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Review

Anticancer Activity of Diosgenin and Its Molecular Mechanism*

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ABSTRACT Diosgenin, a steroidal sapogenin, obtained from *Trigonella foenum-graecum*, *Dioscorea*, and *Rhizoma polgonati*, has shown high potential and interest in the treatment of various cancers, such as oral squamous cell carcinoma, laryngeal cancer, esophageal cancer, liver cancer, gastric cancer, lung cancer, cervical cancer, prostate cancer, glioma, and leukemia. This article aims to provide an overview of the *in vivo*, *in vitro*, and clinical studies reporting the diosgenin's anticancer effects. Preclinical studies have shown promising effects of diosgenin on inhibiting tumor cell proliferation and growth, promoting apoptosis, inducing differentiation and autophagy, inhibiting tumor cell metastasis and invasion, blocking cell cycle, regulating immunity and improving gut microbiome. Clinical investigations have revealed clinical dosage and safety property of diosgenin. Furthermore, in order to improve the biological activity and bioavailability of diosgenin, this review focuses on the development of diosgenin nano drug carriers, combined drugs and the diosgenin derivatives. However, further designed trials are needed to unravel the diosgenin's deficiencies in clinical application.

KEYWORDS diosgenin, steroidal sapogenin, anticancer, molecular mechanism, biological activity

Diosgenin, (25R)-spirost-5-en-3β-Ol (Figure 1), is a phytosteroidal compound extracted from the rhizome of dioscorea.[1] Studies have shown that diosgenin has anti-effects on blood lipid,[2] diabetes,[3] thrombus,[4] myocardial ischemia,[5] rheumatoid arthritis,[6] inflammation,[7] allergy,[8] immune regulation.[9] Concurrently, it also has a resisting effect on neoplasm, nephropathy,[10] encephalomyelitis,[11] osteoporosis,[12] Parkinson's disease,[13] Alzheimer's disease,[14] and plays a beneficial role in pulmonary hypertension.[15] Furthermore, diosgenin is also an important basic raw material for the preparation of steroids and contraceptives in the pharmaceutical industry.[4] Recently, diosgenin is likely to become one of the new drugs to prevent corona virus disease 2019 (COVID-19).[16]

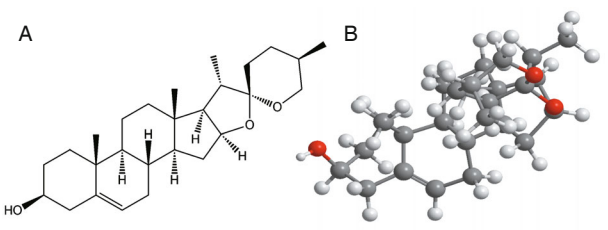

Figure 1. Chemical Structure (A) and Three-Demensinal Structure (B) of Diosgenin

As an important material in the pharmaceutical industry, diosgenin is used to manage various medical conditions such as cancer because of its multiple bioactivities.[17] it has a certain therapeutic effect on head and neck, chest, abdominal and limb tumors. Thus, it is the preferred compound of natural anti-tumor drugs. However, natural diosgenin often has the problems of low yield and low bioavailability, and some dioscoreaceae have become high-value endangered medicinal plants.[18] Consequently, it is extremely important to transform diosgenin into a more efficient and selective utilization way. In the present study, we put focus on the antitumor effect and action pathway of diosgenin, as well as its antitumor effects as nano carriers, combined drugs and the structural modification of diosgenin, in order to provide more clues for the deeply development of diosgenin with higher antitumor activity and lower toxicity.

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Antitumor Effects of Diosgenin

Digestive System Tumors

Oral Squamous Cell Carcinoma

Oral squamous cell carcinoma (OSCC) is the most common oral cancer,[19] accounting for about 90% of oral malignancies.[20] Noticeably, the mortality rate after 5 years is still about half after surgery, chemotherapy and radiotherapy.[21] A series of studies have shown that diosgenin was a key drug in anti-OSCC *in vitro* and *in vivo*. It had an obvious inhibitory effect on OSCC cells PE/ca-pj15 with the dosage 100 μmol/L diosgenin.[22] Diosgenin decreased the oral tumor incidence significantly in male Syrian golden hamster cheek pouch at the dose of 80 mg/kg by oral administration every other day for 16 weeks.[23]

Esophageal Cancer

Esophageal cancer is a common malignant tumor of digestive system. Diosgenin mainly have an obvious role on esophageal cancer cells Eca109. It could inhibit esophageal cancer cells proliferation, migration and invasion, and induce apoptosis with an optimal measure of 50 g/mL on Eca109.[24,25]

Gastric Cancer

Previous studies have shown that diosgenin have effects on human gastric adenocarcinoma cells mainly include BGC823, MGC803, SGC7901, MNK45 and AGS cells, which inhibited the proliferation, growth and invasion, promoted apoptosis of BGC823 and MGC803 gastric cancer cells,[26,27] inhibited the growth of MGC803 and SGC7901 cells and increased the apoptosis rate of MGC803 cells to 30.08% with the dosage at 60 μg/mL.[28] Furthermore, diosgenin caused a dose-dependent decrease in the AGS and SGC7901 cells viability, induced significant increases in G_0/G_1 cell cycle arrest and apoptosis.[29]

Colon Cancer

Colorectal cancer (CRC) is one of the most lethal gastrointestinal tumors.[30] Results from the *in vitro* experiments indicated that diosgenin can inhibit the growth of human colon cancer cells SWILLC, the activity of SW480 cells and cell proliferation, migration and invasion, as well as the proliferation of colorectal cancer cells SW1116 and RKO in a time- and dose-dependent manner. Moreover, diosgenin can induce the apoptosis of colon cancer cell lines, HT-29 and HCT-116, in a dose-dependent manner.[31-34] Results from the *in vivo* experiments indicated that diosgenin could inhibit preneoplastic colonic lesions aberrant crypt foci in azoxymethane-induced rat colon carcinogenesis.[33]

Liver Cancer

Liver cancer is one of the most common malignant tumors occurred worldwide, which has emerged as a main health trouble and accounts for leading cancer-related death.[17] Diosgenin has obvious inhibitory effect on liver cancer, it significantly inhibited the growth of Bel7402, SMMC7721 and HepG2 cells in a concentration-dependent manner, induced G_2/M cell cycle arrest and apoptosis of hepatoma cells, and inhibited cell migration and invasion.[17,35,36] Moreover, a study also found that when rat hepatoma cells CBRH7919 were treated with 50 μ/L diosgenin, the apoptosis rate was 29.67%, the inhibition rate was 89.3%, and the cell cycle was blocked.[37]

Pancreatic Cancer

Pancreatic cancer, one of the most aggressive and lethal malignancies worldwide, is a refractory and intractable disease. It had been found that diosgenin induced apoptosis and G_2/M phase recovery of pancreatic cancer cells Patu8988 and Panc-1, and significantly inhibited the proliferation of pancreatic cancer cells in a dose- and time-dependent manner. Additionally, diosgenin could also significantly inhibit the migration and invasion of pancreatic cancer cells.[38]

Others

Diosgenin was found to have efficient antitumor potential of human cholangiocarcinoma cells *in vitro* and *in vivo*, it could inhibit the progression of HUCCT1 cells and tumor growth in xenografts of nude mice *in vivo* tumor studies.[39] Except above, diosgenin was sensitive to human salivary adenocarcinoma cells.[40]

Respiratory System Tumors

Laryngeal Carcinoma

Squamous cell carcinoma is common in laryngeal cancer. Diosgenin had an effect on not only inhibiting the proliferation of human laryngeal cancer cells Hep-2, but also the invasion of human laryngeal cancer cells Hep-2 and TU212, causing apoptosis and DNA damage, increasing the level of reactive oxygen species, and inducing S-phase arrest.[41,42]

Lung Cancer

Lung cancer is one of the malignant tumors with the highest mortality worldwide, among which non-small cell lung cancer (NSCLC) makes up about 80%.[43] A previous study had shown that diosgenin attenuated the lung histopathological changes such as pulmonary edema, coagulation and infiltration of inflammatory cells,[44] and further experiments indicated that diosgenin played a significant role in anti-lung cancer activities, it could

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inhibit the expression of telomerase reverse transcriptase gene,[45] suppress the A549 cell proliferation and increased apoptosis in a dose-dependent manner,[46] and it could inhibit the growth of human SCLC cells NCI-H446.[47]

Genitourinary System Tumors

Prostate Cancer

Prostate cancer is the second most common malignant tumor in men,[48] especially in elderly men.[49] Recently, plenty of research indicated that diosgenin is important in the treatment of prostate cancer, it could inhibit the proliferation, migration and invasion of prostate cancer cells PC-3, mediate autophagy and increase the apoptosis of prostate cancer cells DU145 and LNCaP,[50,51] and concentration-dependently decreased prostate cancer cells viability.[48,52] In addition, diosgenin also decreased the level of carcinogenic protein in transgenic mice and inhibited the development of prostate tumor.[49]

Breast Cancer

Breast cancer is the most common cancer among women, affecting about 12% of the world's female population.[53] Diosgenin could not only inhibit the growth of human breast cancer cells MCF-7 and MDA-MB-231, but also inhibit its survival and proliferation. Meanwhile, diosgenin inhibited cell viability and stimulated apoptosis, reduced cell invasion.[41,54,55] Moreover, diosgenin treatment resulted in cell MCF-7 and Hs578T growth inhibition, cell cycle arrest, and apoptosis, and all the results are in concentration- and time-dependent manners.[56]

Cervical Cancer

Cervical cancer is the second leading cause of death among women in the world. Diosgenin had been corroborated antitumor effect on cervical cancer *in vivo* and *in vitro*. It had an inhibitory effect on the growth of human cervical cancer cells HeLa, and inhibited cell proliferation and induced apoptosis of cervical cancer cells HeLa and SiHa, as well as CaSki cervical cancer cells.[41,57,58] Additionally, diosgenin inhibited U14 mice transplant tumor with inhibition rates of 30%–50%.[59]

Bladder Cancer

Bladder cancer is the most common malignancy of the urinary tract and the incidence of bladder cancer ranks first among urinary tract malignant tumors in China.[60] Diosgenin could inhibit the proliferation of bladder cancer cells 5637 and T24, prevent cell migration and invasion, induce apoptosis, and kill bladder tumor cells in a dose- and time-dependent manner.[61,62]

Hematopoietic System Tumors

Leukemia is a malignant disease of hematopoietic system. Diosgenin had an inhibitory effect on human chronic myeloid leukemia cell K562, it could inhibit the proliferation of K562 cells and induced cell cycle G_2/M phase arrest and apoptosis and it also induced WEHI-3 murine leukemia cells cell cycle arrest and apoptotic.[63,64] Furthermore, diosgenin could produce reactive oxygen species, induce autophagy, enhance the apoptosis of leukemia cells K562 and BaF3-WT, and achieved anticancer effect.[65] Besides, the *in vivo* study demonstrated that diosgenin had marked antitumor efficacy against tumors in the WEHI-3 cell allograft model.[64]

Others

Except above, diosgenin played a crucial role in human astrocytoma cells U251.[47] Meanwhile, diosgenin showed anti-tumor effects in rat C6 and human glioblastoma cells T98G by induction of differentiation and apoptosis and inhibition of migration, invasion, and angiogenesis.[32] In addition, diosgenin had similar inhibitory effects on the proliferation of melanoma cells M4B, inhibited the growth of cells A375-S and it was more dependent on anti-tumor immunity and improved the composition of gut microbiome in melanoma-bearing C57BL/6 mice.[47,66,67]

Osteosarcoma is a kind of osteogenic malignant tumor, which has a high incidence in primary malignant bone tumors.[68] Diosgenin could inhibit the invasion and migration of osteosarcoma cells MG63 and U2OS, the proliferation of human osteosarcoma cell 1547, as well as osteosarcoma cell S180, and it could block the cell cycle in G_1 phase and induce apoptosis.[59,68,69]

Anticancer Pathways of Diosgenin

Plenty studies have shown that the anti-tumor occurrence and development of diosgenin is often accompanied by multiple mechanisms at the same time, with multi-targets and multi-pathways (Appendix 1). It mainly includes inhibiting tumor cell proliferation and growth, promoting tumor cell apoptosis, inducing tumor cell differentiation, inhibiting tumor cell metastasis and invasion, blocking cell cycle, inhibiting tumor signal transduction, improving immunity and improving gut microbiome through the mechanism of cell autophagy (Figure 2).

Inhibiting Proliferation and Growth of Tumor Cells, Promoting Apoptosis, Inducing Differentiation and Autophagy

Extensive researches have shown that diosgenin inhibits the proliferation and growth of tumor cells,

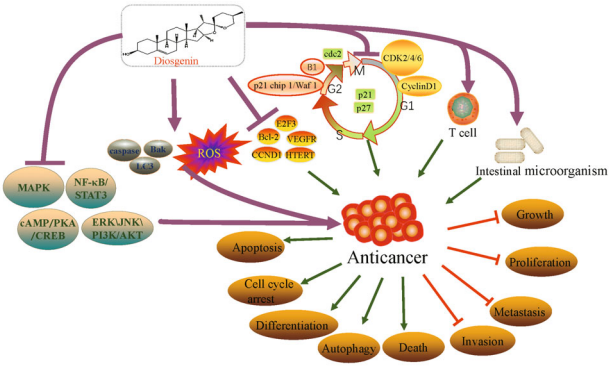

Figure 2. Anticancer Mechanism of Diosgenin

Notes: ROS: reactive oxygen species; MAPK: mitogen-activated protein kinase; NF-κB: nuclear factor kappa B; ERK: extracellular signal-regulated kinase; JNK: c-jun N-terminal kinase; PI3K: phosphatidylinositol-3 kinase; cAMP:cyclic adenosine monophosphate; PKA:protein kinase A; CREB: cAMP-response element binding protein

promotes apoptosis, and induces cell differentiation and autophagy mainly by regulating signal pathways, regulating the expression of genes and proteins, increasing the level of reactive oxygen species (ROS), reducing intracellular Ca^{2+} concentration and inhibiting angiogenesis.

Regulating Signal Pathway

The signal pathway plays an important part in the anti-tumor effect of diosgenin, it mainly involves mitogen-activated protein kinase (MAPK) signal pathway, extracellular signal-regulated kinase (ERK) and c-jun N-terminal kinase (JNK) signal pathway, phosphatidylinositol-3 kinase (PI3K), Akt, nuclear factor kappa B (NF-κB)/STAT3 pathway, Wnt/β-catenin signal pathway and cAMP/PKA/CREB pathway. MAPK signal pathway is shared by four distinct cascades, including the extracellular signal-related kinases (ERK1/2), Jun amino-terminal kinases (JNK1/2/3), p38-MAPK and ERK5, and MAPK/ERK pathway is reported to be associated with the cell proliferation, differentiation, migration, senescence and apoptosis.[70] Diosgenin may regulate the proliferation, apoptosis, migration and invasion of esophageal, colon cancer and osteosarcoma cells through inhibiting the initiation of epithelial-mesenchymal transition (EMT) and then inhibiting p38-MAPK signal pathway with EMT-related proteins such as transforming growth factor β1, E-cadherin and vimentin, and induce apoptosis of A549 cells via related MAPK signal pathway proteins caspase-8, -9 and -3.[68,71]

Noticeably, the anti-tumor effect of diosgenin may also be carried out through multiple pathways at a same time. Diosgenin could inhibit the phosphorylation of NF-κB/p65, IKK-β, Akt, ERK, and JNK, which indicated the anti-tumor effect of diosgenin through downregulation of the MAPK, Akt and NF-κB signaling pathways.[72] Equally, diosgenin could effectively inhibit ERK, JNK and PI3K/AKT signaling pathways against pancreatic tumor cells. In addition, diosgenin inhibited the activity of NF-κB through NF-κB signal pathway, the activation of NF-κB/STAT3, resulting in a significant decrease in the expression of many oncogene products, thus inhibits the proliferation of human hepatocellular carcinoma cells.[49,50,73] Diosgenin may promote the proliferation and differentiation of MG-63 cells by inhibiting the Wnt/β-catenin signal pat hway thereby reducing the number of calcified nodules and inhibiting the expression of osteopontin and osteocalcin in the cells.[12] Moreover, diosgenin inhibited aerobic glycolysis by mediating glucose transporters (GLUT) such as GLUT3 and GLUT4, and inhibits CREB phosphorylation through cAMP/PKA/CREB pathway, thus inducing apoptosis in colorectal cancer cells.[30]

Mitochondria-Mediated Apoptosis Pathway

Mitochondrial apoptosis pathway plays a central role in the process of apoptosis. Diosgenin induced apoptosis with the increased expressions of cytosol cytochrome C, cleaved-caspase-3, cleaved-PARP1 and the Bax/Bcl-2 ratio.[39] Similarly, diosgenin induced apoptosis in HepG2 cells through Bcl-2 protein family-mediated mitochndria/caspase-3-dependent pathway. It is particularly known that the proto-oncogene BcL-2 was discovered with the cloning of the t(14;18) chromosomal translocation responsible for human follicular lymphoma. Other members of the Bcl-2 family have been identified to be important in tumor occurrence and development, and altered expression levels of various members serve as prognostic markers in many lymphoid malignancies.[74] Caspases are cysteinyl aspartate specific proteinase and members of the interleukin-1beta-converting enzyme family containing cysteine, which is closely related to eukaryotic cell apoptosis and participates in the regulation of cell growth, differentiation and apoptosis.[75] The activation and function of caspases, involved in the delicate caspase-cascade system, are regulated by various kinds of molecules, such as the inhibitor of apoptosis protein, Bcl-2 family proteins, calpain, and Ca^{2+}. The p53 gene is a typical tumor suppressor gene, mutations and inactivation of p53 tumor suppressor gene are common events in the development of all or most types of human cancers.[76,77]

Diosgenin could induce DNA damage and then induce apoptosis of K562, bladder cancer, rectal cancer, glioblastoma cancer neoplasm cells by significantly up-regulating the protein levels of Bak, Bax, Bid, p53, caspase-3

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and -9, and down-regulating the protein levels of Bcl-2, Bcl-xL and Survivinm RNA.[30,32,41,57,63] Calcium homeostasis is one of the main targets of endoplasmic reticulum stress, the destruction of Ca^{2+} homeostasis and mitochondrial dysfunction play an important role.[10] Diosgenin was awakened by erythrocyte contraction and disturbance of erythrocyte membrane phospholipids, which was parallel to Ca^{2+} influx, oxidative stress and ceramide.[9] Diosgenin can play a role in mitochondrial-dependent cell apoptosis through calcium homeostasis regulation, cause Ca^{2+} release, reduce the concentration of Ca^{2+}, and induce cell cycle arrest and apoptosis.[52]

Regulating Expression of Genes and Proteins

Diosgenin can up-regulate autophagy marker LC3 protein, reduce the ratio of LC3-Ⅰ to LC3-Ⅱ, induce autophagy of hepatoma cell.[78] Moreover, diosgenin regulated p21 mRNA expression, which is related to the activation of nuclear factor UUB and the synthesis of prostaglandin E_2, miR-145 expression level and methylation status, down-regulated cyclooxygenase (COX)-2 and microsomal prostaglandin E synthase (MPGES)-1, and significantly up-regulated miR-34a expression and down-regulated the expression level of miR-34a target genes E2F1, E2F3 and CCND1, which could inhibit gastric,[79] lung[80] and breast cancers proliferation.[81] Interestingly, telomerase activity is not detected in typical healthy cells, while in cancer cell telomerase expression is reactivated, therefore providing a promising cancer therapeutic target.[82] Doubtlessly, diosgenin inhibited telomerase activity by down-regulating the expression of telomerase reverse transcriptase gene HTERT in A549 lung cancer cells and increased the expression of glial fibrillary acidic protein GFAP to induce glioblastoma cell differentiation, and decreased the abundance of dedifferentiation markers Id2, N-Myc, TERT and Notch-1 to reduce cell dedifferentiation.[32,45] Diosgenin exerts anti-cancer effect through inhibiting mesoderm posterior 1 (MESP1) expression in gastric cancer cells, promotes the cell proliferation, apoptosis, and growth.[26]

Increasing Level of ROS

ROS are the main molecules produced by the body in the process of oxidative stress, and the accumulation of ROS in cells can lead to cell death.[30] A series of studies have shown that diosgenin strongly generated ROS and this oxidative stress might induce apoptosis through activation of ASK1, which are critical upstream signals for JNK/p38 MAPK activation in HepG2 cancer cells.[83] The production of ROS was observed and autophagy and apoptosis were induced by regulating ROS-mediated DNA damage and mitochondrial signal pathway in the experimental studies of diosgenin against human chronic myeloid leukemia,[63] laryngeal cancer,[42] cervical cancer[57] and colorectal cancer.[65]

Inhibiting Angiogenesis

Angiogenesis is one of the main factors in tumor growth, metastasis and invasion.[84] Vascular endothelial growth factor (VEGF) is the most important regulator of angiogenesis and differentiation, which mediated signal transduction pathway participating in all processes of angiogenesis.[85] VEGF not only provides nutrition and waste excretion pathway for tumor, but also provides a pathway for tumor cells to enter the circulatory system and binds to the corresponding receptor VEGFR to play a biological role. Otherwise, the vascular endothelium plays a vital role in coordinating angiogenesis and vascular tone. The vascular endothelium is one of the largest organs in the body and consists of a single layer of highly specialized cells with site-specific morphology and functions.[86] VEGF can induce endothelial cell division, proliferation and migration by promoting endothelial cell germination, so as to accelerate the degradation of vascular basement membrane, promote the formation of foot ring, enhance the activity of endothelial cells, promote angiogenesis, tumor growth and invasion. Diosgenin could inhibit the anti-angiogenesis of HIF-1α, GRP78, VEGFFR, PI3K/AKT, ERK1/2 and FAK signaling pathways, inhibit the expression of VEGF and tubular formation of endothelial cells in cancer cells and inhibit angiogenesis by reducing the protein levels of VEGF and fibroblast growth factor 2 (FGF2).[32,50] Diosgenin significantly reduced IKKβ and NF-κB phosphorylation with inhibition of TNF-α and IL-6 production in endothelial cells. Meanwhile, diosgenin also remarkably inhibited endothelin-1 (ET-1) and plasminogen activator inhibitor 1 (PAI-1) production in the endothelial cells, and markedly restored the loss of insulin-mediated vasodilation.[87] Therefore, inhibiting VEGF and vascular endothelium of tumor angiogenesis is an important way to inhibit tumor growth and metastasis.

Inhibiting Tumor Cell Metastasis and Invasion

Clinically, many patients with malignant tumors have metastasis at the time of diagnosis, tumor cells can escape from the primary tumor site, and metastasis and invasion often take place at the same time.[88] Therefore, the prevention and inhibition of tumor metastasis is a key issue that needs to be paid attention to. A large number of studies have shown that the main mechanism of tumor cell migration and invasion is related to the reduction of matrix metalloproteinase (MMPs) expression, including MMP-2 and -9. The literature show

that diosgenin inhibited the transcription of MMP-2, -9, -7 mRNA gene and extracellular MMP inducer and reduced their activity.[30,32] In addition, diosgenin can significantly up-regulate the expression of E-cadherin, integrin α5 and β6, zona occludens 1 (ZO-1) and Cludin-1 proteins,[29] and down-regulate the expressions of N-cadherin, NMMC-cadherin, vimentin, ZEB1, EZH2, β-catenin protein[38] and PI3K/AKT signal pathway proteins PI3K, AKT, P-AKT,[27] regulate miR-145-5p/KLF5/HIF-1α pathway and Rho/ROCK signaling pathway,[89] thus inhibit the migration and invasion of human lung adenocarcinoma, gastric, bladder, pancreatic, prostate and colorectal cancer.

Blocking Cell Cycle

The anti-tumor mechanism of diosgenin is related to blocking tumor cell cycle. The cell cycle is a complex process referred to as G_1, S, G_2, and M phases that involves numerous regulatory proteins that direct the cell through a specific sequence of events culminating in mitosis and the production of two daughter cells. Central to this process are the cyclin-dependent kinases (CDKs), which complex with the cyclin proteins. These proteins regulate the cell's progression through the stages of the cell cycle and are in turn regulated by numerous proteins, including p53, p21, p16, and cdc25. Downstream targets of cyclin-CDK complexes include pRb and E2F.[90] Diosgenin caused G_2/M, S and G_0/G_1 phase arrest by decreasing cyclin B1 and p21chip1/Waf1 levels, increasing cdc2 levels, inhibiting PI3K-Akt and up-regulating the expression of p21 and p27, increasing the number of G_2 phase cells, regulating cycle-related gene CDK2/4/6, CyclinD1, which inhibited the proliferation of human chronic myeloid leukemia,[63] osteosarcoma,[69] OSCC,[22] hepatoma and pancreatic cancer.[38]

Regulating Immunity and Improve Gut Microbiome

Recently, gut microbiome, as a research hotspot human health, is a kind of microorganism with a large number in the human intestinal tract, which is considered as one of the important factors affecting the tumor.[91,92] In fact, many concepts of Chinese medicine (CM) coincide with modern research results of gut microbiome, and CM is also widely used to regulate gut microbiome disorders, and plays a very important role in restoring the dysfunctional gut microbiome.[93,94] Recent studies indicated that composition of the gut microbiome affects immune system development and modulates immune mediators.[95] Reasonably, as an active ingredient of CM, diosgenin had been found an important role in regulating gut microbiome.[96] Certainly, it could exert its anti-tumor effect by regulating immune balance and improving the composition of gut microbiome, the response to anti-PD-1 immunotherapy is related to gut microbiome and effector T cells in tumor microenvironment. Diosgenin can be used as a microecological regulator to induce anti-tumor immunity and improve the efficacy of immune checkpoint antibodies. In anti-bearing melanoma, diosgenin depends on anti-tumor immune effect and improves the composition of gut microbiome.[67] However, the research on the gut microbiome mechanism of anti-tumor effect of diosgenin is in the ascendant. In terms of regulating immunity, diosgenin also stimulated the expression of cytokine genes essential for the functioning of the human immune system IL-10, IL-1, IL-12, IL-4 and TNF-α in breast cancer and prostate cancer.[97,98] On the other hand, diosgenin significantly inhibited the expression of Ptgs2 and Ptges and decreased the number of COX-2 and membrane-bound prostaglandin e2 synthase 1 (mPGES-1) immunoreactive cells in hepatic sinusoid.[81]

Clinical Studies

Diosgenin has been used in clinical treatment of tumor to study drug resistance, pharmacokinetics and safety (Appendix 2). A clinical pilot study was conducted to investigate the efficacy and safety profile of Fuzheng Jiedu Xiaoji Formula (扶正解毒消积方, FJXF) in the cure of hepatocellular carcinoma (HCC). A total of 291 participants (mean age 54.45 years) were selected and treated orally with 300 mL of the herb decoction, one dose granule (25.5 g) of FJXF was added to 150 mL herbs once in the morning and once in the evening. One course of the FJXF treatment lasted for 12 weeks. HCC patients took the FJXF for one or two courses. The FJXF combined with transcatheter arterial chemoembolization (TACE) treatment significantly prolonged the 1-year overall survival (OS) and progression-free survival (PFS) of patients with HCC. After a literature search, 8 main components of FJXF, which may be related to the AKT/CyclinD1/p21/p27 pathway, were found: diosgenin, formononetin, chlorogenic acid (CGA), caffeic acid, luteolin, gallic acid, ergosterol endoperoxide, and lupeol.[99]

A randomized study was performed on 64 patients diagnosed with tumor cachexia (age: 35–75 years) and treated with Shenling Baizhu Powder (参苓白术散, SLP) for 4-weeks. SBP combined with nutritional support can improve Karnofsky Performance Score, decrease TNF-α, TWEAK, Fn14 expressions of the tumor cachexia patient. SBP control cancer cachexia maybe due to its regulation of these cytokines.[100] Diosgenin from Chinese yam has been confirmed to be one of the quality markers of the classic prescription SBP, it also may be one of the core active ingredients of SBP in treatment.[102]

A pharmacokinetic study with 24 cancer patients as subjects revealed that diosgenin has poor oral absorption rate (about 5 h to peak) and long t1/2 (average 30 h). Bioavailability decreased after taking diosgenin of the higher dose, the single oral of 1,200–2,400 mg showed the process of nonlinear dynamics. The single oral of 600–2,400 mg has good security in clinical.[103] A total of 34 tumor patients were randomly divided into 5 groups to observe the safety and tolerance of diosgenin in cancer patients for establishing the effective clinical dosage. In patients with dose range of 400–2,600 mg, there are not obvious adverse drug reactions. Therefore, 2–13 tablets (200 mg/tablet) of diosgenin are in the scope of safety in clinical practice.[104]

Further Study on Anticancer Effect of Diosgenin

More efficient, non-toxic and selective pathways of diosgenin anticancer are needed to solve the few sources of diosgenin products and its low bioavailability. The construction of targeted nano-drug carriers, the drug combination, and the diosgenin derivative are established to give full play to the anticancer effect of diosgenin (Figure 3).

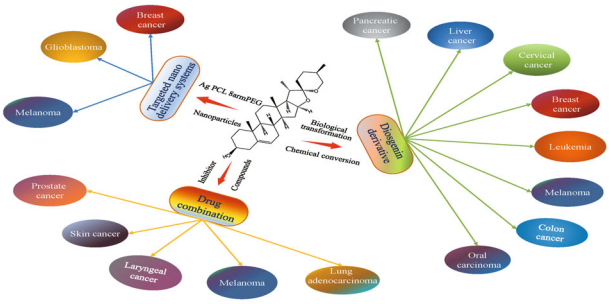

Figure 3. Highly Effective Anticancer Pathway of Diosgenin

Targeted Nano Drug Delivery System in Anticancer of Diosgenin

Nano-drug delivery systems (DDSs) hold immense potential in enhancement of drug therapeutic efficacy for their capability of preventing drug degradation and sustaining drug release.[105] Nano-drug delivery has good biocompatibility, high drug loading, good flexibility and controlled release, and has been widely used to deliver insoluble drugs to inhibit tumor. Numerous nano-DDSs carrying therapeutic agents are springing up unprecedentedly and adopted in promoting cancer healing of diosgenin, mainly including nanohydrogel and nanofbrous structures. Nanohydrogel is the 3-dimensional polymeric networks, the biocompatibility properties and uses of polycaprolactone (PCL) nanoparticles have been approved by the US Food and Drug Administration. PCL is a hydrophobic polymer formed by ring-opening polymerization of ε-caprolactone to form PCL and PCL-based nano-carriers can effectively inhibit the proliferation of breast cancer cells, while the diosgenin polycaprolactone nanoparticles (PCL-F68-D-NPs) with PluronicF68 as surfactant inhibit glioblastoma and it was found that the continuous release of diosgenin was due to the release of diosgenin wrapped in polymer matrix and the hydrophobic interaction of polymer-diosgenin in nanoparticles.[106,107]

Moreover, a novel pH-sensitive polymer prodrug of diosgenin and methoxy-polyethylene glycol-4-formylbenzoate (mPEG-CBA) nanoparticles (NPs) and an octagonal polyethylene glycol-diosgenin conjugate 8armPEG-DGN/HCPT polymer nanoparticles were synthesized to improve the synergistic therapeutic efficiency of diosgenin on tumor cells.[108,109] Inorganic nanoparticles refer to nanoparticles deprived from inorganic materials, including the metallic nanoparticles, carbon-based nanoparticles, ceramic nanoparticles, etc.[110] Diosgenin to synthesize silver nanoparticles could improve ascites lymphoma by regulating blood indexes and inhibiting peroxidation.[111] Iron oxide nanoparticles (IONPs) have gained immense importance recently as drug nanocarriers due to easy multifunctionalization, simultaneous targeting, imaging and cancer hyperthermia. Diosgenin functionalized IONPs can be considered as first diosgenin functionalized novel magnetic nanomedicine with antiproliferative, migration inhibiting and apoptosis inducing properties against breast cancer.[112]

Drug Combination

The anti-tumor effect of diosgenin combined with diosgenin played a stronger inhibitory activity than diosgenin alone, and its mechanism is consistent with the anti-tumor mechanism of diosgenin. Previous researches have shown that diosgenin could combinate with tumor necrosis factor-related apoptosis-inducing ligand,[46] PD-1 antibody,[67] GSK126,[29] autophagy inhibitor 3-methyladenine[51] and HIF-1α specific short hairpin RNA (shRNA)[27] which aggravate tumor necrosis and apoptosis of NSCLC, gastric cancer and prostate cancer via increasing the activation of caspase-8, -9, -3, Bid, PARP cleavage and Bax expressions, decreasing the expression of Bcl-2, inducing enhanced T cell response, inhibiting MAPK signal pathway, PI3K/Akt/mTOR signal pathway, targeting EZH2 via Rho/ROCK signaling mediated EMT. GSK126 is a highly selective EZH2 inhibitor and also a newly synthesized S-adenosylmethionine competitor. Moreover, diosgenin could play a role in application of compound CM with active ingredients,[2] for instance, diosgenin with thymoquinone could inhibit the proliferation and induce apoptosis in common skin cancer and laryngeal cancer though Akt and JNK phosphorylation.[100]

Diosgenin combined with formononetin, chlorogenic acid, caffeic acid, luteolin, gallic acid, ergosterol endoperoxide, and lupeol may function through the AKT/CyclinD1/p21/p27 pathways inhibiting hepatocellular carcinoma.[99]

Diosgenin Derivatives

Natural products derivatives are a major source of newly developed drugs, the current two main methods for generating diosgenin derivatives of antitumor are through standard organic synthesis or using biocatalysts.[113,114] As the most widely used method, chemical semi-synthesis rapidly produces a series of derivatives that are used for structure–activity relationship analysis by conjugating different substituent groups to the modifiable site via esterification, acylation, etherification, or click chemistry. A set of diosgenin derivatives that are substituted with various amino acids, glucoside, esters were synthesized from diosgenin. Glucosamine derivatives were common synthesized. (25R)-spirost-5-en-3β-ylO-β-D-glucopyranoside(3GD), (25R)-spirost-5-en-3β-yl O-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranoside (3GRD); and (25R)-spirost-5-en-3β-yl O-α-L-rhamnopyranosyl-(1→2)-O-[α-L-rhamnopyranosyl-(1→4)]-β-Dglucopyranoside), also known as dioscin, a natural, bioactive steroid saponin, has been widely used in anti-tumor research and it can inhibit proliferation of cervical cancer cells, suppresses TGF-β1-induced EMT and suppresses lung cancer migration and invasion, etc.[115-117] A potential anti-cancer compound from the leaf extract of *Lagerstroemia speciosa*, as a derivative of diosgenin, which can be a possible candidate for cancer therapeutics in future, against pancreatic cancer cells (PANC-1), and induce apoptosis.[118] 22-Oxo-26-selenocyanocholestanic steroids based on diosgenin synthesized including diosgenin 2-acetamido-2-deoxy-b-D-glucopyranoside, hecogenin 2-acetamido-2-deoxy-b-D-glucopyranoside, diosgenyl 2-amino-2-deoxy-b-D-glucopyranoside hydrochloride, exhibited remarkable antiproliferative activity against HeLa cells.[119]

Otherwise, carbamate derivatives have been synthesized at C26 of furostene ring after opening spiroketal bond (F-ring) of diosgenin. Furostene carbamate derivative 10 inhibited triple negative breast cancer cells significantly by arresting cell cycle at G_1 phase and induced apoptosis by activating caspase-3.[53] The necessity of the 1,4-quinones at C-3 position as well as the opening of the cyclic spiroketal revealed that the activities depended on the type of 1,4-quinone moiety, hybrid 11a upregulated Bax, Cl-caspase-3/9, and Cl-PARP levels, and downregulated Bcl-2 level of HepG2 cell line and hybrid 11a could increase the generation of intracellular reactive oxygen species.[120] DG-8d, bearing 1,3,4-oxadiazole/thiadiazole moieties, could down-regulate Bcl-2, PI3K, p-Akt and p-FOXO3a signal molecules, up-regulate Bax and Bim signal molecules, inhibit lung cancer cell proliferation and induce apoptosis by inhibiting PI3K/Akt signal pathway.[121] FZU-0021-194-P2 (P2) showed more potent cytotoxic activities against human NSCLC.[122] In addition, a fluorophore-appended derivative of diosgenin [Glc/CNHphth-diosgenin (GND)] was synthesized, starting from diosgenin and glucosamine hydrochloride in overall yields. GND induced autophagy to activate caspase-8-dependent liver cancer apoptosis.[123] A set of diosgenin derivatives substituted with various amino acids, a dipeptide or levulinic and 3,4-dihydroxycinnamic acid at C-3 position that have displayed a high antiproliferative activity towards breast and prostate cancer.[97] Azasteroids from the modification of the A and B rings of diosgenin inhibited the proliferation of breast cancer cells.[124] Recently, diosgenin derivatives spung up and these successful examples demonstrated that the steroidal structure of diosgenin is promising for the discovery of new anticancer drugs.

Summary

Tumor, a threat to human health, is a large group of diseases and the second leading cause of death worldwide. Lung, prostate, colorectal, stomach, and liver cancers are the most common types of cancer in men, whereas breast, colorectal, lung, cervical, and thyroid cancers are the most common among women.[125] Diosgenin, which arises from *Dioscorea*,[45] *Trigonella foenum graecum* (fenugreek),[33] *Dioscorea opposita* Thunb,[126] *Dioscorea zingiberensis* wright,[43] *Dioscorea villosa* Linn,[43] *Colanum* and *Costus* species,[27] *Rhizoma paridis*, legumes and yam,[39] *Solanum incanum* and *Solanum xanthocarpum*,[73] *Dioscorea nipponica*,[51] *Rhizoma polgonati*,[83] *Solanum lyratum* Thunberg (Solanaceae), has a certain therapeutic effect on both malignant and benign tumors. This review focuses on reviewing the anticancer species and related mechanisms of diosgenin, revealing the anticancer effects of different sources of diosgenin on tumors at different doses through multiple pathways, such as inhibition of tumor cell proliferation and growth, promotion of apoptosis, induction of differentiation and autophagy, inhibition of metastasis and invasion, cell cycle blockade, regulation of immunity and improvement of the gut microbiome, showing potential for the treatment of cancer.

These mechanisms are closely related to signaling pathways and oncogenic factors. MAPK is an important

transmitter that carries signals from the cell surface to the interior of the nucleus, phosphorylated extracellular signal-regulated kinase (pERK) is part of the MAPK network and the ERK MAPK pathway is one of the most important for cell proliferation.[70] Equally, PI3Ks are crucial coordinators of intracellular signaling in response to the extracellular stimulators, the hyperactivation of PI3K signaling pathway is one among the most ordinary events in human cancers.[127] Akt is a serine/threonine kinase and it participates in the key role of the PI3K signaling pathway, once activated, Akt modulates the function of many downstream proteins involved in cellular survival, proliferation, migration, metabolism, and angiogenesis.[128] Furthermore, the typical tumor suppressor p53 is often mutated in regulating cell proliferation, senescence, DNA repair, and cell death.[77] Moreover, NF-κB signal pathway is increasingly recognized as a crucial player in many steps of cancer initiation and progression and it is one of the best understood immune-related pathways. Above all, NF-κB plays a major role in the host response to microbial infection through orchestrating innate and adaptive immune functions.[129-131]

Noticeably, gut microbiome is receiving significant attention and has been found to be closely related to a variety of tumors, evidence suggesting that modulating the gut microbiome may affect responses to numerous forms of cancer therapy.[132] Hence, in view of the above results we speculate that MAPK, p53, AKT and NF-κB may be targets for cancer treatment by diosgenin. Clinical investigations have revealed diosgenin's clinical dosage and safety property. However, there are also some problems in clinical research, such as small amount of data, incomplete and insufficient research scope. Previous study has shown that diosgenin has poor water solubility, short circulation time *in vivo*, low biocompatibility, and unsatisfactory pharmacokinetic properties, which limit the clinical application of diosgenin.[108] To improve the bioavailability of diosgenin in tumors, their efficacy can be further improved by nano-drug carriers, combination with other drugs and design of higher active diosgenin derivatives. However, participating in the construction of targeted nano-drug carriers and combined drug use are still in their infancy and need to be further studied, and the diosgenin derivatives are much more than what is mentioned in this review. Certainly, diosgenin could synthesize labeled probe with biotin and exert the same anti-tumor activity as diosgenin.[133] Nonetheless, complete knowledge about the biosynthesis pathway of diosgenin is still elusive, which needs to be further investigated.[134]

In conclusion, diosgenin is an important anticancer natural product, its mechanisms have multiple targets, multiple pathways, and multiple effect nature. Presently, some more efficient anticancer pathways of diosgenin have been excavated. However, the antitumor microbial mechanism of diosgenin is in the ascendant and further well-designed clinical trials are needed to observe the gut microbiome's effect in clinical application of diosgenin.

Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Author Contributions

Ren QL, Liu JG and Wang Q designed the study and wrote the initial draft. Ren QL, Zhang XQ, Wang M, Hu H and Li XL drafted the article or revised it critically for important intellectual content. Ren QL, Tang JJ, Yang XT, Ran YH, Liu HH, and Song ZX participated in final approval of the version to be submitted. All authors were involved in reviewing and editing of the manuscript.

Data Availability

The datasets used during the current study are available from the corresponding author upon reasonable request.

Electronic Supplementary Material: Supplementary material (Appendixes 1–2) is available in the online version of this article at https://doi.org/10.1007/s11655-023-3693-1.

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