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Anticancer effect and molecular mechanism of ginsenoside Rg3 in various cancer types

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HIGHLIGHTS

- The underlying anticancer mechanisms of Rg3 based on existing studies are described in this review.
- Rg3 exhibits significant anticancer activities through multiple molecular mechanisms.
- Rg3 has two stereoisomeric pairs, 20(S)-Rg3 and 20(R)-Rg3 with different pharmacological actions because of their different chemical structures.
- Rg3 is a promising agent for cancer prevention and treatment.

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ABSTRACT

Ginsenoside Rg3 (Rg3), extracted from the root of *Panax ginseng*, is one of the most abundant ginsenosides. Rg3 exhibits anticancer activity in various cancer models *in vitro* and *in vivo* by modulating several signaling pathways, such as those of phosphatidylinositol 3-kinase, epidermal growth factor receptor, mitogen-activated protein kinases, p53, nuclear factor kappa-B, and reactive oxygen species. Besides, Rg3 can be used as adjunctive with conventional anticancer therapies, enhancing therapeutic effects and reversing drug resistance in cancer therapy. So, the purpose of this review is to provide a systematic summary and analysis the anticancer effects and the potential mechanisms of Rg3 extracted from ginseng.

1. Introduction

Cancer is considered as the second leading cause of mortality globally, which seriously threatens public health. Cancer treatment may mainly include surgery, radiotherapy and chemotherapy. Because of these distinctive therapeutic effects and the fewer side effects, natural products are widely used in the treatment of many diseases in recent years. The root of the plant *Panax ginseng*, commonly known as ginseng, belonging to Chinese traditional medicine, has been widely used in Asian countries for thousands of years. Ginseng, well known for its disease-preventing and therapeutic effects, has a variety of pharmacological effects including enhancing immune function and cardiovascular health, improving memory and learning, and inhibiting tumor growth and so on.[1]

Ginseng is extensively practiced as medicine due to the presence of ginsenoside saponin which accounts for the pharmacological efficacy.[2] The general structure of ginsenosides is a four-ring steroid backbone with hydrophobic properties that is connected to sugar molecules on the C20 position. Based on the positioning of hydrogen on C20, ginsenosides have two stereoisomers, 20(S) and 20(R) epimers. Ginsenoside Rg3 (Rg3) is

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one of the well-studied members of saponins. Rg3, like other members, also has two epimers, 20(S)-ginsenoside Rg3 and 20(R)-ginsenoside Rg3 (Fig. 1). White fresh ginseng by steam heating for several hours transfers to red ginseng that has higher medicinal properties and is well enriched for some ginsenosides including Rg3.[3] The process mainly produces 20(S)-ginsenoside Rg3 as the major epimer. Moreover, enzymatic hydrolysis are other methods of preparation of 20(S)-ginsenoside Rg3. However, production of 20(R)-ginsenoside Rg3 requires procedures that are more complex.

To date, more than 100 ginsenosides have been isolated and identified from *P. ginseng*. Among them, Rg3 exhibits various biological activities, such as promotion of immunity, cardiovascular protection, neuroprotection, hepatoprotection, as well as antioxidant, anti-inflammatory, antifatigue, and antitumor effects.[4–9] In recent years, the antitumor mechanisms of Rg3 have been investigated in various cancer types, including the induction of apoptosis, inhibition of tumor growth, proliferation, migration and invasion, angiogenesis, oncogenesis and development, and cell cycle arrest (Table 1). Besides, Rg3 can be used as adjuvant. Combining Rg3 with chemotherapeutic drugs can enhance drug sensitivity and reverse drug resistance in various cancers, even though its potential mechanism has not yet been fully illustrated. Therefore, this review systematically summarizes the cancer-preventive effects of Rg3 so as to promote further research involving Rg3.

2. Anticancer effects and underlying mechanisms of Rg3 in *in vitro* studies

In previous studies, Rg3 exhibited significant anticancer activities through multiple molecular mechanisms. These mechanisms are mainly associated with proliferation, apoptosis, cell cycle arrest, migration, invasion, metastasis, angiogenesis, autophagy and immunity, which are summarized in Table 1. Although the anticancer activities of Rg3 have been widely illustrated, the exact molecular mechanisms remain unclear. The underlying anticancer mechanisms of Rg3 based on existing studies are described in this review.

2.1. Inhibiting cancer cell proliferation

Cytotoxic agents that inhibit tumor development mainly through suppressing the growth and viability of cancer cells are the most commonly used chemotherapeutic drugs for cancer patients. In Mz-ChA-1, QBC939 and GBC-SD human gallbladder cancer cells, Rg3 was shown to have potent cytotoxic effect that was associated with endoplasmic reticulum stress activation.[10] And, Rg3 inhibited hepatoma carcinoma SMMC-7721 and SK-Hep-1 cells proliferation by reducing the expression of lncRNA HOTAIR and suppressing the PI3K/AKT pathway.[11] Another study on lung cancer revealed that 20(S)-Rg3 inhibited the proliferation of A549 lung cancer cells by blocking the cell cycle at G0/G1 phase via the EGFR/Ras/Raf/MEK/ERK pathway. Moreover, Rg3 inhibited the proliferation of human osteosarcoma MG-63, U–2OS, and SaOS-2 cells by reducing the protein expression of PI3K/AKT/mTOR.[12] Besides, Shan X et al[13] found that 20(R)-Rg3 inhibited melanoma cell proliferation by deactivating EGFR/MAPK pathway and decreasing the fucosyltransferase IV (FUT4) and its synthetic product Lewis Y (LeY) expression. Rg3 also inhibited the proliferation of prostate cancer PC3 cells through ROS-induced cell cycle arrest.[14] Genome-wide methylation analysis showed that Rg3 suppressed the proliferation of MCF-7 breast cancer cells by downregulating hypermethylated TRMT1L, PSMC6 and NOX4 level.[15] Furthermore, Rg3 inhibited cancer cell proliferation by suppressing PI3K/Akt signaling pathway[11,16] and Wnt/β-catenin pathway,[17] activating VRK1/P53BP1 pathway,[18] regulating the Hedgehog signaling pathway,[18] downregulating HDAC3,[19] ERK and Akt,[20] and lncRNA H19 expression,[21] and suppressing glycolysis through PI3K/AKT/miRNA-21 pathway.[22] However, interestingly, Liu W et al found that Rg3 at concentrations lower than 50 μM stimulated the proliferation of human cervix adenocarcinoma HeLa cells in a concentration-dependent manner through activation of mTORC1.[23]

2.2. Inducing cancer cell apoptosis and autophagy

Apoptosis, also called programmed cell death, is generally characterized by distinct morphological characteristics and energy-dependent biochemical mechanisms. As shown in Table 1, Rg3 induces apoptosis via two major pathways in the mammalian system: the mitochondrial-dependent intrinsic apoptotic pathway and the extrinsic pathway initiated by death receptors. Mitochondrial-dependent pathway is involved in a reduction of mitochondrial membrane potential, which results in the activation of caspase-3, release of mitochondrial cytochrome *c*, and eventually apoptosis.[12] Zhang F et al revealed that treatment of gallbladder cancer cells with 20(S)-Rg3 activated p53 pathway, and subsequently induced cellular senescence and mitochondrial-dependent apoptosis.[24] Rg3-triggered intrinsic apoptosis was associated with the production of reactive oxygen species, mitochondrial cytochrome *c*, caspase-3 and Bax protein activation, and Bcl-2 protein inhibition in Hep3B cells.[25] And, 20(S)-Rg3 induced the apoptosis of HeLa cells by regulating autophagy.[26] Cotreatment with 20(S)-Rg3 and TRAIL induced apoptosis of human hepatocellular carcinoma cells via CHOP-mediated DR5 upregulation.[27] Moreover, the occurrence of extrinsic apoptosis with the activation of endoplasmic reticulum stress mediated Rg3-induced apoptosis.[28] In addition, the increases of reactive oxygen species (ROS) production,[25,26,28–31] inactivation of TRPM7 channel,[32] MNNG-induced DNA damage,[33] the inhibitions of EGF-EGFR-ERK1/2-HIF-1α,[34] PI3K/Akt,[12,16,35–37] XIAP[36] and MEK[38] signaling pathways might also contribute to Rg3-induced apoptosis.

Autophagy is a conserved self-degradation mechanism, which forms bilayer autophagosomes to envelop cytoplasmic proteins and organelles, fuse with lysosomes, afterwards to form autolysosomes, eliminate the accumulation of cytotoxic waste products and provide energy to

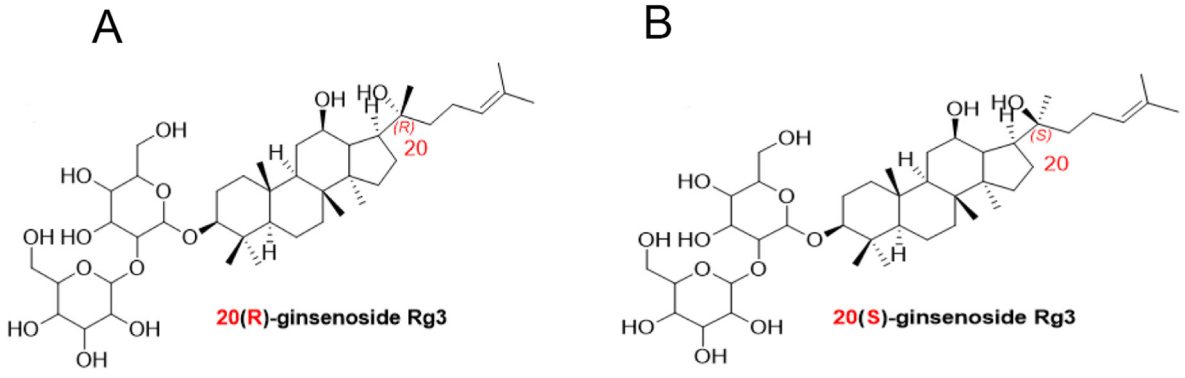

Fig. 1. Chemical structure of the two forms of Rg3 (A) Chemical structure of 20(R)-ginsenoside Rg3 (B) Chemical structure of 20(S)-ginsenoside Rg3.

Table 1
In vitro anticancer effects and molecular mechanisms/targets of Rg3.

cancer	cell type	Ginsenoside Rg3	IC_{50} values or effective dosage	molecular mechanisms	molecular targets	Ref
Liver cancer	Hep3B	Rg3	Dosages of 1, 3, 10 and 30 μM	Induction of apoptosis	mitochondrial cytochrome *c*↑; caspase-3↑; Bax↑; Bcl-2↓; ROS↑	(25)
Liver cancer	SMMC-7721 HepG2	Rg3	Dosages of 25, 50, 75, and 100 μg/ml	Inhibition of proliferation Induction of apoptosis	caspase-3 and Bax↑; Bcl-2↓	(102)
Liver cancer	HepG2	Rg3	Dosages of 15.63, 31.25, 62.5, 125, 250 and 500 μg/ml	Inhibition of proliferation and angiogenesis Induction of apoptosis	global genomic DNA methylation↓; DNMT1↑; DNMT3a↓ and DNMT3b↓; VEGF↓; p53↑; Bcl2↓	(106)
Liver cancer	Bel-7402 HCCLM3	Rg3	Dosages of 25, 50, and 100 μM	Inhibition of viability Induction of apoptosis Induction of cell cycle arrest	NHE1↓; EGFR↓; EGF↓; HIF-1α↓; pERK1/2↓	(34)
Liver cancer	HepG2 MHCC-97L	20(R)- Rg3	Dosages of 1.25, 2.5 or 5 μg/ml	Inhibition of proliferation Inhibition of migration and invasion	ARHGAP9↑	(43)
Liver cancer	SMMC-7721 SK-Hep-1	Rg3	Dosage of 8 μg/ml	Inhibition of proliferation Inhibition of migration and invasion	lnc RNA-HOTAIR↓; MMP2↓; MMP9↓; p-AKT↓; p-PI3K↓	(11)
Lung cancer	A549	20(R)- Rg3	Dosages of 12.5, 25, 50 and 100 μg/ml	Inhibition of proliferation Inhibition of migration, invasion and anoikis resistance	E-cadherin↑; Snail↓; Vimentin↓; MMP2↓; activation of Smad2 and P38 mitogen activated protein kinase↓	(44)
Lung cancer	A549 H1299 H358	Rg3	Dosages of 25, 50 and 100 μg/ml	Inhibition of epithelial–mesenchymal transition and invasion	E-cadherin↑; Snail↓; N-cadherin↓; Vimentin↓; FUT4↓; EGFR↓; pp38↓; p-ERK↓; p-IKB↓	(45)
Lung cancer	A549 H23	Rg3	Dosages of 25, 50, 100 and 200 mg/ml	Inhibition of cell viability Induction of apoptosis	p-PI3K/PI3K↓; p-Akt/Akt↓	(16)
Lung cancer	A549 HCC827	Rg3	IC_{50} value of 65.3 μM	Inhibition of growth regulation of DNA damage	P53BP1↑; VRK1↑	(18)
Lung cancer	A549	20(S)- Rg3	IC_{50} values of 253.101 and 73.618 μg/mL at 48 and 72 h respectively	Inhibition of proliferation, migration, invasion, and epithelial mesenchymal transition	PTCH1↑; GLI1↓	(46)
Lung cancer	A549	20(S)- Rg3	Dosages of 20, 30, 40, 50, 60, 70, 80, 90 and 100 μM	Inhibition of proliferation induction of cell cycle arrest	CDK2, Cyclin A2, and Cyclin E1↓; EGFR↑; BRAF↑; MEK2↑; ERK2↑; Raf1↓; ERK1↓; ERK2↓	(41)
Lung cancer	A549 H460	Rg3	Dosages of 100 and 200 mg/ml	Inhibition of proliferation Induction of apoptosis and mitophagy	PINK1↓; Parkin↓; caspase3↑; PARP cleavage↑; ROS↑	(29)
Colon cancer	HCT-116	Rg3	Dosage of 100 μM	Inhibition of proliferation	AKAPA8L, PMPCB and PDE5A↑; PITPNA, DUS2L and RIC8A↓	(107)
Colon cancer	HT29	20(S)- Rg3	IC_{50} values of 600 μM and 100 μM at 24h and 48h respectively	Inhibition of proliferation Induction of apoptosis	Caspase-3 activity↓; glutathione s-transferase p 1↑; PCNA↓; STRAP↓; ANXA5↑; TM1↑; clathrin↑; RbP 4↑	(108)
Colon cancer	HCT116, HT29 SW480	Rg3	Dosages of 100 and 200 μM	Inhibition of proliferation	C/EBPβ↓; NF-κB transcriptional activity↓	(69)
Colon cancer	LoVo SW620 HCT116	Rg3	Dosage of 200 μM	Inhibition of growth and migration inhibition of stemness of colorectal cancer cells	Unclear	(65)
Colon cancer	Caco-2	Rg3	Dosages of 25, 50 and 100 μM	Inhibition of viability Induction of apoptosis Inhibition of migration and invasion	lnc RNA CCAT1↓; Cyclin D1↓; MMP9↓; vimentin↓; p53↑; Bax↑; Cleaved-Caspase-3↑; Bcl-2↓; phosphorylation of PI3K and AKT↓	(35)
Colon cancer	HT29 SW620	20(R)- Rg3	Dosages of 10, 50, and 100 μM	Inhibition of cancer stem cell properties Inhibition of migration and invasion	p-EGFR and p-AKT↓; *SNAIL*↓; *OCT4*↓; *MMP2*↓	(47)
Colon cancer	HCT15 SW48	Rg3	Dosages of 100 and 200 μM	Inhibition of migration and invasion	E-cadherin↑; vimentin and snail↓; NICD↓; Hes1↓	(48)
Ovarian cancer	SKOV-3	Rg3	Dosages of 2.5 and 5.0 μg/ml	Inhibition of metastasis	MMP9↓	(49)
Ovarian cancer	SKOV3 3AO	20(S)- Rg3	IC_{50} values of 146.8 and 242.6 mg/ml respectively for SKOV3 and 3AO	Inhibition of migration and invasion	HIF-1a↓; PHD1 and VHL↓; E-cadherin↑; vimentin↓; Snail↓;	(50)
Ovarian cancer	HO-8910	20(S)- Rg3	Dosages of 25, 50, and 100 μg/ml	Induction of apoptosis	Bax↑; Bcl-2↓; XIAP, cIAP1 and cIAP2↓; p-Akt↓; p-mTOR↓; p-p70S6K↓; caspase- 3 and caspase-9↑	(36)
Ovarian cancer	A2780	20(S)- Rg3	Dosages of 12.5, 25, 50,100 and 200 μg/ml	Inhibition of proliferation Induction of apoptosis	cleaved caspase-3, caspase-8, caspase-9, and PARP↑; BID↓	(109)
Ovarian cancer	SKOV3 3AO	20(S)- Rg3	Dosages of 80 and 160 μg/ml	Inhibition of migration	HIF-1α↓; NF-κB↓; PHD1↑; VHL↑	(51)
Ovarian cancer	SKOV3	20(S)- Rg3	IC_{50} value of 146.8 μg/ml	Inhibition of viability, migration and invasion Induction of autophagy	LC3 II, ATG5 and ATG7↑	(40)
Ovarian cancer	SKOV3 A2780	20(S)- Rg3	Dosages of 40 and 80 μg/ml	Inhibition of proliferation Inhibition of Warburg effect	PKM2↓	(110)
Ovarian cancer	HOSEpiC HTB-77	Rg3	Dosages of 25, 50, 100 and 200 μg/ml	Inhibition of proliferation Inhibition of metastasis and invasion Induction of apoptosis	DNMT1, DNMT3a and DNMT3b↓; acetylated H3 K14 and K9↑; p53, p16 and hMLH1↑	(111)

(*continued on next page*)

Table 1 (*continued*)

cancer	cell type	Ginsenoside Rg3	IC_{50} values or effective dosage	molecular mechanisms	molecular targets	Ref
Ovarian cancer	SKOV3 3AO	20(S)- Rg3	unclear	Inhibition of proliferation Inhibition of migration and invasion Inhibition cancer progression	miR-4425↓; FDFT1↑	(52)
Ovarian cancer	SKOV3 A2780	Rg3	IC_{50} values of 119.8 μg/ml and 64.9 μg/ml for SKOV3 and A2780	Inhibition of proliferation Inhibition of migration and invasion	lncRNA H19↓	(21)
Breast cancer	MDA-MB-231	Rg3	Dosages of 20 and 60 μg/ml	Inhibition of migration	CXCR4↓	(112)
Breast cancer	MDA-MB-231	Rg3	Dosage of 30 μM	Induction of apoptosis	the ratio of Bax to Bcl-2↑; cleaved caspase-3 and PARP↑; ROS↑	(30)
Breast cancer	MCF-7	Rg3	Dosages of 20 and 50 μM	Inhibition of growth	Hypermethylated TRMT1L, PSMC6 and NOX4↓; hypomethylated ST3GAL4, RNLS and KDM5A↑	(15)
Breast cancer	MDA-MB-231 HCC1143	Rg3	Dosage of 40 μM	Inhibition of proliferation Induction of apoptosis	phosphorylation of EEF2 on Thr57↑; RPS27↑; phosphorylation of MYCBP2, PAWR, RANBP2, UFL1, MPRIP, PPP1R12A and SEPT9↑; phosphorylation of HIRIP3 and MGED2↓	(113)
Breast cancer	MDA-MB-231 MCF-7	Rg3	Dosages of 20, 50 and 100 μM	Inhibition of viability Inhibition of breast cancer stem cell-like properties	Sox-2 and Bmi-1↓; p-AKT↓; HIF-1α↓	(114)
Breast cancer	FM3A	Rg3	Dosages of 3, 6 and 12 μg/ml	Inhibition of invasion Inhibition of progression	NOTCH, β-catenin, and Vimentin↓; CXCL1, CCL3, CXCL2, TIMP-1, and TNF-α↓; p-STAT3↓; G-CSF↓	(53)
Breast cancer	MCF-7	Rg3	Dosages of 20 and 50 μM	Inhibition of proliferation Induction of apoptosis	EYA1, DACH1, and CHRM3↓; ATXN8OS↓; miR-424-5p↑	(115)
Breast cancer	MDA-MB-231	Combination of 20(R) and 20(S) Rg3	Dosage of 25 μM RRg3+50 μM SRg3	Inhibition of proliferation Inhibition of migration Inhibition of cancer cell stemness	IGF-1R; CD44+ stem cell marker↓	(54)
Gastric cancer	AGS cell	Rg3	Dosages of 100, 200, 300, 400 and 500 μM	Inhibition of growth and survival Induction of apoptosis	TRPM7 channel activity↓; sub-G1↑; caspase-3 activity↑; cleaved PARP↑	(32)
Gastric cancer	AGS cell	20(S)- Rg3	IC_{50} value of 24.4 μg/ml	Inhibition of proliferation Induction of apoptosis	cleaved PARP, caspase-3, caspase-8, and caspase-9↑; Bax↑; Bcl-2↓	(101)
Gastric cancer	SGC-7901	Rg3	Dosage of 50 μg/ml	Induction of apoptosis	FUT4↓; SP1↓; HSF1↓;	(116)
Gastric cancer	BGC823	Rg3	Dosage of 100 μM	Inhibition of metastasis and angiogenesis	HIF-1α and VEGF↓	(117)
Gastric cancer	SGC-7901	Rg3	Dosages of 10, 20 and 40 μg/ml	Inhibition of proliferation Inhibition of migration and invasion Induction of apoptosis Induction of cell cycle arrest	PTEN and P53↑; p-PI3K and AKT↓	(37)
Melanoma	B16	Rg3	IC_{50} value of 72.13 μg/ml	Inhibition of metastasis Induction of cell cycle arrest Induction of apoptosis	caspase-3 and Bcl-2↓	(71)
Melanoma	A375 C8161	Rg3	Dosages of 25 and 50 μg/ml	Inhibition of proliferation Induction of cell cycle arrest	HDAC3↓; acetylation of p53↑; PRB, cyclin E, cyclin D1, CDK2, CDK4↓; p21↑	(19)
Melanoma	B16F10	Rg3	Dosages of 25, 50 and 100 μM	Inhibition of migration and invasion	MMP-13↓	(56)
Melanoma	A375	20(R)- Rg3	Dosages of 25, 50 and 100 μg/ml	Inhibition of proliferation	FUT4 and LeY↓; p-EGFR↓; p-ERK1/2↓	(13)
Melanoma	A375.S2	Rg3	IC50 value of 20 μM	Inhibition of viability Inhibition of migration Induction of apoptosis	Bax↑; Bcl-2↓	(57)
Melanoma	B16	Rg3	IC_{50} value of 7.76 μg/ml	Inhibition of growth Inhibition of metastasis Inhibition of angiogenesis	PCNA↓; VEGF↓; p-ERK and p-Akt↓; MMP-2 and MMP-9↓	(20)
Osteosarcoma	MG-63 OS732 U–2OS HOS	Rg3	Dosages of 50, 100 and 150 μM	Induction of DNA double-strand breaks Induction of apoptosis	unclear	(33)
Osteosarcoma	MG-63 U–2OS SaOS-2	Rg3	156 nM, 1250 nM and 156 nM for MG-63 cells, SaOS-2 cells and U–2OS respectively	Inhibition of proliferation Inhibition of migration Induction of apoptosis	Bcl-2, PI3K, AKT and mTOR↓; cleaved-caspase3↑	(12)
Osteosarcoma	MG63 143B	Rg3	IC_{50} values of 51 μM and 57.07 μM for 143B and MG63 cells respectively	Inhibition of proliferation Inhibition of migration and invasion	MMP2, MMP7 and MMP9↓; E-cadherin↑; N-cadherin and Vimentin↓; Snail, Twist and ZEB1↓; β-catenin, c-Myc and Cyclin D1↓	(17)
Osteosarcoma	MG63 Saos2	Rg3	Dosages of 40, 80 and 120 μM	Inhibition of proliferation Inhibition of migration and invasion Induction of apoptosis	PCNA and MMP9↓; cleaved caspase-3↑; circ_0003074↓; miR-516b-5p↑; KPNA4↓	(58)
Cervical cancer	ATCC CCL-2	20(S)- Rg3	IC_{50} value of 50 μM and 75 μM at 24 and 48 h respectively	Inhibition of proliferation Induction of cell cycle arrest Induction of apoptosis and autophagy	AIF release and nuclear translocation↑; LC3-II, c-PARP and p62↑; ROS↑	(26)
Cervical cancer	human cervix adenocarcinoma HeLa cells	Rg3	Dosages of 5, 10 and 20 μM	Promotion of cell growth	p-mTORC1and p-ERK1/2↑; p-S6↑; ATP↑	(23)
Glioma	U87	20(S)- Rg3	Dosages of 10, 20, 50 and 100 μM	Induction of senescence-like growth arrest or apoptosis	ROS↑; p-AKT↑; p53 and p21↑	(31)

(*continued on next page*)

Table 1 (*continued*)

cancer	cell type	Ginsenoside Rg3	IC_{50} values or effective dosage	molecular mechanisms	molecular targets	Ref
Glioma	U87MG	Rg3	Dosage of 10 μM	Induction of apoptosis	activation of MEK1/2↓; DCFH-DA↑; Bax↑; pro-caspase3 and Bcl-2↓	(38)
Pancreatic cancer	PANC-1 SW1990	Rg3	Dosage of 25, 50, 100 and 200 μM	Inhibition of vasculogenic mimicry	VE-cadherin, EphA2, MMP-2 and MMP-9↓	(67)
Prostate Cancer	LNCaP PC3	Rg3	IC_{50} values of 14.1 and 8.4 μM for LNCaP and PC3 respectively	Inhibition of proliferation	P42/44 MAP kinase↓	(118)
Prostate Cancer	PC-3M	Rg3	Dosages of 1 and 10 μM	Inhibition of migration	AQP1↓; P38 MAPK↓	(62)
Prostate Cancer	PC3	Rg3	Dosage of 25, 50 and 100 μM	Inhibition of proliferation Induction of cell cycle arrest	ROS↑	(14)
Gallbladder cancer	Mz-ChA-1 QBC939 GBC-SD	Rg3	Dosage of 10, 50 and 100 μM	Induction of apoptosis	caspase-12↑; CHOP↑; P-PERK↑; p-IRE1↑	(10)
Gallbladder cancer	NOZ GBC-SD	20(S)- Rg3	Dosages of 25, 50, 100, 200, and 400 μM	Inhibition of viability and proliferation Induction of cell cycle arrest Induction of apoptosis	p53 and p21↑; MDM2↓; Bad and Bax↑; cleaved caspase 3↑; Bcl-2 and Bcl-XL↓	(24)
Gallbladder cancer	GBC-SD	Rg3	Dosages of 50 μM and 100 μM	Induction of apoptosis	p-eIF2α, ATF4 and Lcn2↑; CHOP↑; ROS↑; GSH-PX and SOD↓; lincRNA-p21↑;	(28)
Thyroid Cancer	TPC-1 BCPAP C643 Ocut-2c	Rg3	Dosage of 50 μM	Inhibition of proliferation Inhibition of migration and invasion	Rac-1 and Cdc42↓; MMP-2 and MMP-9↓; VEGF-C and VEGF-A↓	(61)
Nasopharyngeal carcinoma	HNE1 CNE2	20(S)-Rg3	Dosages of 25, 50 and 100 μg/m	Inhibition of migration and invasion	MMP-2 and MMP-9↓; E-cadherin↑; Vimentin and N-cadherin↓; ZEB1↓	(59)

maintain metabolism.[39] Autophagy may be one of the effective ways to inhibit migration and invasion of tumors.[40] Selective targeting autophagy in cancer have attracted more and more attention. Treatment of SKOV3 cells with 20(S)-Rg3 was found to increase autophagy through upregulating the autophagy-related proteins ATG5, ATG7 and LC3.[40] Another study revealed that Rg3 repressed starvation-induced autophagic flux in human HeLa ATCC and CCL-2 cells and significantly increased LC3-II and p62 protein levels.[26]

2.3. Induction of cell cycle arrest

Cell cycle arrest is a stopping point in the cell cycle, resulting in cell growth inhibition. Cells undergoing cell cycle arrest lose their ability to replicate and divide. Many researches demonstrated that Rg3 could block and arrest several cancer cells at G0/G1 phase including gallbladder cancer, lung cancer, melanoma, prostate cancer and gastric cancer cells.[14,19,24,37,41] Zhang F et al revealed that 20(S)-Rg3 inhibited murine double minute 2 (MDM2) to activate the p53 pathway and subsequently induce cell cycle arrest at G_0/G_1 phase in gallbladder cancer NOZ and GBC-SD cells.[24] And, 20(R)-Rg3 induced G_0/G_1 cell cycle arrest through downregulating histone deacetylase 3 (HDAC3) expression and increasing the p53 acetylation and transcription activity in melanoma A375 and C8161 cells.[19] In addition, another study revealed that Rg3 induced cell cycle arrest at the G_1 phase in SGC-7901 gastric cancer cells via regulation of the PTEN/p-PI3K/AKT pathway.[37]

2.4. Suppressing cancer cell invasion, migration, and metastasis

Invasion, migration and metastasis are general features of cancer cells that spread from a primary tumor site to a distant part of the body. Tumor metastasis is a major cause of tumor recurrence and mortality. Inhibiting these processes in cancer treatment was considered as a successful strategy.[42] Rg3 not only inhibited the migratory and/or invasive ability of liver cancer HepG2, MHCC-97L, SMMC-7721 and SK-Hep-1 cells,[11,43] lung cancer A549, H1299 and H358 cells,[44–46] colon cancer Caco-2, HT29, SW620, HCT15 and SW48 cells,[35,47,48] ovarian cancer SKOV3, 3AO, HPSEpiC and A2780 cells,[21,40,49–52] breast cancer FM3A and MDA-MB-231 cells,[53,54] gastric cancer BGC823 and SGC-7901 cells,[37,55] melanoma B16, B16F10 and A375.S2 cells,[20,56,57] osteosarcoma MG-63, U–2OS, SaOS-2, and 143B cells,[12,17,58] but also suppressed the metastasis of nasopharyngeal carcinoma HNE1 and CNE2 cells.[59]

The epithelial-to-mesenchymal transition (EMT) plays a crucial role in the formation of cancer metastasis.[60] As previously demonstrated, 20(R)-Rg3 inhibited the invasion of osteosarcoma, lung cancer, nasopharyngeal carcinoma Cells and colon cancer cells by suppressing EMT.[17,45,48,59] And, Rg3 significantly suppressed the EMT process by decreasing the expression of EMT-related proteins including Snail, vimentin, and TGF-β1.[17,44–48,50,53] Meanwhile, Tian LL et al revealed that Rg3 inhibited EMT and invasion of lung cancer via down-regulating FUT4 mediated EGFR inactivation and suppressing MAPK and NF-κB signal pathways.[45]

In the previous study, Rg3 apparently inhibited the migration and invasion in four papillary thyroid cancer (PTC) cells (TPC-1, BCPAP, C643, and Ocut-2c cells) through suppressing cells actin skeleton function and decreasing the expression levels of matrix metalloproteinase-2(MMP-2) and MMP-9 proteins.[61] Matrix metalloproteinases (MMPs) also played an important role in tumor metastasis. Lee SG et al found that Rg3 could effectively inhibit metastasis of B16F10 melanoma cancer cells by suppressing MMP-13 expression.[56] Moreover, 20(S)-Rg3 inhibited migration and invasion of SKOV3 ovarian cancer cells by the induction of autophagy.[40] Besides, Rg3 effectively suppressed the migration of PC-3M prostate cancer cells by downregulating the expression level of the aquaporin 1 (AQP1) protein through p38MAPK pathway and some transcription factors acting on the AQP1 promoter.[62] Zhao LQ et al revealed that Rg3 suppressed the migration and invasion of human ovarian cancer SKOV3 and A2780 cells by partially inhibiting the expression of lncRNA H19.[21] Therefore, Rg3 played an inhibitory role in the process of cancer metastasis.

2.5. Anti-angiogenesis and other activity

Angiogenesis plays a critical role in tumor growth and metastasis by providing the necessary nutrients and oxygen.[63] Antiangiogenic therapy is one of the most promising approaches to control tumor growth.[64] One study revealed that Rg3 inhibited colorectal cancer growth by impeding angiogenesis via decreasing the expression of 22 pro-angiogenic genes, including *ANGPT1*, *ANGPT2*, *CCL13*, *COL18A*, *CSF3*, *CXCL1*, *EGF*, *FGF-2*, *IL1A*, *IL1B*, *IL8*, *KDR*, *MMP1*, *PGF*, *PIGF*, *PLAUR*, *TEK*, *THPO*, *TIMP1* and *TIMP2* in cancer cells.[65] Moreover, Rg3 inhibited tumor angiogenesis by decreasing CD34+ microvessel density and VEGF expression, and downregulating Glut1 and Glut4 expression in gastric precancerous lesions.[66] On the other hand, Rg3 exerted anti-ovarian cancer activity by downregulating oncogenic miR-4425 that inhibited the expression of the tumor suppressor gene *FDFT1* to suppress ovarian cancer progression.[52]

3. Anticancer effects and potential mechanisms of Rg3 in *in vivo*

As showed in Table 2, the *in vivo* antitumor effect and potential mechanism of Rg3 have been investigated in many researches which involves in many types of cancer xenograft models.[2,10,14,16–18,20,22,24,28,30,34,40,43,45,48,50,51,55,61,65–70] The underlying mechanisms are documented as well. In colon cancer xenograft nude mice, 10 ~ 40 mg/kg/d Rg3 via intraperitoneal injection significantly inhibited SW480 xenograft tumor growth, and further research demonstrated that anti-tumor effect of Rg3 was mediated by inhibition of C/EBPβ/NF-κB signaling *in vivo*. In hepatocellular carcinoma HCCLM3 xenograft mice models, Rg3 significantly inhibited liver cancer cell growth by inhibiting EGF-EGFR-ERK1/2-HIF-1α-NHE1 pathway *in vivo*.[34] Another study revealed that treatment with Rg3 (intraperitoneally injected with 5,10, 20 mg/kg/day, respectively) significantly inhibited vasculogenic mimicry through downregulating the expression of VE-cadherin, EphA2, MMP9 and MMP2 in SW-1990 xenograft nude mice.[67] Regulation of ER stress are also observed.[10,28] The activations of TGF-β1[44] and VRK1/P53BP1,[18] the alleviation of tumor-associated depression,[64,71] and the promotion of immune activation,[68,72] as well as the inhibition of EGFR,[13,34] PI3K/Akt,[16] NF-κB,[51] ERK,[20] and Notch-Hes1[48] signaling pathway, metastasis process,[43,47,53,61] and cancer vascularization[67] were also associated with the antitumor activity of Rg3. In addition, Rg3 has poor oral absorption in tumor-bearing rats. *In vivo* pharmacokinetics studies demonstrated that normal rats had better absorption than walker 256 tumor-bearing rats after oral administration of Rg3, and ginsenoside Rh2, as a glycosylation hydrolysis metabolite product, was found in plasma.[73] Thus, modification of the chemical structure of Rg3 to enhance oral bioavailability may be as one of the future research directions.

Among these studies, Rg3 was administered in a variety of ways such as intraperitoneal injection, oral administration, oral gavage, gastric perfusion, tail vein injection, orogastric gavage, subcutaneous injection and intragastric administration. when Rg3 was administration by oral at the dosage of 300 mg/kg, still no measurable toxicity was detected,[72] which meant that Rg3 had a wide tolerance range for animal models. Most of the studies had reported about the inhibitory effect of Rg3 on tumor growth. Due to the great difference between *in vitro* and *in vivo* experiments, so these *in vitro* mechanisms do not actually work *in vivo*. Interestingly, for the results of Rg3 on human non-small cell lung carcinoma, Park D et al[72] found that Rg3 did not inhibit the growth of transplanted nude mice, whereas Xie QP et al[16] revealed that Rg3 reduced tumor volumes and growth.

4. Effects of Rg3 in combinational cancer therapy

Table 3 summarizes the combinational anticancer effects of Rg3 with other chemotherapy drugs. Drug resistance is a major reason for the failure of cancer chemotherapy, because chemoresistance limits ideal outcomes of chemotherapy, and finally leads to cancer recurrence, metastasis and death. Some studies have illustrated that Rg3 can reverse drug resistance and improve treatment efficacy. One study revealed that Rg3 reversed cisplatin resistance in lung cancer cells through down-regulating PD-L1,[74] and reversed drug resistance in cisplatin-resistant bladder tumor T24R2 cells.[75] Another study reported that Rg3 effectively reversed cisplatin resistance in gastric cancer cells through inhibiting SOX2 and the PI3K/Akt/mTOR signaling axis by upregulating miR-429.[76] Besides, Rg3 could attenuate the Osimertinib resistance by reducing the stemness of non-small cell lung cancer cells, which was dependent on Hippo pathway.[77] These reports suggested that Rg3 had an adjunctive effect in cancer chemotherapy, and could delay the occurrence of drug resistance.

The adverse side effects of chemotherapy drugs seriously affect the life quality of cancer patients, which limits its wider application. The anticancer effects of Rg3 in combinational therapy have been verified in many types of cancer cells or animal models such as HCT116,[78] SW620,[78,79] LNCaP, PC-3 and DU145,[80] SPCA1,[81] A549,[81–83] SK-Hep1,[27,84] Hep1-6,[85] HepG2,[27,84–87] Hep3B and HT-29,[27] H322,[78] SPC-A1,[81,83] H1299,[82] C6 glioma cells,[88] T98G and U118,[89] BxPC-3 and AsPC-1,[90] MDA-MB-231,[91] MDA-MB-453, BT-549,[91] Huh7,[86] Bel 7404,[87] SMMC-7721,[92] HCC827 and PC-9,[93] H1975 and H1975-OR,[77] and LOVO[79] cells, as well as Lewis lung tumor xenograft C57/BL6 mice,[94,95] athymic mice bearing SKOV-3,[96] mice bearing Hep1-6 cells,[85] mice bearing 4T1 breast cancer cells,[97] A549 xenograft nude mice,[81] Huh-7 xenograft nude mice,[78,86] rat bearing C6 glioma cells,[88] mice bearing MCF-7 cells,[98] BxPC-3 xenograft nude mice,[90] MDA-MB-231 xenograft nude mice,[91] U118 xenograft nude mice[89] and PC-9 xenograft nude mice.[93] And this combinational effects of Rg3 mainly included chemosensitive, synergistic, additive, and the reduction of side effects. In addition, a meta-analysis involving 2202 non-small cell lung cancer patients in China also suggested that Rg3 combined with first-line chemotherapy could enhance drug efficacy and reduce drug-induced toxicity from chemotherapy.[99]

The combinational anticancer effects of Rg3 with other chemotherapy drugs among these reports are attained by inhibiting cellular viability and proliferation, migration, invasion and metastasis, and inducing apoptosis, autophagy and cell cycle arrest, reversing multidrug resistance, and prolonging the survival. The underlying mechanisms also have been illustrated. Rg3 treatment resulting in an increase of Bax,[78,80,82,85,91] caspase 3,[75,78,80,82,85] caspase 9,[78,80] cytochrome *c*,[75] DR5,[27] LC3-II/LC3-I,[98] E-cadherin82 and miR-429,[76] and a decrease of VEGF,[83,88,94–98] PCNA,[92,96] Bcl-2,[75,78,80,85,91] Cox-2, c-Fos and c-Jun,[78] cyclin D1,[78,92] MMP2,[98] MMP9,[98] PD-L1,[74] PTX3,[81] SNAIL and SLUG,[82] as well as the inhibition of YAP/TAZ expression,[77] SOX2,[76] NF-κB activity,[74,78,80,81,83,91,100] PI3K/Akt/mTOR,[76,98] EGFR/PI3K/Akt,[90] PTEN/Akt[86] and PI3K/Akt[79,87] signaling pathways contributed to the combinational effects of Rg3.

5. Signaling pathway of Rg3 in cancer

Based on current studies, the anticancer signaling pathway of Rg3 remains unclear. Shan X et al revealed that targeting FUT4/LeY mediated fucosylation by Rg3 inhibited the activation of EGFR/MAPK pathway and prevented melanoma growth.[13] Rg3 inhibited colorectal cell growth via downregulation of C/EBPβ/NF-κB signaling.[69] Another study suggested that Rg3 inhibited lung cancer cell proliferation by targeting EGFR-mediated Ras/Raf/MEK/ERK[41] and Hh[46] pathway. Rg3 induced melanoma cell cycle arrest by downregulating HDAC3 expression and increasing p53 acetylation,[19] as well as inducing apoptosis by decreasing noncleaved caspase-3 and Bcl-2. Moreover, Rg3 induced cancer cell apoptosis via activation of caspase-3, -8 and -9 and upregulation of Bax,[101] and suppressing PI3K/Akt/mTOR[12,36] and MEK[38,57] signaling pathway. Besides, Rg3 inhibited cancer cell migration and invasion by downregulating the expression levels of MMP2[20,44,67] and MMP9,[20,67] and by regulating p38 MAPK and Smad,[44] Notch-Hes1[48] and NF-κB[51] pathway. In hepatocellular carcinoma, Rg3 decreased NHE1 expression via inhibiting EGF/EGFR/ERK1/2/HIF-1α pathway.[34] Rg3 exhibited anticancer activity in lung cancer cells,[16] human hepatocellular carcinoma cells[102] and gastric cancer cells[37] by suppressing the PI3K/Akt signaling pathway. Furthermore, Rg3-induced DNA damage in non-small cell lung cancer cells was dependent on VRK1/P53BP1 pathway.[18] Therefore, we hypothesized based on these reports that PI3K/Akt could be an important signaling pathway for Rg3 to exert its anticancer activity. As shown in Fig. 2, the potential signaling pathways of anticancer activity of Rg3 have been demonstrated.

6. The stereospecific effects of two epimers of Rg3

Rg3 has two stereoisomeric pairs, 20(S)-Rg3 and 20(R)-Rg3 with different pharmacological actions because of their different chemical structures. Compared with 20(S)-Rg3, 20(R)-Rg3 had more potent adjuvant activity in terms of the stimulation of the immune response.[8]

Table 2
In vivo anticancer effects of Rg3.

cancer	Models	Ginsenoside Rg3	Administration	Dosage	Main findings	Ref
Liver cancer	H22-bearing mice	Rg3	intraperitoneal	3 mg/kg/day	Inhibit tumor growth at least partly by improving the host's cellular immunity	(68)
Liver cancer	HCCLM3 xenograft mice	Rg3	intraperitoneal	10 mg/kg/two days	Inhibit tumor growth by inhibiting EGF-EGFR-ERK1/2-HIF-1α-NHE1 pathway	(34)
Liver cancer	Hep1-6 HCC tumor-bearing C57BL/6 mice	Rg3	oral	10 mg/kg/day	Prolong the survival of Hep1-6 HCC tumor-bearing mice, reduce HCC tumor growth, induce HCC apoptosis *in vivo* and decrease angiogenesis quantification (MVD) in HCC tumors	(64)
Live cancer	BABL/c nude mice bearing HepG2 or MHCC-97L	20(R)- Rg3	oral gavage	Dosages of 2.5, 5 and 10 mg/kg	Inhibit the migration and invasion of liver cancer cells by upregulation the protein expression of ARHGAP9	(43)
Lung cancer	Mice bearing H460	Rg3	oral	Dosages of 100 and 300 mg/kg	Inhibit of tumor growth by indirect immunomodulatory actions	(72)
Lung cancer	A549 xenograft nude mice	Rg3	intraperitoneal	10 mg/kg/three days	Inhibit tumor growth, EMT and metastasis	(45)
Lung cancer	A549 or H23 xenograft nude mice	Rg3	unclear	20 mg/kg	Inhibit tumor growth by down-regulating PI3K/Akt signaling pathway	(16)
Lung cancer	urethane-induced NSCLC model in mice	Rg3	Oral gavage	3 mg/kg/day	Inhibit tumorigenesis and viability by activating VRK1/P53BP1 pathway	(18)
Colon cancer	SW480 xenograft nude mice	Rg3	intraperitoneal	10–40 mg/kg/d	Inhibit growth via down-regulation of C/EBPβ/NF-κB signaling	(69)
Colon cancer	mice bearing LoVo and or SW620 orthotopic xenografts	Rg3	Gastric perfusion	25 mg/kg/day	Inhibit growth and stemness of colorectal cancer downregulate the expressions of angiogenesis-related genes and repress the vascularization	(65)
Colon cancer	NSG mice bearing HT29 colorectal cancer cells	20(R)- Rg3	intraperitoneal	5 mg/kg/three times weekly	Inhibit tumor metastasis	(47)
Colon cancer	HCT15 xenograft nude mice	Rg3	intraperitoneal	10 mg/kg/once every two days	Inhibit Epithelial–Mesenchymal Transition via downregulating Notch-Hes1 signaling	(48)
Ovarian cancer	mice bearing SKOV-3	Rg3	intraperitoneal	0.3, 1.0 and 3.0 mg/kg/day	Inhibit lung metastasis of SKOV-3 cells and tumor-induced angiogenesis	(49)
Ovarian cancer	SKOV3 xenograft nude mice	20(S)- Rg3	intraperitoneal	5 mg/kg/other day	Inhibit hypoxia-induced Epithelial Mesenchymal Transition and tumor growth	(50)
Ovarian cancer	SKOV3 xenograft nude mice	20(S)- Rg3	tail vein	5 mg/kg/other day	Inhibit migration via downregulating HIF-1α expression	(51)
Ovarian cancer	SKOV3 xenograft nude mice	20(S)- Rg3	intraperitoneal	5 mg/kg/other day	Induce autophagy to inhibit migration and invasion	(40)
Ovarian cancer	SKOV3 xenograft nude mice	20(S)- Rg3	unknown	unknown	Inhibit the Warburg effect in ovarian cancer cells via H19/miR-324-5p/PKM2 pathway	(110)
Ovarian cancer	SKOV3 xenograft nude mice	20(S)- Rg3	unclear	unclear	Upregulate FDFT1 via reducing miR-4425 to inhibit ovarian cancer progression	(52)
Breast cancer	mice bearing FM3A	Rg3	orogastric gavage	2.5, 5, and 10 mg/kg	Inhibit cancer progression	(53)
Breast cancer	mice bearing MDA-MB-231	Combination of 20(R) and 20(S) Rg3	subcutaneous	23 mg/kg SRg3 + 11.5 mg/kg RRg3 or 46 mg/kg SRg3 + 23 mg/kg RRg3	shrink the primary tumor and decrease the load of metastasis in mice	(54)
Gastric cancer	Atp4a−/− C57Bl/6 mice	Rg3	intragastric	Dosages of 5 and 10 mg/kg/day	Ameliorate gastric precancerous lesions in Atp4a−/− Mice via inhibition of glycolysis through PI3K/AKT/miRNA-21 Pathway	(22)
Gastric cancer	gastric precancerous lesion rat model	Rg3	gavage	1.8, 3.6 and 7.2 mg/kg	Induce apoptosis and inhibit proliferation by down-regulating TIGAR	(55)
Gastric cancer	gastric precancerous lesion rat model	Rg3	gavage	50 and 100 mg/kg	Inhibit angiogenesis via downregulation of Glut1 and Glut 4	(66)
Melanoma	mice bearing B16	Rg3	tail vein	1.5 mg/kg/every other day	Inhibit tumor metastasis with lighter lung weight, lower density of microvessels, fewer metastasis nodules and longer survival time	(71)
Melanoma	A357 xenograft nude mice	Rg3	intraperitoneal	20 mg/kg	Inhibit tumor growth via downregulation of HDAC3 and increasing of p53 Acetylation	(19)
Melanoma	A357 xenograft nude mice	20(R)- Rg3	subcutaneous	20 mg/kg	Inhibit tumor growth via downregulation of FUT4	(13)
Melanoma	mice bearing B16	Rg3	intraperitoneal	0.3, 1.0 and 3.0 mg/kg/day	Inhibit tumor growth, inhibit metastasis and angiogenesis, downregulate ERK and Akt signaling pathways	(20)
Osteosarcoma	MG63 xenograft nude mice	Rg3	unclear	20 mg/kg/day	Inhibit tumor formation by regulating circ_0003074/miR-516b-5p/KPNA4 axis	(58)
Pancreatic cancer	SW-1990 xenograft nude mice	Rg3	intraperitoneal	5, 10 or 20 mg/kg/day	Inhibition of vasculogenic mimicry through downregulation of VE-cadherin/EphA2/MMP9/MMP2 expression	(67)
Gallbladder cancer	GBC-SD xenograft nude mice	Rg3	oral	20 mg/kg/day	Inhibit tumor growth	(10)
Gallbladder cancer	NOZ xenograft nude mice	20(S)- Rg3	intraperitoneal	20 and 40 mg/kg/day	Inhibit tumor growth by causing cell senescence and apoptosis	(24)
Gallbladder cancer	GBC-SD xenograft nude mice	Rg3	oral	20 mg/kg/day	Inhibit tumor growth through the ER stress-mediated pathway	(28)
Thyroid Cancer	TPC-1 and C643 xenograft nude mice	Rg3	intragastric	10 mg/kg/day	Inhibit tumor growth and metastasis	(61)

Table 3
Effects of Rg3 in combinational cancer therapy.

Drugs	Combinational effects	Cell lines or models	Results	Ref
Cyclophosphamide	antiangiogenic	Lewis lung tumor xenograft C57/BL6 mice	Combination treatment inhibits tumor growth, has no treatment side effect, improves survival and has much lower Ki-67 LI, VEGF, Bcl-2 or P53 expression	(94)
Cyclophosphamide	synergistic antiangiogenic	Athymic mice bearing SKOV-3 cells	Combination treatment reinforces the antitumor effect and improves the living quality and survival time of mice with tumor	(96)
Cyclophosphamide	synergistic	Hep1-6 and HepG2 cells mice bearing Hep1-6 cells	inhibit tumor growth and prolong mouse survival time by inducing HCC cell apoptosis via intrinsic pathway by expression alterations of Bcl-2 family proteins	(85)
Capecitabine	antiangiogenic	Mice bearing 4T1 breast cancer cells	Combination treatment enhances antiangiogenic effect and prolongs the survival time	(97)
Docetaxel	Chemosensitive	HCT116 and SW620 cells	Combination treatment inhibits cell growth and induces apoptosis by significant inhibition of NF-κB activity	(78)
Docetaxel	Chemosensitive	LNCaP, PC-3 and DU145 cells	Enhance the inhibition of cancer cell growth and induction of apoptosis as well as G0/G1 arrest, and inhibit NF-κB activity	(80)
Gemcitabine	antiangiogenic	Lewis lung tumor xenograft C57/BL6 mice	inhibit angiogenesis and tumor growth, improve survival and quality of life of tumor-bearing mice, and decrease VEGF expression and MVD as well as signals of blood flow and PSV in tumors	(95)
Gemcitabine	synergistic	A549 and SPCA1 A549 xenograft nude mice	Inhibit migration and invasion through ROS-dependent, NF-κB- and HIF-1α-mediated downregulation of PTX3	(81)
Doxorubicin	Chemosensitive/ synergistic	SK-Hep1, HepG2, A549, and H322 cells Huh-7 xenograft nude mice	Induce apoptosis via suppression of autophagic flux, promote caspase-independent cell death, and inhibit tumor growth in HCC xenografts *in vivo*	(84)
Cisplatin	synergistic	T24R2 cells	Induce cell cycle arrest at the G2/M phase, increase Cleaved PARP, caspase-3, -8 and -9, cytochrome c, Bad and Cyclin B1 expression, and decrease Bcl-2 expression	(75)
Cisplatin	Chemosensitive	A549 and A549/DDP	Alleviate the resistant to cisplatin of A549/DDP cell, and decrease PD-L1, p-P65 and p-Akt expression	(74)
Cisplatin	Chemosensitive	SPC-A1 and H1299	Increase the sensitivity to cisplatin via blocking of NF-κB mediated epithelial–mesenchymal transition and stemness in hypoxic lung cancer cells	(100)
Cisplatin	Chemosensitive	AGS GC and AGSR-CDPP cells	Alleviated Cisplatin resistance, enhance chemosensitivity in AGSR-CDDP GC cells partly through upregulating miR-429, targeting SOX2 and modulating downstream PI3K/AKT/mTOR signaling	(76)
Temozolomide	antiangiogenic	HUVEC and C6 glioma cells Rat bearing C6 glioma cells	Inhibit proliferation, induce cell cycle arrest and apoptosis and reduced VEGF-A and Bcl-2 expression in HUVECs; inhibit tumor growth, decrease VEGF-A expression and MVD in rat bearing C6 glioma cells	(88)
Temozolomide	synergistic	T98G and U118 cells U118 xenograft nude mice	Augment TMZ-mediated chemotherapy by downregulating the expression of MGMT, and restrain the epithelial–mesenchymal transition progression of glioma cells *in vitro* and *in vivo*	(89)
Endostar	antiangiogenic	Mice bearing MCF-7 cells	Inhibit tumor growth, decrease *VEGF-A*, *VEGF-B* and *VEGF-C* as well as *MMP2* and *MMP9* mRNA expression, increase *LC3-II/LC3-I* mRNA expression, and decrease mRNA mTOR, PI3K, Akt, JNK and Beclin-1 expression	(98)
Erlotinib	Chemosensitive	BxPC-3 and AsPC-1 BxPC-3 xenograft nude mice	Enhance the anti-proliferation, apoptosis, and sensitivity of pancreatic cancer cell lines to erlotinib through downregulation of EGFR/PI3K/Akt signaling, enhance the sensitivity of pancreatic cancer xenograft to erlotinib	(90)
Paclitaxel	Chemosensitive	MDA-MB-231, MDA-MB-453 and BT-549 cells MDA-MB-231 xenograft nude mice	Promote cytotoxicity and apoptosis of Paclitaxel by inhibiting NF-kB signaling and modulating Bax/Bcl-2 expression	(91)
Sorafenib	synergistic	HepG2 and Huh7 Huh7 xenograft nude mice	Enhance the antiproliferation and apoptosis of hepatocellular carcinoma cells to Sorafenib, decrease tumor volumes and weight *in vivo*, increase PTEN, Bax and cleaved caspase-3 expression, decrease p-PDK1 and p-Akt expression	(86)
Sorafenib	synergistic	HepG2 and Bel7404	Combination treatment exhibits a better effect, and reduces cell viability, glucose consumption, lactate levels, and protein expression of HK2, PI3K, and Akt	(87)
Sorafenib	synergistic	HepG2-SR HepG2-SR xenograft nude mice	Inhibit activation/phosphorylation of Src and STAT3, decrease the STAT3 nuclear level and induce ROS production	(119)
Gefitinib	Chemosensitive	A549 and H1299	Increase the cytotoxic effect, inhibit migration, increase Bax, cleaved caspase-3 and E-cadherin expression, and decrease Bcl-2, SNAIL and SLUG expression	(82)
Oxaliplatin	synergistic	SMMC-7721	Suppress the proliferation and promote the apoptosis of SMMC-7721, inhibit the expressions of PCNA and cyclin D1	(92)
Icotinib	Chemosensitive	HCC827 and PC-9 cells PC-9 xenograft nude mice	Inhibit autophagic flux and sensitize parental and resistant cells to icotinib, reverse the resistance to icotinib in *vivo*, increase LC3 II expression	(93)
Osimertinib	Chemosensitive	H1975 and H1975-OR	Attenuates osimertinib resistance of H1975 spheroids and enhance osimertinib sensitivity of H1975 cells, Attenuate the stemness and resistance of H1975-OR cells, increase MST1/2 and LATS1/2 expression	(77)
5-FU	synergistic	SW620 and LOVO cells	Combination treatment enhances the inhibition of the proliferation, colony formation, invasion and migration, enhances the apoptosis by activating the Apaf1/caspase 9/caspase 3 pathway and arrests the cell cycle in G0/G1 by promoting the expression of Cyclin D1, CDK2 and CDK4, and enhances the anticancer effect of 5-FU by inhibiting the PI3K/AKT pathway	(79)
5-FU	antiangiogenic	A549 and SPC-A-1 cells	Combination treatment enhances the suppression on proliferative, invasive, and migratory abilities and angiogenesis, and presents the optimal inhibitory effects on VEGF-A expression and NF-κB Signaling Pathway	(83)
TRAIL	Chemosensitive	SK-Hep1, HepG2, Hep3B and HT-29	20(S)-Ginsenoside Rg3 mediates TRAIL-induced cell death via CHOP-mediated DR5 upregulation, and promotes TRAIL-induced caspase-dependent apoptotic cell death	(27)

Moreover, 20(R)-Rg3 had more potency and efficacy antitumor effects than 20(S)-Rg3 in hepatocellular carcinoma H22 transplanted tumors and the immune function of H22-bearing mice.[68] However, Cheong JH et al found that 20(S)-Rg3 in red ginseng showed more potent antitumor activity than 20(R)-Rg3 through inducing the apoptosis of human liver cancer HepG2 cells via downregulation of Bcl2 and upregulation of

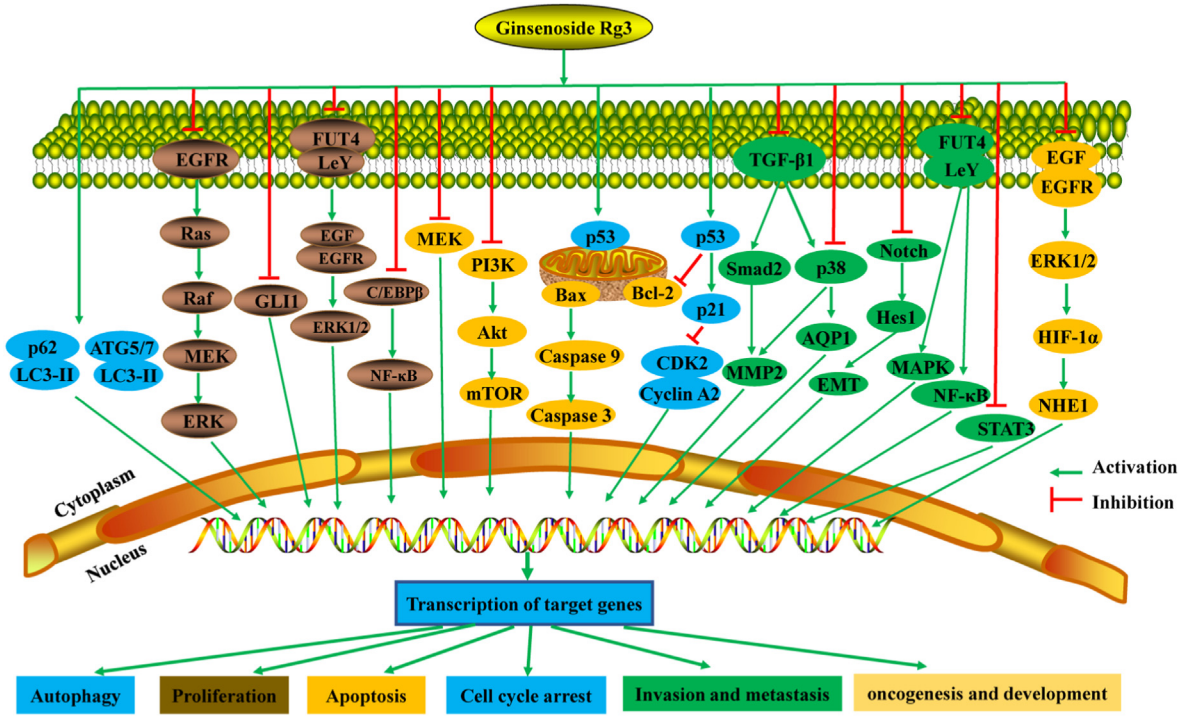

Fig. 2. Potential anticancer signaling pathways of Rg3. Rg3 exhibits anticancer activity by modulating Ras/Raf/MEK/ERK, EGF/EGFR/ERK1/2, C/EBPβ/NF-κB, PI3K/Akt/mTOR, TGF-β1/Smad2 or p38/MMP2, FUT4/LeY/MAPK or p38, NF-κB, MEK, Notch-Hes1, STAT3 and EGFR/ERK1/2/HIF-1α/NHE1.

Fas.[103] Therefore, much more study is required to understand the differences caused by stereoisomer-specific anticancer activity of Rg3.

7. Conclusions

Rg3 is one of the main bioactive ingredients of ginseng, which has varieties a variety of pharmacological effects and plays a therapeutic role in numerous diseases. Many studies have demonstrated that Rg3 exerts excellent anticancer activity *in vitro* and *in vivo*. The molecular mechanisms of anticancer effects of Rg3 mainly involve the induction of apoptosis, autophagy, cell cycle arrest, and the inhibition of proliferation, metastasis and angiogenesis, and the promotion of immunity. Moreover, a number of experiments have been investigated that Rg3 is relatively safe for application in the heart, liver, lung, kidney, marrow, and central nervous system. The long-term toxicological studies on beagle dogs and rats also have confirmed that 20(S)-ginsenoside Rg3 is non-toxic and well-tolerated.[104,105] Rg3 has also been reported to significantly decrease some environmental carcinogen-induced DNA damage, including N-methyl-N′-nitro-N-nitrosoguanidine (MNNG).[33] These results make Rg3 an attractive candidate for preventing cancer. Combinational cancer therapy, such as chemotherapy and surgery has been suggested to be more effective. Furthermore, Rg3 promotes the chemosensitivity of tumor cells to chemical drugs, reverses multidrug resistance and reduces toxicity. Therefore, Rg3 is getting more and more attention as an anticancer adjuvant due to its well safety and efficacy.

However, there are still several aspects that limit the application of Rg3. Firstly, its molecular mechanisms of anticancer action have not yet been fully illustrated. Rg3 induces strong genotoxicity and DNA damage in human osteosarcoma cells, but whether these genotoxic effects are directly associated with cell cycle arrest and apoptosis is not yet clear. Future studies are warranted to demonstrate the mechanisms through which different signaling pathways are verified. Secondly, Rg3 exerts low oral bioavailability and poor aqueous solubility. Compared to the Rg3 solution, liposomal or nanometer targeted Rg3 shows increased anticancer activity. Therefore, it may be possible to develop novel Rg3 analogues with improved bioavailability profiles, pharmacokinetics and efficacy. Finally, the evidences of Rg3 efficacy have not yet conclusive in humans. Therefore, larger cohort clinical studies are necessary to confirm its efficacy for better clinical application in the future.

In summary, Rg3 exerts anticancer effects *in vitro* and *in vivo*, and is a promising agent for cancer prevention and treatment.

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Authors' contributions

W.Y., L.G., and C.T. Collected the literatures. W.Y., L.G., and W.W., analyzed the literatures. W.Y wrote the review. W.Y, L.G., Y.Z., C.T., and L.X gave final approval of the version to be published.

Declaration of competing interest

On the behalf of all the authors in paper, I corresponding author hereby accept that there is no conflicts of interest.

Abbreviations

IC50	Half maximal inhibitory concentration
P38	phosphor-p38
Bcl-2	B-cell lymphoma-2
MAPKs	mitogen-activated protein kinases
NF-kB	nuclear factor kappa-B
MMP	matrix metalloproteinase
DNMT	DNA methyltransferase
p-ERK	phosphorylated extracellular signal-regulated protein kinase
EGF	epidermal growth factor

HOTAIR	HOX antisense intergenic
LC3	microtubule-associated protein light chain 3
EMT	epithelial–mesenchymal transition
NSCLC	non-small cell lung cancer
GLI1	glioma-associated oncogene-1
PINK1	PTEN-induced putative kinase 1
PITPNA	phosphatidylinositol transfer protein alpha
C/EBPβ	CCAAT/enhancer binding protein β
ER	Endoplasmic reticulum
eIF2α	eukaryotic translation-initiation factor 2α
KPNA4	karyopherin subunit alpha 4
PCNA	proliferating cell nuclear antigen
FDFT1	farnesyl-diphosphate farnesyltransferase 1
MAP	mitogen-activated protein
TRPM7	transient receptor potential melastatin 7
HSF1	Heat Shock Transcription Factor 1
NADP	nicotinamide adenine dinucleotide phosphate
GSH	glutathione
CASC2	cancer susceptibility candidate 2
TRAIL	Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand
Bax	Bcl-2-associated X
CHOP	CCAAT/enhancer-binding protein homologous protein
PARP	cleaved poly adenosine diphosphate-ribose polymerase
STAT3	signal transducer and activator of transcription 3
VEGF	vascular endothelial growth factor
ROS	reactive oxygen species
NHE1	Na+/H+ exchanger 1
HIF-1α	hypoxia-inducible factor 1 alpha
ARHGAP9	Rho GTPase activating protein 9
EGFR	epidermal growth factor receptor
TGF-β1	Transforming growth factor-beta 1
FUT4	Fucosyltransferase IV
VRK1	Vaccinia-related kinase 1
TKIs	tyrosine kinase inhibitors
AKAPA8L	A-kinase (PRKA) anchor protein 8-like
AP-1	activator protein-1
CCAT1	Colon Cancer Associated Transcript 1
PERK	PKP-like endoplasmic reticulum
ATF4	activating transcription factor 4
HDAC3	Histone Deacetylase 3
ERK	extracellular signal-regulated kinase
AQP1	aquaporin 1
CXCR4	Chemokine CXC receptor 4
SP1	Specificity protein 1
TIGAR	TP53-induced glycolysis and apoptosis regulator
G6PDH	glucose-6-phosphate dehydrogenase
EphA2	epithelial cell kinase
5-FU	5-Fluorouracil
ATG5	autophagy-related 5

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