Advances in the pharmacological activities and mechanisms of diosgenin

CHEN Yan, TANG You-Mei, YU Su-Lan, HAN Yu-Wei, KOU Jun-Ping*, LIU Bao-Lin, YU Bo-Yang

Jiangsu Key Laboratory of TCM Evaluation and Translational Research, Department of Complex Prescription of TCM, China Pharmaceutical University, Nanjing 211198, China

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[ABSTRACT] Diosgenin, a well-known steroid sapogenin derived from plants, has been used as a starting material for production of steroidal hormones. The present review will summarize published literature concerning pharmacological potential of diosgenin, and the underlying mechanisms of actions. Diosgenin has shown a vast range of pharmacological activities in preclinical studies. It exhibits antitumor, cardiovascular protective, anti-diabetes, neuroprotective, immunomodulatory, estrogenic, and skin protective effects, mainly by inducing apoptosis, suppressing malignant transformation, decreasing oxidative stress, preventing inflammatory events, promoting cellular differentiation/proliferation, and regulating T-cell immune response, etc. It interferes with cell death pathways and their regulators to induce apoptosis. Diosgenin antagonizes tumor metastasis by modulating epithelial-mesenchymal transition and actin cytoskeleton to change cellular motility, suppressing degradation of matrix barrier, and inhibiting angiogenesis. Additionally, diosgenin improves antioxidant status and inhibits lipid peroxidation. Its anti-inflammatory activity is through inhibiting production of pro-inflammatory cytokines, enzymes and adhesion molecules. Furthermore, diosgenin drives cellular growth/differentiation through the estrogen receptor (ER) cascade and transcriptional factor PPARγ. In summary, these mechanistic studies provide a basis for further development of this compound for pharmacotherapy of various diseases.

[KEY WORDS] Diosgenin; Steroid sapogenin; Pharmacological activities; Multiple targets

[CLC Number] Q5

Introduction

Plants provide an extensive reservoir of natural products used as a primary source of medicine throughout the history of civilization. Historical experiences with plants have led to discoveries of many important drugs such as quinine, morphine, paclitaxel, and camptothecin. Saponins, classified into triterpenes and steroids based on their carbon skeletons, are common in a variety of higher plants and possess a wide range of health beneficial properties. The steroidal saponins are mainly found in Agavaceae, Dioscoreaceae, Liliaceae, Solanaceae, Scrophulariaceae, Amaryllidaceae, Leguminosae, and Rhamnaceae. Diosgenin (Fig. 1), firstly discovered by Fujii and Matsukawa in Dioscorea Tokoro Makino in 1935, is a naturally occurring steroidal sapogenin predominantly present in yams, fenugreek, and Costus speciosus. In the pharmaceutical industry, diosgenin is the principal precursor compound in the manufacture of synthetic steroids. Studies in the last few decades have demonstrated that diosgenin has preventive/therapeutic effects not only against several cancers, but also other diseases such as cardiovascular disorders, type 2 diabetes, neurodegenerative disorders, allergic diseases, menopausal symptoms, and skin aging. In this brief review, we focused mainly on the recent progress made in the investigations of pharmacological activities of diosgenin and underlying mechanisms of actions. Some parts of this article have already been reported in previous reviews and briefly represented here for the completeness of this review.
Pharmacological Activities and Mechanisms of Actions

Anticancer effects

It has been reported that diosgenin affects various phases of tumorigenesis including tumor cell proliferation, apoptosis, epithelial-mesenchymal transition, cell migration, and angiogenesis [8-12]. In the comprehensive networks of tumorigenesis, diosgenin is pivotal in inducing apoptotic cell death and halting their malignant transformation [9]. Research on anticancer mechanisms of diosgenin before 2011 mainly focuses on inhibiting growth and inducing apoptosis [8], and there have been limited information on the mechanisms of diosgenin for preventing tumor invasion and metastasis until recent years [11-13].

Effects on cancer cell proliferation and apoptosis

Cell proliferation and apoptosis play major roles in maintaining homeostasis and deregulation of these processes is a hallmark of cancer [9]. Diosgenin has been reported to induce cell cycle arrest in osteosarcoma cells [8], KBM-5 cells [11] and breast cancer cells [16] at the concentration up to 50 μmol L⁻¹. Additionally, diosgenin (at concentrations ranging from 10 to 60 μmol L⁻¹) leads to or independently induces apoptosis through the extrinsic (death receptor (DR)) or the intrinsic (mitochondrial) death pathways converging at caspase-3 [9, 17-18]. A recent study has shown that 5 μmol L⁻¹ of diosgenin suppresses telomerase activity in the A549 lung cancer, which may be responsible for cellular apoptosis induction [19]. Moreover, growth suppressive effect of diosgenin has been also demonstrated in thymocytes with an IC₅₀ of 35 μmol L⁻¹ in vitro and at doses of 20 and 100 mg kg⁻¹ in vivo [20].

Effects on tumor invasion, metastasis, and angiogenesis

One of the initiating steps of primary tumor invasion is the epithelial-mesenchymal transition (EMT), during which tumor cells lose epithelial markers and gain mesenchymal traits that confer stem-like properties and a migratory phenotype [23]. Down-regulation of E-cadherin and up-regulation of mesenchymal markers, such as vimentin, N-cadherin, and α5β1 integrin, are often associated with EMT [24]. Chang et al. has reported that diosgenin (1–10 μmol L⁻¹) effectively inhibits the HGF-induced up-regulation of vimentin and MDM2 in prostate cancer cells, while the expression of E-cadherin is affected [25]. Similarly, diosgenin at 10 μmol L⁻¹ markedly inhibits high glucose-induced increase in α-smooth muscle actin (α-SMA) and decrease in E-cadherin in HK-2 cells, suggesting diosgenin antagonizes high glucose-induced renal tubular fibrosis through the EMT pathway [10]. Furthermore, Mao et al. has demonstrated that 10 μmol L⁻¹ diosgenin inhibits gastric cancer BGC-823 cells invasion in a hypoxic mimic microenvironment, which might be related to the enhanced expression of E-cadherin and integrinα5 and decreased integrinβ6 level [17]. The process of cell invasion is promoted by expressing and secreting various proteolytic enzymes, such as matrix metalloproteinases (MMPs) that can degrade extracellular matrix components [26]. Among the MMPs, MMP-2, MMP-9 and MMP-7 play a critical role in prostate cancer progression. Chen et al. has demonstrated that diosgenin (5–20 μmol L⁻¹) inhibits invasion and migration of human prostate cancer PC-3 cells, through reducing the activities and mRNA levels of MMP-2 and MMP-9 [11].

Abnormal cell migration is a hallmark feature of metastatic cells in cancer [27]. The migration process of cancer cells requires appropriate remodeling of the actin cytoskeleton [28]. Cdc42 is a key member in Rho family of small GTPases which regulate the rearrangement of actin cytoskeleton [29]. In addition, the induction of Cdc42 activation by Vav2 causes invasion and metastasis of breast cancer cells [30]. Recently, He et al. have found that 5 μmol L⁻¹ diosgenin significantly suppresses actin polymerization and Vav2 phosphorylation and reduces Cdc42 activation in human breast cancer MDA-MB-231 cells, which might be attributed to the anti-metastatic potential of diosgenin [12].

Angiogenesis is essential for invasiveness and metastasis of cancer and is dependent on the direct action of angiogenic factors such as VEGF and integrin [11]. It has been reported that the expression of VEGF in PC-3 cells is abolished by diosgenin in a dose-dependent manner, suggesting that diosgenin inhibits angiogenesis by suppressing VEGF expression [11].

Effects on tumorigenesis related signal pathways and targets

The upstream signal pathways such as PI3K-Akt-mTOR and MAPK have gained recognition for their principal role in aggressive, therapy-resistant malignancies [32]. It has been reported that diosgenin suppresses tumorigenesis by disrupting PI3K-Akt-mTOR signaling pathway in squamous cell carcinoma [33] and breast cancer [16, 34]. Among three different groups of MAPKs, namely ERKs, JNKs, and p38 MAPKs [33-36], Kim et al. has demonstrated that diosgenin (40 μmol L⁻¹) strongly generates ROS to induce apoptosis in liver cancer HepG2 cells through activation of ASK1, which is critical upstream signal for JNK/p38 MAPK activation [13]. By contrast, the JNK signaling has been shown to be significantly suppressed by diosgenin (20 μmol L⁻¹) in squamous cell carcinoma [33] and human prostate cancer cells [31]. However, opposing results regarding the effect of diosgenin (40 μmol L⁻¹) on activation of ERK have been found in HEL cells [34] and K562 cells [37]. It is
likely that the effects of diosgenin on MAPK pathway depend on the cell type and dose involved. In addition, nuclear factor-kappaB (NF-xB) and STAT3 have emerged as major regulators of cancer-related inflammation and contribute to tumorigenesis [38]. Previous studies have shown that the inhibitory effects of diosgenin on NF-xB [11, 39] and STAT3 [40] contribute to suppression of tumor cell proliferation, induction of apoptosis, and inhibition of invasion.

Of note, cyclooxygenase (COX)-2 regulated by NF-xB is possibly a vital target of diosgenin [40]. Over-expressed COX-2 participates in carcinogenesis by regulating a wide range of molecules including Bcl-2, p53, Fas, VEGF and E-cadherin [41]. Although there are controversial results concerning the association between COX-2 and survival rate, various studies on selective COX-2 inhibitors have revealed their ability to induce apoptosis in a variety of cancer cell lines [42]. However, it appears that the proapoptotic effect of diosgenin is related to activation of COX-2 in most cases. For instance, diosgenin up-regulates COX-2 in HEL cells at 40 μmol·L⁻¹ [36] or 10 μmol·L⁻¹ [40] and in colorectal cancer cells at 40 μmol·L⁻¹ [44]. Additionally, the study by Lepage et al. [43] has suggested that diosgenin (20 or 40 μmol·L⁻¹) sensitizes TRAIL-induced apoptosis in HT-29 cells. The underlying mechanisms for this sensitization may be the overexpression of functional TRAIL receptors DR5 and increase in COX-2 expression [45]. By contrast, diosgenin (20, 100, or 500 mg·kg⁻¹; p.o.) significantly reduces the expression of COX-2 and the number of colon tumors in a mouse model established by AOM/DSS feeding [46]. The exact reasons for the conflicting results regarding its effects on COX-2 remain to be elucidated.

Cellular transformation is a multistep process that requires a sequence of genetic alterations and changes in intracellular signaling [47]. High level of aerobic glycolysis, along with increased synthesis of protein, DNA, and fatty acids, are hallmarks of the transformed phenotype, which may provide cancer cells growth advantages in the tumor microenvironment [48]. Several studies have shown that regulation of de novo biosynthesis of fatty acids might be one of its proapoptotic mechanisms [49]. For instance, diosgenin (10 μmol·L⁻¹) suppresses the expression of fatty acid synthase (FASN), a key lipogenic enzyme upregulated in most human cancers, in HER2-overexpressing breast cancer cells [46]. Furthermore, it has been demonstrated that the upregulation of candidate genes involved in the cholesterol biosynthetic pathway leads to the stimulation of DNA synthesis during tumorigenesis [50, 51]. Diosgenin (20–40 μmol·L⁻¹) inhibits growth and induces apoptosis in human colon carcinoma HCT-116 cells via downregulating HMG-CoA reductase, which is the rate-limiting enzyme in cholesterol biosynthesis [52]. These results provide impetus to study the cholesterol biosynthetic pathway as a novel molecular target for its efficacy against colon cancer [53].

Overall, diosgenin shows significant anticancer activities, which possibly are linked to modulating P13K/Akt, MAPKs and NF-xB signaling pathways and multiple downstream functional proteins. A schematic diagram to illustrate its anticancer activity and potential mechanisms of actions is shown in Fig. 2

**Effects on cardiovascular disorders**

Previous studies support the potential of diosgenin in the management of cardiovascular disorders, including myocardial damage, vascular disturbances and hyperlipidemia [54, 58]. Important biomarkers of the cardiovascular system related to endothelial dysfunction, coagulation processes, and oxidative stress have been reported to be affected by diosgenin. In the following subsections, we will discuss the activities and possible molecular mechanisms for diosgenin’s role in regulating these events.

**Effects on myocardial damage**

In a recent study, Hadi et al. reported that diosgenin at 0.001 μmol·L⁻¹ protects myocardium against reperfusion injury through activating mitochondrial ATP-sensitive potassium channels and reducing the production of inflammatory mediators [54]. In addition, studies in isoproterenol-induced myocardial infarction (MI) rats have shown that diosgenin protects the myocardial membrane from the oxidative damaging action of lipid peroxides and decreases lysosomal damage and membrane liability at doses of 10 mg·kg⁻¹·d⁻¹ (15 d) and 80 mg·kg⁻¹·d⁻¹ (35 d), respectively [55-56].

**Effects on vascular disturbances**

One of the characteristic features of vasculature dysfunction is impaired vasodilation [57]. Reduced nitric oxide (NO) bioavailability play a causal role in impaired endothelium-dependent vasodilation, and vascular smooth muscle cell (SMC) dysfunction is associated with changes in endothelium-independent vasodilation [58]. Studies have shown that diosgenin (at the dose of 40 mg·kg⁻¹·d⁻¹ (5 weeks) in vivo and 0.1–10 μmol·L⁻¹ in vitro) ameliorates chronic renal failure (CRF) [59] and insulin-resistance-induced endothelial dysfunction by restoring NO production [60]. In addition, it is reported that 0.002–2 μmol·L⁻¹ of diosgenin induces relaxation of superior mesenteric rings in vitro, which appears to involve endothelial muscarinic receptor activation and release of endothelium-derived relaxing factors (EDRFs), mainly NO and cyclooxygenase derivatives [61]. On the other hand, experiments in human umbilical vein endothelial cells (HUVECs) have shown diosgenin (50 μmol·L⁻¹) protects HUVECs against H₂O₂-induced apoptosis partly through regulating mitochondrial dysfunction pathway [62]. Moreover, diosgenin (at concentrations ranging from 0.01 to 50 μmol·L⁻¹ in vitro or 40 mg·kg⁻¹ in vivo) can induce endothelium-independent vascular relaxation, with several underlying mechanisms being involved, such as protecting vasculature against oxidative stress [59], activating protein kinase G and opening iberiotoxin-sensitive BKₐ channels [63], and modulating intracellular calcium release and calcium influx in vascular smooth muscle cells [64].

Inflammation-induced monocyes adhesion to endothelial cells, followed by transmigration into the sub-endothelial intima, is one of the key events in the development of vasculature
Diosgenin inhibits transcriptional factors STAT3, NF-κB, and sterol-regulatory element binding protein (SREBP) regulated genes involved in tumor cell proliferation, phenotypic switching, invasion, migration, and angiogenesis, partly by modulating the PI3K-Akt-mTOR and MAPK pathways. Besides, telomerase is also a target of diosgenin which is important for most cancer cells to grow and proliferate.

Tissue factor (TF) is a glycosylated transmembrane protein, expressed by endothelial and other cells \[71\]. Its best known function is the initiation of plaque instability and subsequent thrombus formation which contributes to the progression of cardiovascular diseases \[72\]. Yang et al. has reported that diosgenin \((0.01–1 \text{ μmol} \cdot \text{L}^{-1})\) significantly inhibits TF procoagulant activity and reduces expression of TF in actin- 

vated THP-1 monocyteic cells \[73\]. Moreover, diosgenin (at doses approximately equivalent to 1, 2, and 4 times of the normal human dose of steroidal saponins in clinical prescription) has been found to possess anti-thrombosis activity on thrombosis model by improving the anticoagulation function, inhibiting platelet aggregation and thrombosis \[74\].

**Effects on hyperlipidemia**

There are abundant evidences supporting that hyperlipidemia has a tight relation with cardiovascular damage \[75-76\]. The underlying mechanisms include lipid peroxidation related oxidative stress \[77-78\]. Oxidative stress has been identified as a primary determinant of cellular damage and dysfunction and contributes to the initiation and progression of the cardiovascular dysfunction \[79-80\]. In studies designed to investigate the vascular protective effects of diosgenin in rats fed with a high-cholesterol diet, diosgenin improves lipid profile and modulate oxidative stress by upregulating antioxidants in hyperlipidemic rats \[82, 81\]. Similarly, diosgenin inhibits chronic renal failure (CRF)-induced vasculature dysfunction.

![Fig. 2 Schematic representation of the potential molecular mechanisms for the anticancer activity of diosgenin.](image-url)
Table 1 Summary of various activities and related potential targets of diosgenin in different cells

<table>
<thead>
<tr>
<th>Activities/cells/references</th>
<th>Related potential targets</th>
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<tr>
<td><strong>Proliferation</strong></td>
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<td>Growth</td>
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<tr>
<td>neuron</td>
<td>nerve growth factor (NGF), c-fos cAMP</td>
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<td>keratinocyte</td>
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<td>mammary epithelium</td>
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<td>Cell cycle arrest</td>
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<tr>
<td>osteosarcoma cell</td>
<td>p53, NF-κB</td>
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<tr>
<td>breast carcinoma cell</td>
<td>cyclin D1, cdk-2, cdk-4</td>
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<tr>
<td>thryocyte</td>
<td>cyclin D1</td>
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<tr>
<td>Apoptosis</td>
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<tr>
<td>lung cancer cell</td>
<td>telomerase, Bcl-2, p53, COX-2, caspase-3, PARP</td>
</tr>
<tr>
<td>breast carcinoma cell</td>
<td>Akt, Raf, ERK</td>
</tr>
<tr>
<td>osteosarcoma cell</td>
<td>Akt, p53, NF-κB</td>
</tr>
<tr>
<td>colon carcinoma cell</td>
<td>p21, caspase-3, Bcl-2, COX-2</td>
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<tr>
<td>erythroleukemia/leukemia cell</td>
<td>Src, Tor, Akt, CREB, MAPK, NF-κB, COX-2</td>
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<tr>
<td>hepatoma carcinoma cell</td>
<td>ASK1, MAPK, STAT3</td>
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<tr>
<td>squamous carcinoma cell</td>
<td>Akt, JNK, Bcl-2, PARP</td>
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<tr>
<td>melanoma cell/laryngocarcinoma</td>
<td>caspase-3, p53, PARP</td>
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<td>Angiogenesis</td>
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<td>induced by PC-3 tumor cell</td>
<td>VEGF</td>
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<td>osteoblast</td>
<td>VEGF-A</td>
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<td>Differentiation</td>
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<td>adipocyte</td>
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<td>oligodendrocyte progenitor cell</td>
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<td>megakaryocyte</td>
<td>PPAR-γ</td>
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<td>T cell</td>
<td>estrogen receptor</td>
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<td></td>
<td>COX-2, thromboxane synthase (TXS)</td>
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<td>Cellular phenotypic switching</td>
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<td>Malignant transformation</td>
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<tr>
<td>breast cancer cell</td>
<td>fatty acid synthase (FASN)</td>
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<tr>
<td>human colon carcinoma cell</td>
<td>HMG-CoA reductase</td>
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<tr>
<td>Epithelial-mesenchymal transition (EMT)</td>
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<tr>
<td>prostate cancer cell</td>
<td>vimentin, Mdm2</td>
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<tr>
<td>renal proximal tubular epithelial cell</td>
<td>α-smooth muscle actin, E-cadherin</td>
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<tr>
<td>gastric cancer cell</td>
<td>E-cadherin, integrin5, integrin8</td>
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<tr>
<td>Cellular function modulation</td>
<td></td>
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<tr>
<td>Migration (−)</td>
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<tr>
<td>breast cancer cell</td>
<td>vav2, cdc42, actin cytoskeleton</td>
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<tr>
<td>vascular smooth muscle cell</td>
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<tr>
<td>Adhesion (−)</td>
<td>ICAM-1 / VCAM-1</td>
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<tr>
<td>vascular smooth muscle cell</td>
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<tr>
<td>endothelial cell</td>
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<tr>
<td>Nitric oxide production (−/+)</td>
<td></td>
</tr>
<tr>
<td>(+) endothelial cell</td>
<td>eNOS</td>
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<tr>
<td>(−) monocyte / macrophage</td>
<td>iNOS</td>
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<tr>
<td>Thrombus formation (−)</td>
<td></td>
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<tr>
<td>monocyte</td>
<td>tissue factor (TF)</td>
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<tr>
<td>Membrane stability (+)</td>
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<tr>
<td>myocardial cell</td>
<td>lipid peroxidation</td>
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<tr>
<td>Contraction (−)</td>
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<tr>
<td>vascular smooth muscle cell</td>
<td>BKCα channel</td>
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<tr>
<td>vascular smooth muscle cell</td>
<td>store-operated calcium channels</td>
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(+ ) enhance; (−) inhibit
by preventing oxidative stress, partly through increasing antioxidant enzymes activity and suppressing lipid peroxidation [59, 82]

**Effects on type 2 diabetes**

Type 2 diabetes mellitus (T2DM) is characterized by defects in insulin secretion and peripheral insulin resistance in the skeletal muscle, the adipose tissues and the liver [83]. Studies support the potential of diosgenin in the management of diabetes by ameliorating oxidative stress and dysfunctional lipid metabolism. In the obese diabetic mice, fenugreek ameliorates hepatic steatosis and hyperlipidemia by suppressing the mRNA expression of lipogenic genes [84]. Being the substance in fenugreek responsible for the inhibitory effect, diosgenin (5 and 10 μmol·L⁻¹) inhibits the accumulation of triglyceride (TG) and the expression of lipogenic genes in HepG2 cells, which contribute to the therapeutic effects of fenugreek on lipid metabolism disorders [84]. Additionally, analysis of lipid accumulation in 3T3-L1 preadipocytes has indicated that diosgenin (at concentrations ranging from 0.1 to 10 μmol·L⁻¹) can promote the expression of PPARγ and the differentiation of adipocyte, which may be helpful in reducing circulating lipids in blood and contribute to the hypolipidemic activity of diosgenin in type 2 diabetes rats [85]. Furthermore, a study on aorta of diabetic rats has also demonstrated the beneficial effects of diosgenin in improving antioxidant status and decreasing lipid peroxidation [86].

It is recognized that chronic inflammation in adipose tissues is involved in the pathogenesis of obesity-related insulin resistance and type 2 diabetes [83]. In a study by Kawada et al., it has been reported that diosgenin (1–10 μmol·L⁻¹) promotes 3T3-L1 adipocyte differentiation and thus enhances insulin-dependent glucose uptake [87]. Co-culturing of 3T3-L1 adipocytes and RAW 264 macrophage in vitro has demonstrated that diosgenin inhibits the production of proinflammatory mediators in the medium and suppresses the inflammation in macrophages through inhibiting NF-κB and JNK pathways [88].

**Neuroprotective effects**

Accumulated data both in vitro and in vivo indicate that diosgenin has a neuroprotective activity. In a purified rat oligodendrocyte progenitor cell (OPC) culture model, diosgenin (10–50 μmol·L⁻¹) has been shown to promote the differentiation of OPC through an estrogen receptor-mediated ERK1/2 activation pathway to enhance remyelination, thus protecting the normal function of neurons [89]. Diabetic neuropathy is one of the most debilitating complications of type 1 and type 2 diabetes [90]. Previous studies have suggested that reduced availability nerve growth factor (NGF) plays a significant role in the pathogenesis of diabetic polyneuropathy [91]. Diosgenin has been shown to increase NGF levels in the sciatic nerve of diabetic rats, neurite outgrowth in PC12 cells as well as enhance nerve conduction velocities in a diabetic neuropathy mouse model [92]. It has been also reported that 0.1 μmol·L⁻¹ of diosgenin ameliorates mitochondrial dysfunction in the presence of dopamine in isolated rat synaptosomes [93]. Furthermore, diosgenin administration (5, 25 and 125 mg·kg⁻¹, p.o.) significantly improves learning and memory abilities of the D-gal treated mice, which may be partly mediated by enhancing endogenous antioxidant enzymatic activities [94]. The study by Chihiro et al. has also demonstrated that diosgenin treatment (10 mmol·kg⁻¹, i.p.) in normal mice promotes object recognition memory, which is mediated by steroid-binding receptor (1, 25D3-MARRS) -triggered axonal growth [95]. Finally, diosgenin could affect ion currents in human cortical neurons (HCN-1A) through modulating large-conductance Ca²⁺-activated K⁺ channel with an EC₅₀ value of 25 μmol·L⁻¹, which may affect the functional activity of cortical neurons [96]. These results provide valuable clues as to the therapeutic effects of diosgenin in neuropathies such as neurodegenerative diseases.

**Effects on immune modulation and inflammation**

It has been found that diosgenin ameliorates allergic diseases mainly through regulating T-cell immune response. Diosgenin suppresses allergen-induced intestinal Th2 responses through enhancing the regulatory T-cell immunity in BALB/c mice sensitized with ovalbumin (OVA) [97]. Similarly, in an OVA-induced intestinal allergic mice model, diosgenin has been found to possess anti-allergic activity, which is associated with the suppression of IgE production and mast cell infiltration and degranulation [98]. Moreover, diosgenin modulates certain aspects of acquired immunity in OVA-sensitized and challenged BALB/c mice, including the enhancement of antigen-specific IgG2α and IFN-γ expression, which might be mediated through the up-regulation of Th1 differentiation [99]. Furthermore, diosgenin treatment inhibits both IL-2 (Th1) and IL-10 (Th2) cytokine production, suggesting that it has an anti-inflammation potential through the inhibition of T-cell immune responses in mouse primary splenocytes [100]. In a study designed to investigate the effect of diosgenin on LPS-induced acute lung injury in mice, diosgenin (0.1, 1.0 and 10 mg·kg⁻¹; i.g.) significantly attenuates the lung histopathological changes and infiltration of inflammatory cells [101]. In part, these in vivo effects correlate with the inhibition of immune response of THP-1 monocytic cells [102]. Similar anti-inflammatory mechanism of diosgenin (0.1–10 μmol·L⁻¹) has also been demonstrated in LPS/IFN-γ-activated Raw264.7 macrophage cells [102]. Besides, diosgenin at concentrations from 5 to 400 μg·mL⁻¹ inhibits the growth of tumor cells by stimulating both specific and non-specific cellular immune responses, instead of direct cytotoxic effect [103].

**Effects on menopausal symptoms**

Estrogens and estrogen receptors regulates the proliferation and differentiation of mammary epithelial cell as well as various aspects of glucose and lipid metabolism [104]. Estrogen can exert protective activity in menopausal women. Many menopausal women take traditional herbal medicines, such as yam or diosgenin, to substitute for the female hormone (es-
trogen)\textsuperscript{[105]}. The estrogenic effect of diosgenin has been previously hypothesized for the molecular structural similarity between diosgenin and estrogen\textsuperscript{[106]}. The estrogenic effect of diosgenin on an ovariectomized (OVX) animal model was firstly reported by Aradhana et al. in 1992\textsuperscript{[107]}. Their results have shown that diosgenin administration (20 or 40 mg·kg\textsuperscript{-1}) for a period of 15 days stimulates the growth of mammary epithelium, and concomitant treatment of estrogen and diosgenin showed synergistic action\textsuperscript{[107]}. Similarly, in an OVX rat model of postmenopausal osteoporosis, it has been demonstrated that diosgenin prevents bone loss induced by estrogen deficiency\textsuperscript{[108–109]}. Furthermore, it has been demonstrated that 2 μmol·L\textsuperscript{-1} of diosgenin up-regulates VEGF-A and promotes angiogenesis in an estrogen receptor-dependent manner\textsuperscript{[109]}. Conversely, a recent study in the immature rat has suggested that diosgenin does not manifest estrogenic effect as an estrogen agonist\textsuperscript{[110]}. Diosgenin does not affect the uterine wet weight, epithelium height, and volume densities of endometrium, nor does estrogen receptor α or progesterone receptor immunostaining intensity altered by diosgenin\textsuperscript{[111]}. Additionally, Yam, but not its extract diosgenin, can regulate calpain isoforms in OVX rats\textsuperscript{[112]}. Taken together, it remains unclear whether diosgenin has an estrogenic effect, and its relation with estrogen receptor merits further study.

Effects on skin aging

A study designed to investigate the efficacy of diosgenin against skin aging has revealed that diosgenin may enhance DNA synthesis and improve the proliferation of keratinocytes via inducing cAMP signals, without the involvement of estrogen receptors\textsuperscript{[113]}. Administration of diosgenin (0.01%, 0.02%, and 0.04% mixed in basal diet) improves the epidermal thickness in a climacteric mouse model\textsuperscript{[113]}. Additionally, diosgenin (1–50 μmol·L\textsuperscript{-1}) inhibits melanogenesis through the activation of the PI3K pathway in B16 melanoma cells, suggesting that diosgenin may be an effective inhibitor of hyperpigmentation in the treatment of skin diseases such as acquired hyperpigmentation conditions\textsuperscript{[114]}. 

Discussion and Conclusion

Diosgenin is a biologically active constituent of sanyaku, a traditional Chinese medicine that is the freeze-dried powder of the yam tuber (Dioscorea), and Yam has been used as a botanical dietary supplement to maintain or improve health for a long time\textsuperscript{[115]}. Natural foods/drugs containing diosgenin have been marketed and promoted as being effective in treating various diseases such as osteoporosis, premenstrual syndrome\textsuperscript{[116]}, a coronary artery disease, and angiogenesis. This review indicates that diosgenin can have multiple beneficial effects against cancer, cardiovascular disorders, diabetes, neurodegenerative diseases, allergic diseases, menopausal symptoms, and skin aging. It interacts with various target molecules and related signaling pathways. Diosgenin is potentially a antitumor agent for various types of cancers, including breast carcinoma, osteosarcoma, colon carcinoma, leukemia, erythroleukemia, laryngocarcinoma, and prostate cancer. Moreover, diosgenin is beneficial in improving the function of endothelial cells, neurons, myocardial cells, vascular smooth muscle cells, and epithelium cells. However, these preclinical data should be confirmed in future clinical studies.

In conclusion, diosgenin shows multiple activities against various diseases, but the underlying mechanisms of actions remain to be fully elucidated. Determination of specific targets of diosgenin is required in order to further validate its applications in the prevention and treatment of human diseases.

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