumor cells (3). Much of this reprogramming depends on mitochondria as functional biosynthetic organelles. The metabolic signatures of cancer cells are not restricted to passive responses to damaged mitochondria, but result from oncogene-directed metabolic reprogramming, which is required for support of anabolic growth (8).

Current cancer research focuses on targeting cancer-associated defects in apoptosis. Mitochondria are not only the major center of cell respiration and oxidative phosphorylation, but also the control center of apoptosis. Apoptosis is regulated by two major mechanisms. The extrinsic pathway, or death-receptor pathway, is mediated by the transduction of extracellular death receptor ligand signaling (9). The intrinsic pathway, also referred to as the mitochondrial pathway, is governed by a specific mitochondrion-localized signaling cascade. The activation of the mitochondrial death pathway includes changes in mitochondrial outer membrane permeabilization, membrane potential ($\Delta\Psi_m$) collapse, assembly of the permeability pore complex and the activation of pro-apoptotic B-cell lymphoma 2 (Bcl-2) family members apoptotic regulator BAX (Bax) and Bcl-2 homologous antagonist/killer (10).

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The anti-apoptotic Bcl-2 family members, including Bcl-2 and B-cell lymphoma-extra large, mediate signaling in normal physiology to prevent cell death (11). Pro-apoptotic proteins oligomerize at the outer membrane of the mitochondria to mediate mitochondrial outer membrane permeabilization that works in tandem with the voltage dependent anion channel (VDAC) and adenine nucleus translocator (ANT) protein (10). This complex activation of different target proteins results in pore formation and the release of cytochrome C from mitochondria into the cytosol (12). Cytochrome C activates caspases, the executors of programmed cell death which trigger a caspase cascade reaction cleaving >100 substrates in cells and leading to cell apoptosis (12,13).

Selective targeting of cancer metabolism and apoptosis-associated signaling may provide an alternative strategy for the development of anticancer drugs that have minimal adverse effects on normal cells. The current review focuses on the role of ursolic acid (UA) as a potential anticancer drug that influences mitochondrial function.

2. Structure and function of ursolic acid

UA is a pentacyclic triterpenoid (14). UA-associated compounds include oleanolic acid, betulinic acid, uvaol and α - and β -amyrin (14). Triterpenoids have been used as ingredients of herb extracts employed in traditional medicine. The presence of UA has been confirmed in numerous classes of medicinal plants including the peels of the orchard apple (*Malus domestica*), marjoram (*Origanum majorana*) leaves, oregano (*Origanum vulgare*) leaves, rosemary (*Rosmarinus officinalis*) leaves, sage (*Salvia officinalis*) leaves, thyme (*Thymus vulgaris*) leaves, lavender (*Lavandula angustifolia*) leaves and flowers, eucalyptus (*Eucalyptus*) leaves and bark, black elder (*Sambucus nigra*) leaves and bark, hawthorn (*Crataegus* spp.) leaves and flowers, coffee (*Coffea arabica*) leaves as well as the wax layer of numerous edible fruits (15-17). The chemical structure of UA (β -hydroxy-urs-12-en-28-oic-acid) is presented in Fig. 1. UA has the molecular formula $C_{30}H_{48}O_3$, a molecular weight of 456.70032 g/mol and a melting point of 283-285°C. UA can be dissolved in methanol, pyridine and acetone, but is insoluble in water and petroleum ether (18).

UA and its derivatives exhibit potent biological and pharmaceutical effects (18). The anti-inflammatory effect of UA was linked to attenuation of production of proinflammatory cytokines including tumor necrosis factor α , interleukin (IL)-6 and/or IL-17 (19,20). UA was associated with suppression of the nuclear factor- κ B (NF- κ B) pathway, inhibition of expression of cyclooxygenase-2 (COX-2) and nitric oxide synthase and the reduction of perhydrides including nitric oxide and hydrogen peroxide (21). Furthermore, UA demonstrated an antidiabetic function and inhibited the activity of pancreatic α -amylase, succinate dehydrogenase, and glucose-6-phosphatase and aldose reductase (22). UA also induced fatty acid synthetase activity and glucokinase activity, and upregulated glucose transporter (GLUT) 2 mRNA levels, thereby reducing the blood glucose levels of diabetic mice (22-25).

UA stimulated an increase in the level of plasma total cholesterol, low-density lipoprotein cholesterol, very low-density lipoprotein cholesterol, free fatty acids and phospholipids in high fat diet-fed mice or rabbits (26). By

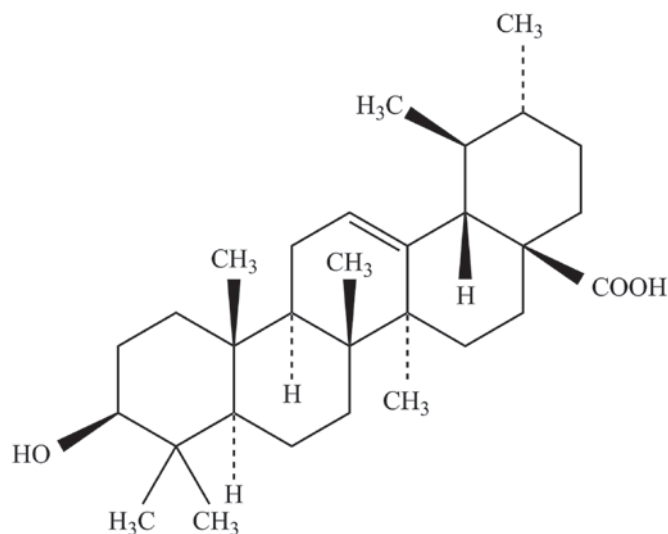


Figure 1. Structure of ursolic acid.

contrast, UA reduced the expression of sterol regulatory element binding protein 1c, Fas cell surface death receptor, acetyl-coenzyme A carboxylase and carnitine palmitoyltransferase-1 (27). UA induced the phosphorylation of adenosine 5'-monophosphate (AMP)-activated protein kinase (AMPK) and stimulated expression of sirtuin 1, thus serving an anti-hyperlipidemic role (26). UA can provide hepatoprotective activity against several liver diseases, including fatty liver, liver fibrosis, hepatocellular carcinoma and other types of liver cancer by influencing multiple metabolic factors (28). For instance, UA reduced the serum/plasma levels of alanine transaminase and aspartate transaminase, which are liver disease biomarkers (27,29,30).

Numerous studies in rodents and humans have investigated the beneficial effects of UA that targeted cancer cell metabolism. Data demonstrated that UA inhibited tumorigenesis and cancer cell proliferation, modulated apoptosis and cell cycle progression and promoted autophagy (18,31-36). Luo *et al* (37) reported that treatment with UA inhibited the viability and migration of T47D, MCF-7 and MDA-MB-231 breast cancer cells by targeting phosphoinositide-3-kinase/protein kinase B (PI3K/Akt)-regulated glycogen synthase kinase 3 β phosphorylation levels and caspase-3 activation via the NF- κ B signaling pathway. Lewinska *et al* (38) analyzed the effects of low doses of UA in breast cancer cell lines with different hormone and growth factor receptor status. The authors revealed that UA promoted autophagy, apoptosis and induced gap (G)0/G1 cell cycle arrest. Additionally, UA affected glycolysis. The effect was accompanied by decreased levels of ATP, lactate, hexokinase 2 and pyruvate kinase. It was suggested that these effects were mediated by Akt-5'-AMP-activated protein kinase signals, activation of phospho-extracellular signal-regulated kinases1/2 and/or by the oxidative stress pathway. Yeh *et al* (39) revealed that UA suppressed the migration and metastasis of the MDA-MB-231 breast cancer cell line by modulating c-Jun N-terminal kinase (JNK), Akt and mechanistic target of rapamycin mTOR signaling. UA may down-regulate the expression of COX-2 (40,41). The effect has been observed in various types of cancer cells and was directly proportional to

tumor aggressiveness and metastasis in gastric cancer SGC7901 cells (34) and hepatic cancer HepG2 cells (42). UA upregulated COX-2 in colorectal cancer HT-29 and prostate cancer DU145 cells (43). Furthermore, it was reported that treatment with UA suppressed the metastasis of HeLa cells, fibrosarcoma HT1080 cells and C6 glioma cells through the downregulation of matrix metalloproteinase 9 (33,36,44). Furthermore, it has been indicated that UA induced pro-apoptotic signaling in human liver cancer cell lines as well as gastric cancer cell lines, including HepG2, Hep3B, Huh7, AGS, BGC823 and SGC7901 (39,45-48). UA significantly enhanced proapoptotic effects and stimulated mitochondrial dysfunction by activating caspases 3, 8 and 9, and downregulated Bcl-2 expression in these cancer cells.

3. Anticancer effect of ursolic acid via mitochondrial energy metabolism

Mitochondria are involved in oxidative phosphorylation and ATP formation in cells; furthermore, 95% of the energy required for cellular activity is generated in these organelles (49). The regulation of mitochondrial metabolism is associated with the activities of key enzymes in different energy-linked metabolic pathways (50).

Hexokinases (HKs) are irreversible, rate-limiting enzymes in the first step of glycolysis (7). HKs catalyze the conversion of glucose to glucose-6-phosphate with concomitant dephosphorylation of ATP (8,51). Among the four known HK isoforms, HK2 has the greatest association with tumors (52). It is strategically located on the mitochondrial outer membrane, interacts with the VDAC and provides preferential access to the mitochondrial ATP via ANT in the mitochondrial inner membrane (53,54). This interaction results in a shift in the susceptibility of mitochondria to proapoptotic signals that are mediated through Bcl-2-family proteins. The upregulation, or increased activity of HK accelerate glycolysis in tumor cells and increase their energy metabolism. The enhancement of glycolysis increases the production of lactate, which acidifies the tumor microenvironment (53). The acidified extracellular fluid decomposes and destroys the cell matrix, thus facilitating the invasion of tumor cells into surrounding tissues (8).

Several studies indicated that UA may modulate the expression and function of mitochondria-associated enzymes, resulting in antiproliferative and apoptosis-promoting effects in various experimental cancer models *in vitro* and *in vivo* (55-59). Lewinska *et al* (38) demonstrated that UA downregulated the Akt signaling in three breast cancer cell lines with different phenotypes including MCF-7 estrogen receptor (ER) positive; progesterone receptor (PR) positive or negative; human epidermal growth factor receptor 2 (HER2) negative, MDA-MB-231 (ER negative; PR negative; HER2 negative) and SK-BR-3 (ER negative; PR negative; HER2 positive) cells. The Akt inhibition affected glycolysis and markedly decreased levels of HK2, pyruvate kinase M2, ATP and lactate.

Wang *et al* (60) synthesized a series of UA diamine derivatives to evaluate their biological activities. The authors demonstrated that the carbon chains of the modified UA derivatives competed strongly with glucose for binding sites in glucokinase (GK). Furthermore, the combination

of 2-deoxy-D-glucose (2-DG) and UA derivative US597 (UA-4) induced cell cycle arrest at the G2/mitotic (M) phase, promoted caspase-dependent cell death, reduced HK activity, aggravated depletion of intracellular ATP, decreased lactate production and synergistically inhibited cancer cell growth *in vitro* (HepG2) and *in vivo* (H22). Another study indicated that the UA derivative UP12 could bind to the active sites of GK, GLUT1 and ATPase in hepatocellular carcinoma (61). Combined with 2-DG, UP12 depleted intracellular ATP, decreased lactate production and arrested an increased number of cancer cells at the synthesis and G2/M cell cycle phases (61).

4. Anticancer effect of ursolic acid via reduction of mitochondrial oxidative stress

In addition to energy production, mitochondria are involved in the regulation of a number of processes including the generation of reactive oxygen species (ROS), redox molecules and metabolites (7). The majority of these by-products are removed by free radical scavenger molecules, including superoxide dismutase (SOD), in order to maintain balance under normal physiological conditions and counteract initiation of cell death (62). Oxidative stress is induced when the functional domain of mitochondria is exposed to high concentrations of ROS (63). Mild oxidative stress often has protective effects including promoting cell survival, proliferation and differentiation. However, severe oxidative stress causes irreversible damage, decreases proliferation, and induces aging, apoptosis and necrosis (64). In cancer cells, mitochondrial ROS amplify the tumorigenic phenotype and accelerate the accumulation of additional mutations that lead to metastatic behavior (65). Targeting of mitochondrial metabolism that contributes to redox regulation presents a promising avenue for future anticancer therapy (66,67).

Synthesized UA derivatives 5, 17 and 23 inhibited cell growth in the human bladder cancer cell line NTUB1 (68). Derivative 17 significantly increased the production of ROS for 24 h, while 5 and 23 did so for 48 h. Wu *et al* (32) evaluated ROS generation in osteosarcoma U-2 and MG-63 cells exposed to a combination of UA and zoledronic acid (ZOL) using the fluorescent dye hydroethidine. The results indicated that the UA/ZOL combination increased intracellular ROS levels more effectively than either of the compounds alone. Furthermore, N-acetyl-L-cysteine, a ROS scavenger, suppressed this effect, and significantly reduced the apoptosis induced by the UA/ZOL combination. Mitochondrial oxidative damage is further aggravated by accumulation of high ROS level. A high level of ROS inhibited the activity of respiratory enzymes and electron transport through the respiratory chain (67). ROS adversely impacted oxidase and antioxidant enzyme activity, stimulated depolarization of mitochondrial membrane potential and facilitated 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine pore opening (66). These actions increased mitochondrial membrane permeability and resulted in changes in calcium metabolism, leading to mitochondrial morphological destruction, loss of mitochondrial function and cell death (66).

In the human breast cancer cell line MDA-MB-231, UA decreased the mitochondrial $\Delta\Psi_m$, demonstrated by affecting the level of 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolyl-

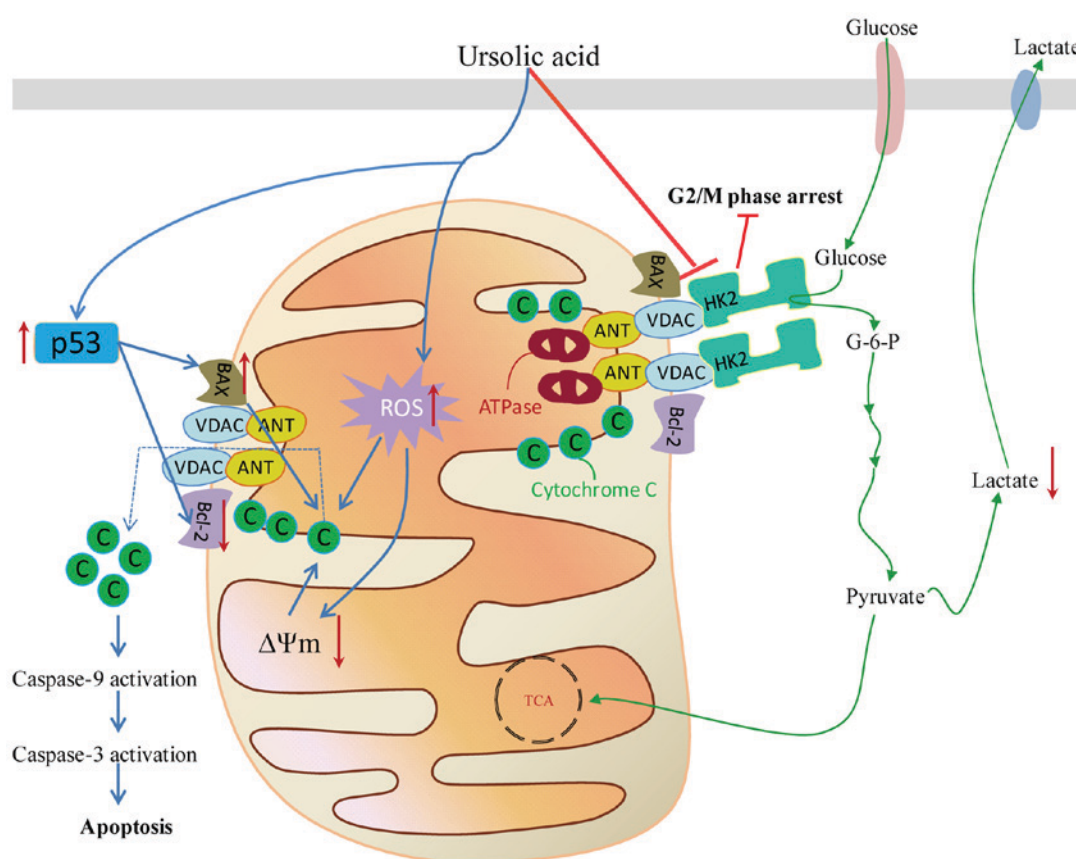


Figure 2. Schematic presentation of the antiproliferative effect of the UA targeting the mitochondrial pathway in cancer cells, including the associated metabolic enzymes, the promotion of ROS production and accumulation in mitochondria under stress conditions, destabilization of mitochondrial membrane potential and activation of the p53 pathway to decrease proliferation or promote apoptosis. G2, gap 2; M, mitosis; G-6-P, glucose 6-phosphate; HK2, hexokinase 2; Bcl-2, B-cell lymphoma 2; VDAC, voltage dependent anion channel; ANT, adenine nucleus translocator; ATP, adenosine triphosphate; ROS, reactive oxygen species; C, cytochrome C; TCA, tricarboxylic acid; $\Delta\Psi_m$, membrane potential.

carbocyanine iodide (JC-1) which gathered in the mitochondrial matrix to form aggregates (69). Another study also revealed that after 24 h of treatment with 15 μM of UA, human melanoma M4Beu cells had a low mitochondrial membrane potential, identified by staining with JC-1/TOTO-3 iodide. Therefore, UA may participate in ROS-mediated oxidative stress, activate metabolic disturbances in mitochondria in cancer cells and induce proapoptotic cascade reactions (55). The detailed mechanisms of the anticancer effects of UA require further research.

5. Anticancer effect of ursolic acid mediated by the p53-modulated mitochondrial pathway

Inactivation of tumor suppressor genes is an important initiating factor for tumor reprogramming. One of the established tumor suppressors, p53 protein, encoded by human tumor protein p53 (TP53) gene, controls tumorigenesis through various cellular mechanisms including DNA damage repair, cell cycle regulation and cell death (70). According to the Cancer Gene Census mutation database, TP53 is the most frequently mutated gene (36.1%) in different types of cancer (71). p53 serves an important role in modulating the balance between mitochondrial respiration and glycolysis (70). Matoba *et al* (72) reported that p53 regulated the expression of the synthesis of cytochrome C oxidase 2 (SCO2) protein, which is required for regulating the cytochrome C oxidase complex. The authors revealed that

oxygen consumption was reduced in the liver mitochondria of p53^{-/-} knockout mice, as well as in p53^{-/-}HCT116 deficient cells compared with the wild type. In addition, lactate production was increased in p53^{-/-}knockout cells. Further study verified that SCO2 links p53 to mitochondrial respiration, providing a possible explanation for the Warburg effect (73). In addition to regulating SCO2 transactivation and expression, p53 regulates mitochondrial biogenesis genes, including mitochondrial transcription factor A and apoptosis-inducing factor which encode for major apoptosis-affecting proteins involved in complex 1 assembly (72). p53 regulates expression of ferredoxin reductase protein responsible for the maturation of iron-sulfur proteins essential in electron transfer reactions. p53 also modulates fatty acid metabolism through sphingosine kinase 1 and lipin 1, which can promote cell growth and mediate nutritional and genotoxic stress signals (74).

p53 may induce growth arrest or programmed cell death through transcriptional activity. Nam and Kim (75) reported that treatment with UA in human colon adenocarcinoma SW480 cells significantly increased the expression level and transcriptional activity of p53, as well as that of NF- κ B and Bax. UA also enhanced p21 transcriptional activity and induced caspase3-dependent apoptosis (76). Manu and Kuttan (77) suggested that the activation of p53 by treatment with UA mediated activation of proapoptotic pathways in B16F-10 melanoma cells. Yu *et al* (78) demonstrated that treatment with UA induced

cycle arrest and apoptosis in the human hepatoma cell line SMMC-7721. The authors also verified that the proapoptotic and regulatory proteins p53 and Bax were upregulated while the antiapoptotic protein Bcl-2 was downregulated following treatment with UA. Furthermore, the mRNA level of growth differentiation factor 15, SOD2 and activating transcription factor 3 were increased, while Fos levels were decreased. The p53 inhibitor pifithrin- α blocked these effects, supporting the role of p53. The aforementioned studies provide a preliminary interpretation of UA signaling via activation of the p53 pathway and the induction of tumor cell apoptosis. However, p53 can regulate the expression of a variety of metabolism-associated enzymes in mitochondria and, thus, its role may not be limited to the induction of apoptosis (78). The metabolic mechanism of the anticancer effect of UA via the p53 pathway requires further investigation.

6. Conclusions and perspectives

Reprogrammed energy metabolism is an emerging hallmark of cancer phenotyping. In the search for new anticancer agents, phytochemicals have drawn an increasing amount of attention. Clinicians are searching for natural drugs with a high efficiency, which are less toxic to normal cells compared with conventional treatments. Mitochondrial metabolism was investigated as a target for cancer therapy, owing to the reemergence of mitochondria as central metabolic organelles adversely affected by tumorigenesis. Numerous studies suggest that UA regulates mitochondrial function through the activation of multiple pathways. UA regulates the expression of associated metabolic enzymes, decreases tumor proliferation, promotes ROS production and accumulation in mitochondria under stress conditions, destabilizes mitochondrial membrane potential, activates the p53 pathway and promotes apoptosis in various types of cancer cells (Fig. 2). Further research investigating the cellular and molecular mechanisms underlying the effects of UA is required for the development of new therapeutic agents.

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Availability of data and materials

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Authors' contributions

XMF conducted the literature review and wrote the manuscript. XLS was involved in drafting and revising the manuscript. Both authors read and approved the final manuscript.

Ethics approval and consent to participate

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Competing interests

The authors declare that they have no competing interests.

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