

Anticancer effects of ginsenoside Rg3 (Review)

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Abstract. Cancer is a life-threatening disease with an alarmingly increased annual mortality rate globally. Although various therapies are employed for cancer, the final effect is not satisfactory. Chemotherapy is currently the most commonly used treatment option. However, the unsatisfactory efficacy, severe side-effects and drug resistance hinder the therapeutic efficacy of chemotherapeutic drugs. There is increasing evidence indicating that ginsenoside Rg3, a naturally occurring phytochemical, plays an important role in the prevention and treatment of cancer. The suggested mechanisms mainly include the induction of apoptosis, and the inhibition of proliferation, metastasis and angiogenesis, as well as the promotion of immunity. In addition, ginsenoside Rg3 can be used as an adjuvant to conventional cancer therapies, improving the efficacy and/or reducing adverse effects via synergistic activities. Ginsenoside Rg3 may be a widely applied natural medicine against cancer. To date however, there is no systematic summary available of the anticancer effects of ginsenoside Rg3. Therefore, in this review, all available literature over the past 10 years was reviewed and discussed in order to facilitate further research of ginsenoside Rg3.

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1. Introduction

Cancer, characterized by uncontrolled cell growth, is considered to be the second leading cause of mortality (30% of total mortality) worldwide, which seriously threatens public health (1). A report released by the World Health Organization on the global situation of cancer in 2012 indicated that the annual new cancer cases reached 14,000,000 globally, with 8,200,000 deaths, and the number of new cases is expected to increase to 24,000,000 in the year 2035 (2). The global cancer burden is currently growing at an alarming rate, and there is no effective available treatment to date to curb the spread of cancer.

At present, the clinical management of cancer always involves several conventional modalities. Surgical resection is an effective method for the treatment of tumors in the early stages and for the clinical treatment of local tumors. However, surgery is often ineffective once tumors have spread or are diagnosed at an advanced and/or late stage of the disease. The prognosis remains poor due to tumor recurrence, diffusion and even metastasis following surgery (3). Radiotherapy is more effective than surgery in many cases. However, its application is limited by tumor metastasis and various side-effects. Recently, biotherapies such as immunotherapy, gene therapy and monoclonal antibody therapy have become a hotspot and provide a new method with which to prevent and treat tumors. Since many aspects are still unclear, tumor biotherapy has not been widely used (4). Chemotherapy is currently the most commonly used treatment option for cancer. However, the administration of traditional chemotherapeutic agents at high doses always induce significant non-selective toxicities, such as a reduction in bone density and immunosuppression, while low or moderate doses of these agents usually does not exert significant antitumor effects (5); in addition, there is the issue of acquired drug resistance. Therefore, the development of novel agents which can selectively induce cancer cell death without threatening normal cells is of utmost importance.

It has been generally recognized that natural products play a unique therapeutic role in the treatment of a number of diseases. Ginseng, the root of *Panax ginseng* C.A. Meyer, has been widely used in East Asia countries for thousands years as a natural tonic (6). Ginsenosides, extracted from *Panax ginseng* C.A. Meyer, are the main active components with a wide range of pharmacological activities. More than 100 types of ginsenosides have been isolated and determined

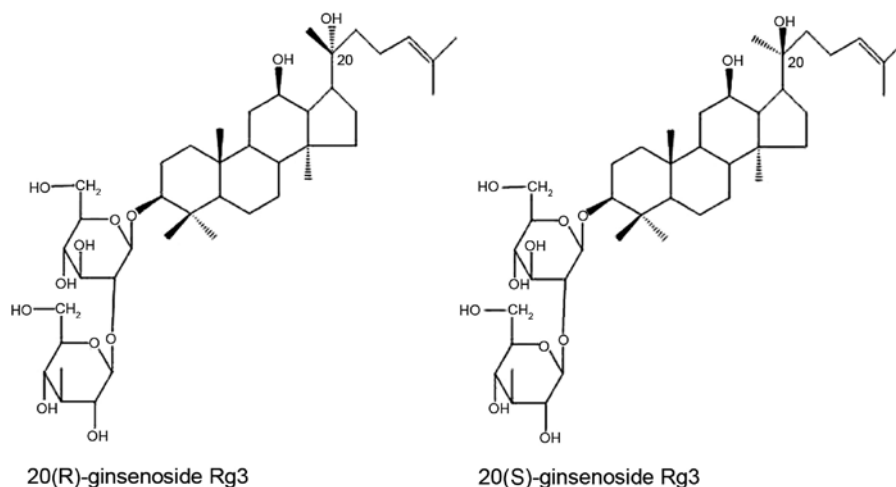


Figure 1. Chemical structures of 20(R)-ginsenoside Rg3 and 20(S)-ginsenoside Rg3.

from ginseng (7). Among these ginsenosides, ginsenoside Rg3 has been shown to have significant physiological activities (8), such as hepatoprotection, neuroprotection, cardiovascular-protection, promotion of immunity, as well as anti-fatigue, antioxidant, and most importantly, antitumor effects (9-14). There is increasing evidence indicating that ginsenoside Rg3 exerts antitumor effects in a number of cancer models, such as lung, liver and breast cancer (15-17). Furthermore, ginsenoside Rg3 may be a beneficial supplement, and the combined administration of ginsenoside Rg3 and conventional chemotherapeutic drugs may be more effective than either one being administered alone. The use of ginsenoside Rg3 in cancer therapy may aid in the prevention of toxicity and morbidity associated with conventional chemotherapy, even though the underlying mechanisms have not yet been fully elucidated (18). Thus, in this review, we aimed to provide a systematic summary on the cancer-preventive effects of ginsenoside Rg3.

2. Chemical structure

Ginsenoside Rg3 is a type of tetracyclic triterpenoid saponin, rich in red ginseng. The major ginsenosides, such as Rb1, Rb2, and Rd can be readily converted into ginsenoside Rg3, the production of which may increase from 0.37 to 1.32% (w/w) by heating (19). Due to the different spatial structures from C20 positions, there are two enantiomers, 20(R) and 20(S)-isomer (Fig. 1) (20). With differential configuration, their antitumor activities exhibit certain differences (21).

3. Effects of Rg3 in cancer models

Natural products have always been a very good source of drugs against cancer and have gained much attention lately. For example, paclitaxel is one of the most important antitumor natural agents isolated from the bark of Pacific yew tree (22). A study carried out in 1978 demonstrated the inhibition or prevention of carcinogenesis induced by various chemical carcinogens for ginseng (23). Ginsenoside Rg3 has been found to be an effective ingredient contributing to the anticarcinogenic activity of ginseng. To provide a summary of the

antitumor effects of ginsenoside Rg3, we collected data from previous scientific studies published over the last 10 years. Ginsenoside Rg3 has been shown to possess significant anticancer activity and it may be used alone (Table I) or as a supplement to chemotherapeutic drugs in order to improve the therapeutic efficacy and minimize or eliminate drug-induced toxicity and chemotherapeutic resistance (Table II).

Treatment with Rg3 significantly has been shown to inhibit the growth of cancer in various cancer models. Ginsenoside Rg3 has been shown to exert cancer-preventive effects in both *in vitro* and *in vivo* studies in a dose- and time-dependent manner. The protective effects of Rg3 are mainly related to the induction of apoptosis, and the inhibition of proliferation, metastasis and angiogenesis (Table I). Table II displays the combined treatment effects of both Rg3 and several existing chemical drugs on cancer models. Ginsenoside Rg3 has been shown to enhance the antitumor effects of conventional chemotherapeutic agents and to reduce drug-induced toxicity and chemotherapeutic resistance *in vitro* and *in vivo*. Combination therapies using chemotherapeutic agents and ginsenoside may be an innovative and promising therapeutic strategy for the treatment of human cancer. However, the pharmacodynamic interactions between chemotherapeutic drugs and Rg3 warrant further investigation.

In addition, the hepatic arterial administration of Rg3 combined with local transarterial embolization (TAE) was shown to more effectively inhibited VX2 liver tumor growth than any mono-therapies, with the inhibition of angiogenesis and the induction of caspase-dependent apoptosis (24).

4. Suggested mechanisms of action of Rg3

Cancer is a class of diseases involving genetic damage which alters several intracellular biochemical signals and eventually results in uncontrolled cell growth (25). Drugs with antitumor activity, such as Rg3 may be able to alter these abnormal alterations through certain mechanisms. Although the anticancer activities of Rg3 have been widely investigated, the exact molecular mechanisms are not yet clear. The possible mechanisms of action of Rg3 based on the existing studies are described as follows:

Table I. Summary of the anticancer activities of ginsenoside Rg3.

Cancer	Ginsenoside Rg3	Observation	Cell type	Effects	Mechanisms of action	Refs.
Breast cancer	Rg3	<i>In vitro</i> (30 μ M)	MDA-MB-231	Induction of apoptosis	Inhibition of mutant p53 and NF- κ B signaling via possibly inactivation of ERK and Akt to activate mitochondrial death pathway	(17,35)
Breast cancer	20(S)-ginsenoside Rg3	<i>In vitro</i> (100-300 μ M)	MCF-7 MDA-MB-231	Inhibition of proliferation	Arrested the cells in the G1-phase	(46)
Breast cancer	20(S)-ginsenoside Rg3	<i>In vitro</i> (20-60 μ g/ml)	MDA-MB-231	Inhibition of metastasis	Inhibition of the expression of CXCR4	(48)
Colon cancer	20(S)-ginsenoside Rg3	<i>In vitro</i> (10-100 μ M)	HT-29	Inhibition of proliferation, induction of apoptosis	Activation of caMKK β /AMPK mediated apoptosis mainly by regulating the mitochondrial pathway involving p53/Bcl-2/Bax/cytochrome <i>c</i> /caspase-3, caspase-9/PARP	(36)
Colon cancer	20(S)-ginsenoside Rg3	<i>In vitro</i> (\geq 100 μ M)	HT-29	Inhibition of proliferation, induction of apoptosis	Reduced PCNA, STRAP and other protein related to mitosis and DNA repair, downregulated Rho-GDI while upregulated TM1, GSTP1 and Annexin	(42)
Colon cancer	20(S)-ginsenoside Rg3	<i>In vitro</i> (100-300 μ M)	HCT116	Inhibition of proliferation	Mostly by regulating the Eph/ephrin pathway and gene expression of AKAPA8L and PTPNA	(44)
Colon cancer	Rg3	<i>In vitro</i> (above 100 μ M) <i>in vivo</i> (20 mg/kg)	HCT116 SW480	Inhibition of proliferation, inhibition of tumor growth in xenograft model	Blocked the nuclear translocation of β -catenin and then inhibited β -catenin/Tcf signal pathway	(43)
Colon cancer	Rg3	<i>In vitro</i> (200 μ M)	SW480	Inhibition of metastasis	Inhibited NF- κ B signaling pathway and the NF- κ B-regulated gene expression such as c-Myc, COX-2 and MMP-9	(52)
Esophageal carcinoma	Rg3	<i>In vitro</i> (25-200 μ M)	Eca-109	Inhibition of proliferation	Suppressed VEGF expression by blocking multiple signaling pathways including HIF-1 α , COX-2, NF- κ B, STAT3 and MAPKs	(56)
Renal cancer			786-0	and angiogenesis		
Gallbladder	20(S)-ginsenoside Rg3	<i>In vitro</i> (25-400 μ M) <i>in vivo</i> (20 and 40 mg/kg)	GBC-SD NOZ	Cycle arrest, induction of apoptosis, inhibition of the growth of xenografts	Suppressed MDM2 to activate the p53 pathway, induced cycle arrest at G ₀ /G ₁ and mitochondrial-dependent apoptosis	(34)
Gallbladder	Rg3	<i>In vitro</i> (10-100 μ g/ml) <i>in vivo</i> (20 mg/kg)	GBC-SD QBC939 Mz-ChA-1	Induction of apoptosis, inhibition of the growth of tumor xenografts	Activation of ER stress to regulate apoptosis related proteins such as caspase-12, CHOP	(38)

Table I. Continued.

Cancer	Ginsenoside Rg3	Observation	Cell type	Effects	Mechanisms of action	Refs.
Gastric cancer	20(S)-ginsenoside Rg3	<i>In vitro</i> (25-100 μ M)	AGS	Induction of apoptosis, inhibition of proliferation	Upregulation of caspase-3, caspase-8, caspase-9 and Bax while downregulation of Bcl-2	(32)
Gastric cancer	Rg3	<i>In vitro</i> (50 μ g/ml)	SGC-7901	Induction of apoptosis	Inhibition of the expression of FUT4 via SP1 and HSF1 transcriptional regulation and eventually, activation of caspase-3, caspase-8 and caspase-9	(37)
Gastric cancer	Rg3	<i>In vitro</i> (200-500 μ M)	AGS	Induction of apoptosis	Blocked TRPM7 channel activity	(39)
Glioblastoma multiforme	Rg3	<i>In vitro</i> (≥ 10 μ M)	U87MG	Induction of apoptosis	Suppression of MEK/MAPK signaling pathway, activation of ROS by antioxidant enzyme system	(30)
Glioma	20(S)-ginsenoside Rg3	<i>In vitro</i> (20 μ M)	U87	Inhibition of proliferation	Induced cell senescence by activating Akt and p53/p21 dependent signaling pathways	(45)
Lung cancer	Rg3	<i>In vitro</i> (50-100 μ M) <i>in vivo</i> (30 mg/kg)	A549	Induction of apoptosis, inhibition of proliferation, inhibition of tumor growth	Inhibited EGFR activation and its downstream signal transduction, induced caspase-dependent apoptotic pathway	(15)
Lung cancer	20(R)-ginsenoside Rg3	<i>In vitro</i> (25 and 50 μ g/ml)	A549	Inhibition of metastasis	Inhibited TGF- β 1 induced EMT via downregulation of Snail and inactivation of MMP-2, p38 MAPK and Smad2	(53)
Liver cancer	20(S)-ginsenoside Rg3	<i>In vitro</i> (≥ 10 μ M)	Hep3B	Induction of apoptosis	Upregulation of ROS, Bax, the release of cytochrome <i>c</i> and caspase-3, down-regulation of Bcl-2, MMP	(16)
Liver cancer	Rg3	<i>In vitro</i> (50-200 μ g/ml) <i>in vivo</i> (3.0 mg/kg)	Hep1-6 HepG2	Induction of apoptosis, inhibition of tumor growth and increased survival time of tumor-bearing mice	Decrease of Bcl-2 and Bcl-xL while increase of Bax, caspase-3 and the release of cytochrome <i>c</i>	(29)
Liver cancer	Rg3	<i>In vitro</i> (25-100 μ g/ml)	SMMC-7721 HepG2	Inhibition of proliferation, induction of apoptosis	Induction of caspase-dependent endogenous apoptotic pathway	(33)
Melanoma	20(R)-ginsenoside Rg3	<i>In vitro</i> (25-100 μ g/ml) <i>in vivo</i> (20 mg/kg)	A375	Inhibition of, proliferation inhibition of the growth of tumor xenografts	Reduction of FUT4 and LeY to inhibit EGFR/MAPK signaling pathway	(41)
Melanoma	20(R)-ginsenoside Rg3	<i>In vitro</i> (50 μ g/ml) <i>in vivo</i> (20 mg/kg)	A375 C8161	Inhibition of proliferation, inhibition of xenograft tumor volume and weight	Decreased the expression of HDAC3, increasing p53 acetylation and transcription activity, induced cell cycle arrest at G ₀ /G ₁	(47)

Table I. Continued.

Cancer	Ginsenoside Rg3	Observation	Cell type	Effects	Mechanisms of action	Refs.
Melanoma	20(S)-ginsenoside Rg3	<i>In vitro</i> (40-100 μ g/ml) <i>in vivo</i> (1.5 mg/kg)	B16	Inhibition of proliferation, induction of apoptosis, inhibition of metastasis, increased the survival time	Regulation of cell cycle and the expression of caspase and Bcl-2, inhibition of angiogenesis	(31)
Melanoma	20(S)-ginsenoside Rg3	<i>In vitro</i> (25-100 μ M)	B16F10	Inhibition of metastasis	Inhibited MMP-13 expression by the p38 MAPK signaling pathway	(51)
Ovarian cancer	20(S)-ginsenoside Rg3	<i>In vitro</i> <i>in vivo</i>	SKOV-3 (160 μ g/ml) 3AO (80 μ g/ml)	Induction of apoptosis, inhibition of proliferation, inhibition of the growth of tumor xenografts	Suppression of Warburg effect though the STAT3/HK2 pathway	(91)
Ovarian cancer	20(S)-ginsenoside Rg3	<i>In vitro</i> (25-100 μ g/ml)	HO-8910	Induction of apoptosis	Regulation of PI3K/Akt and XIAP pathways to activate caspase-3 and caspase-9	(28)
Ovarian cancer	20(S)-ginsenoside Rg3	<i>In vitro</i> <i>in vivo</i> (5 mg/kg)	SKOV-3 (80 μ g/ml) 3AO (160 μ g/ml)	Inhibition of metastasis, inhibition of tumor growth and metastasis	Blocked hypoxia-induced EMT by decreasing HIF-1 α expression	(54)
Ovarian cancer	Rg3	<i>In vitro</i> (2.5, 5.0 μ g/ml) <i>in vivo</i> (0.3-3 mg/kg)	SKOV-3	Inhibition of metastasis, and angiogenesis, inhibition of lung metastasis of ovarian cancer	Inhibition of angiogenesis, tumor cell invasive ability, as well as the expression of MMP-9	(50)
Pancreatic cancer	Rg3	<i>In vitro</i> (25-200 μ M) <i>in vivo</i> (5, 10, 20 mg/kg)	SW-1990	Inhibition of VM and the growth of tumor xenografts	Inhibition of the expression of particular genes including VE-cadherin, EphA2, MMP-2 and MMP-9	(60)
Prostate cancer	20(S)-ginsenoside Rg3	<i>In vitro</i> (above 100 μ M)	PC3 LNCaP	Inhibition of proliferation	Inhibition of DNA synthesis and cancer cells attachment, regulation of three MAP kinase activity, including ERKs, p38 MAP kinases and JNK	(40)
Prostate cancer	20(S)-ginsenoside Rg3	<i>In vitro</i> (1 and 10 μ M)	PC-3M	Inhibition of migration	Transcriptionally inhibited the expression of AQP1 via the p38 MAPK pathway	(55)

NF- κ B, nuclear factor- κ B; ERK, extracellular signal-regulated kinase; PARP, poly (ADP-ribose) polymerase; PCNA, proliferating cell nuclear antigen; STRAP, serine/threonine kinase receptor associated protein; AKAPA8L, A-kinase (PRKA) anchor protein 8-like; PIP3, phosphatidylinositol transfer protein α ; COX-2, cyclooxygenase-2; MMP-2, matrix metalloproteinase 2; HIF-1 α , hypoxia-inducible factor-1; ROS, reactive oxygen species; STAT3, signal transducer and activator of transcription 3; HK2, hexokinase 2; p38 MAPK, p38 mitogen activated protein kinase; MDM2, murine double minute 2; ER, endoplasmic reticulum; CHOP, C/EBP homologous protein; FUT4/LeY, fucosyltransferase IV/Lewis Y; HSF1, heat shock factor protein 1; SP1, specificity protein 1; TRPM7, transient receptor potential melastatin 7; EGFR, epidermal growth factor receptor; TGF- β 1: transforming growth factor- β 1; MMP, mitochondrial membrane potential; HDAC3, histone deacetylase 3; AMPK, AMP-activated protein kinase; EMT, epithelial-mesenchymal transition; PI3, phosphoinositide-3-kinase; CXCR4, CXC receptor 4; VM, vasculogenic mimicry; VEGF, vascular endothelial growth factor; VE-cadherin, vascular endothelial-cadherin; EphA2, epithelial cell kinase; AQP1, aquaporin 1.

Table II. Combination therapy with ginsenoside Rg3 and conventional chemotherapeutic drugs.

Drug	Cancer	Observation	Cell type	Activity	Mechanisms	Refs.
As ₂ O ₃	Lung cancer	<i>In vitro</i> <i>in vivo</i>	NCI-H1299	Enhanced the antitumor efficacy, prolonged the survival time of tumor-bearing mice	Promoted proliferation inhibition and apoptosis induction	(62)
Cisplatin	Bladder cancer	<i>In vitro</i>	T24R2	Enhanced the antitumor activity	Stimulated cell arrest at G2/M, activated intrinsic apoptotic pathway	(61)
Cisplatin	Colon cancer	<i>In vivo</i>		Augmented the anti-neoplastic activity of cisplatin, decreased cisplatin-induced tissue damage in the kidney and liver, inhibited resistance to chemotherapeutics	Reduction of the level of nuclear Nrf2, HO-1/NQO-1 and ROS	(72)
Cyclophosphamide		<i>In vivo</i>		Suppressed CP-induced side effects	Inhibited CP-induced oxidative stress by regulating the activities of SOD and GPx, and MDA contents	(6)
Cyclophosphamide	Ovarian cancer	<i>In vivo</i>		Reinforced each other's antitumor activity, decreased CP-induced side-effects	Not investigated	(64)
Cyclophosphamide	Lewis lung cancer	<i>In vivo</i>		Augmented the antitumor and anti-angiogenesis activity, reduced drug-induced toxicity and prolonged the survival time	Inhibited the expression of Ki-67, VEGF, Bcl-2 and p53	(65)
Docetaxel	Prostate cancer	<i>In vitro</i>	LNCaP PC-3 DU145	Augmented susceptibility of cancer cells to docetaxel	Inactivation of NF-κB and then regulation of its target gene expression such as Bax and caspase-3	(70)
Docetaxel	Colon cancer	<i>In vitro</i>	HCT116 SW620	Potentiated sensitivity of cancer cells to docetaxel	Inhibition of NF-κB to regulate the expression of pro-apoptotic protein and anti-apoptotic protein	(71)
Doxorubicin	Hepatocellular carcinoma	<i>In vitro</i> <i>In vivo</i>	SK-Hep1 HepG2 Huh-7 Hep3B	Sensitized doxorubicin-induced cancer cell death	Inhibited autophagy possibly by blocking lysosomal function via regulating gene expression such as CHOP	(74)
Doxorubicin		<i>In vivo</i> <i>in vitro</i>	CMEC	Suppressed doxorubicin induced cardiotoxicity	Improved cardiac function and endothelial dysfunction at least partially by activating the Nrf2/ARE and Akt pathway	(63)
Gemcitabine	Lung cancer	<i>In vivo</i>		Inhibited gemcitabine induced side-effects, enhanced each other's antitumor activity	Not investigated	(18)

Table II. Continued.

Drug	Cancer	Observation	Cell type	Activity	Mechanisms	Refs.
Paclitaxel and cisplatin	Esophageal squamous cell carcinoma	<i>In vivo</i>		Enhanced the antitumor efficacy of chemotherapy	Lowered the tumor microvascular density and the Ki-67 expression	(66)
Paclitaxel	Breast cancer	<i>In vitro</i> <i>in vivo</i>	Caco-2	Increased the bioavailability and antitumor effect of paclitaxel lowered paclitaxel induced toxicity	Inhibited P-gp in tumor tissue, accelerated the distribution of paclitaxel to tissues and avoided a high plasma concentration	(67)
TRAIL	Hepatocellular carcinoma	<i>In vitro</i> <i>in vivo</i>	HepG2, SK-Hep1, Huh-7, Hep3B	Sensitized TRAIL-induced HCC cell apoptosis	Increased of TRAIL receptors DR5 expression by CHOP upregulation	(73)

CP, cyclophosphamide; CMEC, cardiac microvascular endothelial cells; HO-1/(NQO)-1, heme oxygenase/NAD(P)H quinone oxidoreductase-1; Nrf2/ARE, nuclear factor erythroid 2-related factor/antioxidant response element; TRAIL, TNF related apoptosis-inducing ligand.

Induction of apoptosis. Apoptosis, known as programmed cell death, is one of the principal mechanisms which maintains cellular homeostasis. In human cancers, tumor cells proliferate more rapidly than normal cells and always lose appropriate apoptotic control. This disruption of growth balance can promote tumor development (26). As shown in Table I, Rg3 can induce apoptosis via two major pathways: the mitochondrial-dependent intrinsic apoptotic pathway and the death receptor-dependent extrinsic pathway. Mitochondrial-dependent apoptosis involves a reduction in mitochondrial membrane potential, which results in the release of cytochrome *c* from the mitochondria, and the activation of caspase-9 and caspase-3, and finally, apoptosis (27). It has been shown that 20(S)-ginsenoside Rg3-induced apoptosis is dependent on the activation of caspase-3 and caspase-9, which is mediated by the PI3K pathway in HO-8910 human ovarian cancer cells (28). Reactive oxygen species (ROS) and Bcl-2 family members play an important role in the mitochondrial apoptotic pathway. Ginsenoside Rg3 has been shown to activate intracellular ROS generation and/or increase the ratio of Bax to Bcl-2 protein to induce mitochondrial-dependent apoptosis (16,29-33). p53 and nuclear factor (NF)- κ B, two nuclear transcription factors, play a critical role in mitochondrial membrane potential through the regulation of the expression of apoptosis-related genes, such as inhibitors of apoptosis proteins (IAPs), X-linked inhibitor of apoptosis protein (XIAP) and Bcl-2. p53 is an important pro-apoptotic factor, while NF- κ B is a type of anti-apoptotic factor. 20(S)-ginsenoside Rg3 exerts cytotoxic effects by activating the p53 pathway and subsequently inducing mitochondrial-dependent apoptosis (34). Moreover, ginsenoside Rg3 has been shown to inhibit mutant p53 and NF- κ B signaling, possibly via the inactivation of extracellular signal-regulated kinase (ERK) and Akt to activate the mitochondrial death pathway, including the reduction of the ratio of Bcl-2/Bax expression, the disruption of mitochondrial membrane potential, the activation of caspase-3 and ROS generation (17,35). p53 and Bax have also been shown to play a role in the 20(S)-Rg3-induced apoptosis of

HT-29 colon cancer cells via the activation of 5' AMP-activated protein kinase (36).

The extrinsic apoptotic pathway is activated by specific ligands, such as Fas ligand, tumor necrosis factor- α (TNF- α), and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). These receptors can activate a caspase-8-dependent cascade to induce apoptosis (21). Another previous study demonstrated that ginsenoside Rg3 induced apoptosis through the activation of caspase-8 in the human gastric cancer cell line, AGS (37). The increased expression of Fas has also been observed in Rg3-treated A549 cells (15). In addition, the occurrence of extrinsic apoptosis with the activation of endoplasmic reticulum stress mediates ginseng Rg3-induced apoptosis (38).

In addition, ion channels are crucial to tumor growth and cancer cell survival. Kim *et al* suggested that the blockade of TRPM7 channels played an important role in the Rg3-induced apoptosis of AGS cells, a human gastric adenocarcinoma cell line (39).

Inhibition of proliferation. Ginsenoside Rg3 has been shown to exert significant inhibitory effects on cancer cell proliferation. In LNCaP and PC3 human prostate carcinoma cell lines, 20(S)-ginsenoside Rg3 was shown to exhibit good growth inhibitory activity, which may be associated with the modulation of mitogen-activated protein (MAP) kinases (40). Rg3 inhibited EGFR activation and its downstream signal transduction to suppress the growth and proliferation of lung cancer cells A549 (15). Another study on melanoma suggested that 20(R)-ginsenoside Rg3 inhibited cancer cell proliferation by the deactivation of EGFR and the decrease of fucosyltransferase IV (FUT4)/Lewis Y (LeY) expression (41). In addition, the protein expression of proliferating cell nuclear antigen (PCNA), serine/threonine kinase receptor associated protein (STRAP) and that of proteins related to mitosis and DNA repair was shown to be suppressed in 20(S)-ginsenoside Rg3-treated human colon cancer cell lines (42). Ginsenoside Rg3 has also been shown

to inhibit the nuclear translocation of β -catenin, an oncogene, and the proliferation of colon cancer cell lines (43). Microarray hybridization analysis has shown 20(S)-ginsenoside Rg3 inhibits the proliferation of HCT116 cells, mostly by regulating the Eph/ephrin pathway and the gene expression of anchor protein 8-like (AKAPA8L) and phosphatidylinositol transfer protein alpha (PITPNA) (44). Additionally, chronic treatment with 20(S)-ginsenoside Rg3 at a sub-apoptotic concentration has been shown to induce senescence-like growth arrest in human glioma cells through the Akt and p53/p21 pathways (45).

Cell cycle arrest involves a set of events, resulting in cell growth inhibition. 20(S)-ginsenoside Rg3 has been shown to have an anti-proliferative activity in MCF-7 human breast cancer cells by arresting the cell cycle at the G₁-phase (46). p53, a tumor suppressor, plays an important role in mediating cell cycle arrest and the DNA damage response or apoptosis, as well as p21, a cell cycle inhibitor, downstream of p53. 20(S)-ginsenoside Rg3 has been found to have the ability to inhibit mouse double minute 2 homolog (MDM2), a negative regulator of p53, to activate the p53/p21 pathway and subsequently induce cycle arrest at the G₀/G₁ phase in gallbladder cancer cells (34). Moreover, was previously demonstrated that in both A375 and C816 melanoma cell lines, 20(R)-ginsenoside Rg3 inhibited proliferation and induced cell cycle arrest at the G₀/G₁ phase by decreasing the expression of histone deacetylase (HDAC)3 and increasing p53 acetylation and transcriptional activity (47).

Inhibition of metastasis. Metastasis is a complex process through which cancer cells spread from a primary site to form tumors at other distant parts of the body. Tumor metastasis is a major cause of tumor recurrence and mortality, in many types of cancer, such as breast cancer (48). Matrix metalloproteinases (MMPs), which can degrade the extracellular matrix and basement membrane, play an important role in tumor metastasis, and MMP-2 and MMP-9 are of particular importance in these events (49). As previously demonstrated, the Rg3-induced downregulation of MMP-9 appears to be associated with the decreased invasive capacity of SKOV-3 ovarian cancer cells (50). The suppression of MMP-13 by the p38 MAP kinase signaling pathway has been shown to play a role in the inhibition of the metastasis of B16F10 cells by 20(S)-ginsenoside Rg3 in (51). In addition, ginsenoside Rg3 has been shown to inhibit the migration of SW480 colon cancer cells by suppressing NF- κ B activity and the expression of NF- κ B-regulated gene products, including MMP-9, cyclooxygenase (COX)-2 and c-Myc (52).

EMT is also an important mechanism involved in cancer metastasis. 20(R)-ginsenoside Rg3 and 20(S)-ginsenoside Rg3 possess the ability to inhibit tumor metastasis by the suppression of EMT. In a previous study, 20(R)-ginsenoside Rg3 suppressed lung cancer migration or/and invasion by inhibiting TGF- β 1-induced EMT accompanied by the inactivation of MMP-2, p38 MAPK and Smad2 (53). In another study, 20(S)-ginsenoside Rg3 effectively suppressed hypoxia-induced EMT, inhibiting ovarian cancer metastasis (54).

In the breast cancer cell line, MDA-MB-231, 20(S)-ginsenoside Rg3 was reported to exert anti-metastatic effects by inhibiting the expression of C-X-C chemokine receptor type 4 (CXCR4), which is a vital molecule in migration (48).

Moreover, 20(S)-ginsenoside Rg3 has been shown to decrease the incidence of metastasis by inhibiting the expression of aquaporin 1 (AQP1) in PC-3M prostate cancer cells (55).

Inhibition of angiogenesis. Tumor angiogenesis is a process involving the formation of new blood vessels, which is essential to tumor growth and metastasis by supplying oxygen and nutrients. Rg3 has been found to inhibit tumor angiogenesis via the suppression of vascular endothelial growth factor (VEGF) expression by blocking multiple hypoxia-induced and angiogenesis-related signaling pathways in the human esophageal carcinoma cell line, Eca-109, and in the renal cell carcinoma cell line, 786-0 cells (56). In addition, both *in vitro* and *in vivo* experiments have demonstrated that 20(R)-ginsenoside Rg3 significantly inhibits human umbilical vein endothelial cell (HUVEC) proliferation and VEGF- and basic fibroblast growth factor (bFGF)-stimulated angiogenesis (57). Endothelial progenitor cells (EPCs) are closely related to tumor angiogenesis by promoting angiogenic factors, such as VEGF. In human umbilical cord blood (hUCB)-derived CD34-positive stem cells, treatment with Rg3 was shown to inhibit EPC differentiation and tube formation via the VEGF-dependent Akt/endothelial nitric oxide synthase (eNOS) signaling pathway (58). Rg3 also was found to effectively inhibit EPC proliferation and VEGF-induced angiogenesis *in vivo* through multiple signaling cascades, such as p38 MAP kinase (59).

Furthermore, vasculogenic mimicry (VM), a novel tumor microcirculation system different from classical endothelium-dependent angiogenesis, plays a critical role in tumor progression. Guo *et al* found that ginsenoside Rg3 effectively inhibited VM formation in pancreatic cancer by inhibiting the expression of particular genes, including VE-cadherin, EphA2, MMP-2 and MMP-9, both *in vitro* and in tumor xenografts (60).

Inhibition of multidrug resistance (MDR) and increase of chemosensitivity. Some studies have suggested that combination treatment may be more lead to an improved treatment efficacy with decreased toxicity by exerting synergistic effects, although the exact mechanisms involved remain unclear (18,61). For example, As₂O₃ in combination with Rg3 has been shown to significantly inhibit NCI-H1299 lung cancer cell proliferation and to prolong the survival of tumor-bearing mice (62). Rg3 has been shown to suppress doxorubicin-induced cardio-toxicity both *in vitro* and *in vivo*, possibly by activating the NF-E2-related factor 2 (Nrf2)/antioxidant responsive element (ARE), and PI3/Akt pathways (63). 20(S)-ginsenoside Rg3 has also been shown to protect normal cells against cyclophosphamide (CP)-induced genotoxicity through antioxidant activity (6). In Lewis lung carcinoma and ovarian cancer models, the co-administration of ginsenoside Rg3 was shown to effectively enhance the inhibitory effects of CP on tumors and to reduce the occurrence of side-effects (64,65). Ginsenoside Rg3 has also been shown to improve the antitumor activity of paclitaxel and cisplatin in an animal model of esophageal squamous cell carcinoma (66). In addition, 20(S)-ginsenoside Rg3 increases the oral bioavailability of paclitaxel and then improves the antitumor activity of paclitaxel (67).

Rg3 enhances the chemosensitivity of cancer cells to chemical drugs or/and can help cells to overcome MDR, which may partly explain the mechanisms of combination therapy.

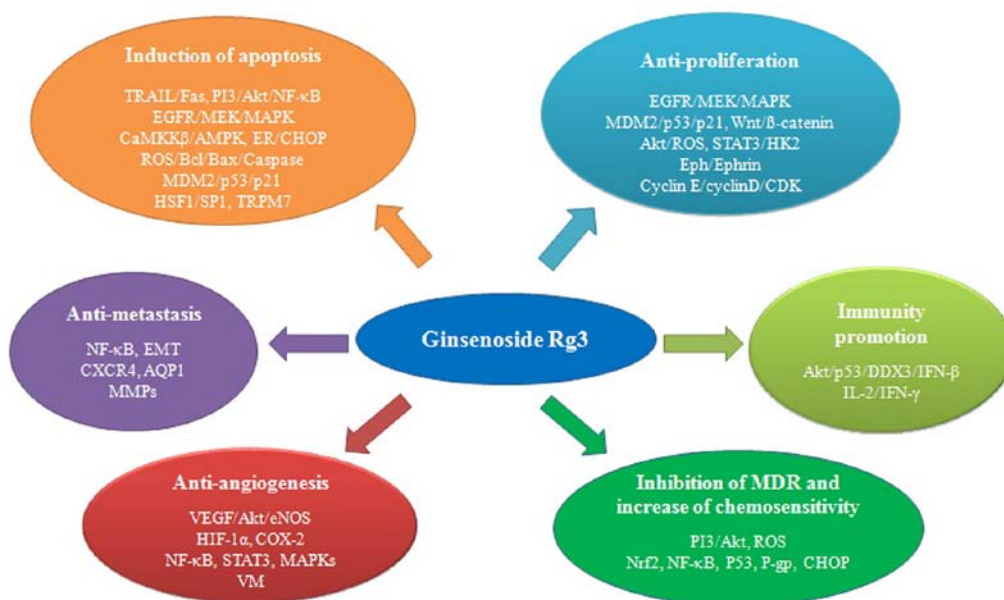


Figure 2. Possible antitumor mechanisms of ginsenoside Rg3. CDKs, cyclin-dependent kinases.

Previous studies have indicated that 20(S)-ginsenoside Rg3 is a highly effective modulating agent in reversing MDR in drug-resistant P388 leukemia cells and human fibrocarcinoma KBV20C cells specifically (68,69). Combination therapy using Rg3 and the conventional chemotherapeutic agent, docetaxel, has been shown to significantly enhance the sensitivity of cancer cells and to decrease drug resistance in prostate cancer and colon cancer cells via the inhibition of NF-κB (70,71). Moreover, Rg3 has been shown to enhance the susceptibility of the colon to cisplatin by inhibiting HO-1/NQO-1 expression. Moreover, Rg3 also decreased cisplatin-induced tissue damage in the kidneys and liver by preventing cisplatin-induced intracellular ROS generation (72). 20(S)-ginsenoside Rg3 has also been shown to increase TRAIL receptor DR5 expression and sensitivity to TRAIL in hepatocellular carcinoma (HCC) cells, such as HepG2 (73). 20(S)-ginsenoside Rg3 is also capable of sensitizing doxorubicin-treated liver cancer cells death by inhibiting doxorubicin-induced autophagy possibly by blocking lysosomal function (74).

Promotion of immunity. Rg3 also plays a role in the improvement of host immunity in tumor-bearing animal models. A previous study suggested that a ginsenoside Rg3-fortified red ginseng preparation significantly suppressed tumor growth in H460 tumor-bearing mice by immunopotentiality (75). In addition, treatment with Rg3, has been shown to markedly enhance ($P < 0.05$) cellular immunity by stimulating ConA-induced lymphocyte proliferation and the secretion of cytokines [interleukin (IL)-2, interferon (IFN)-γ] in the immune organs and serum of hepatoma H22-bearing mice (76). Moreover, Rg3 has been shown to augment innate immunity and IFN-β expression via the upregulation of DDX3 and the activation of the TANK-binding kinase 1 (TBK1)/inhibitor-κB kinase ε (IKKε)/interferon regulatory factor 3 (IRF3) pathway (77). Therefore, Rg3 may be a potent immunomodulatory candidate for the treatment of cancer.

5. Clinical studies

Although many studies have demonstrated a clear anticancer activity of Rg3 in various cancer cell lines or/and xenograft tumor models, the efficacy of Rg3 in human cancer requires further investigation. Shenyi capsule (ginsenoside Rg3 monomer preparation), a class I new drug in traditional Chinese medicine, is being used clinically in the treatment of various types of cancer, such as lung cancer, breast cancer and gastrointestinal tumors in China (78). A prospective, randomized, controlled study using 133 non-small cell lung cancer cases suggested that Shenyi capsule, particularly in combination with chemotherapy, improved the post-operative lifespan of patients mainly by enhancing the immune function and inhibiting angiogenesis (79). Another randomized trial of 60 advanced esophageal cancer cases found that the combination of chemotherapy with Shenyi capsule was effective in improving the quality of life of patients and 1 year survival rates. In addition, a meta-analysis involving 274 female breast cancer patients in China also showed that the combination treatment group exhibited significantly attenuated leucopenia. However, there were no significant differences in the total response rate compared with the chemotherapy groups (80,81).

In general, the published clinical studies suggested that Rg3 was a good antitumor agent by improving the immune function and the quality of life of cancer patients. However, there has been no strong evidence to confirm the efficacy of Rg3. Further, large controlled and highly qualified clinical trials are required in order to better guide clinical applications.

6. Conclusions

Ginsenoside Rg3 displays significant antitumor activities in several types of cancer both *in vitro* and *in vivo*. The molecular mechanisms of tumor inhibition employed by ginsenoside Rg3 mainly involve the induction of apoptosis, and the inhibition

of proliferation, metastasis and angiogenesis, and the promotion of immunity (Fig. 2). Furthermore, experiments have demonstrated that Rg3 is relatively safe for use in the marrow, heart, lung, liver, kidney and nervous system (82). Long-term toxicological studies on beagle dogs and rats also confirmed that 20(S)-ginsenoside Rg3 was non-toxic and well-tolerated (83,84). Ginsenoside Rg3 has also been shown to significantly decrease some environmental carcinogen-induced DNA damage, such as N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and benzo[a]pyrene (85,86). These results make ginsenoside Rg3 an attractive candidate for cancer prevention. Combination therapy with conventional cancer treatments, such as chemotherapy and surgery has been suggested to be more effective. Rg3 improves the chemosensitivity of tumor cells to chemical drugs, reverses MDR and decreases toxicity. Additionally, Rg3 combined with TAE, more effectively inhibits tumor growth. Apart from these, a recent study found that 20(S)-ginsenoside Rg3 induced the apoptosis of HepG2 cells, accompanied by the induction of autophagy via mitochondrial and Ca²⁺-related pathways, which decreased the survival of cancer cells. Thus, co-treatment with autophagy inhibitors and Rg3 would be beneficial (87). Clinical trials still demonstrate that ginsenoside Rg3 is an effective antitumor agent. Therefore, ginsenoside Rg3 is gaining more attention as an anticancer drug due to its favorable safety and efficacy.

However, there are still several aspects limiting the use of Rg3: i) its mechanisms of action have not yet been fully established. For example, ginsenoside Rg3 induces strong genotoxicity and DNA damage in human osteosarcoma cells. However, whether these genotoxic effects are directly associated with cell cycle arrest and apoptosis is not clear (85). Further studies are warranted to determine the mechanisms through which the different signaling pathways are orchestrated and and those through which the synergistic antitumor activities of ginsenosides and conventional cancer treatments are realized in order to fully understand the benefits of Rg3. ii) Its poor aqueous solubility and low oral bioavailability (88). Although studies have been performed to explore a method to solve this problem, for example, 20(S)-ginsenoside Rg3-loaded magnetic human serum albumin nanospheres [20(S)-Rg3/HSAMNP] were created and were shown to markedly enhance the efficiency of HeLa cervical cancer cell inhibition when combined with hyperthermia (89), and liposomal ginsenoside Rg3 showed increased anticancer activity *in vitro* compared to the Rg3 solution (90), it is necessary to evaluate the efficacy of these optimized therapy *in vivo*. It may be possible to develop novel Rg3 analogues with improved efficacy, pharmacokinetics and bioavailability profiles. iii) Currently, the evidence of Rg3 efficacy is not yet conclusive in humans. There is a significant need to perform further, larger cohort clinical studies to confirm its efficacy for better application in the clinic.

In conclusion, Rg3 has great potential for use as a broad-spectrum anticancer drug and an effective adjuvant to cancer therapies in the future.

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