Anticancer effects and potential mechanisms of ginsenoside Rh2 in various cancer types (Review)

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Abstract. Ginsenoside Rh2 (G-Rh2) is a natural bioactive product derived from *Panax ginseng* Meyer (*P. ginseng*). G-Rh2 exhibits anticancer activity in various human cancer cell lines both *in vitro* and *in vivo* by modulating several signaling pathways, such as those of PDZ-binding kinase/T-LAK cell-originated protein kinase, phosphatidylinositol 3-kinase, protein kinase B, mammalian target of rapamycin, epidermal growth factor receptor, p53, and reactive oxygen species. Moreover, G-Rh2 could effectively reverse drug resistance and enhance therapeutic effects in cancer therapy. This review summarizes the chemical properties, *in vitro* and *in vivo* anticancer activity, and underlying molecular mechanisms of G-Rh2 to facilitate cancer chemoprevention studies.

Contents

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1. Introduction

Cancer is the second leading cause of death globally, responsible for ~9.6 million deaths in 2018 (1). The main forms of cancer treatments are surgery, radiotherapy, chemotherapy and hormone therapy. Adjuvant chemotherapy following surgery has been proven to decrease recurrence and improve patient survival time (2,3). Natural products have been major sources for the active ingredients in numerous medicines. Compared with synthetic products, natural products generally have fewer toxic effects and are less expensive. Ginseng, derived from the rhizome and root of *Panax ginseng* Meyer (*P. ginseng*), is a popular herbal medicine that has been used in Asian countries (e.g., China, Japan and Korea) for thousands of years. It is well known for its disease-preventing and therapeutic effects.

Ginsenosides are the main active chemical constituents of *P. ginseng*. To date, >100 types of ginsenosides have been isolated and identified from *P. ginseng* (4). Among them, ginsenoside Rh2 (G-Rh2) exhibits various biological activities, such as improvement in learning and memory (5), promotion of immunity (6), and antioxidant (7), anti-inflammatory (8-11), antihyperglycemic (12) and antitumor (13-21) effects. Increasing evidence shows that G-Rh2 exerts antitumor effects in a variety of cancer models, including human lung (22-26), liver (27,28), gastric (29), colorectal (17,18,30,31), breast (32-34), prostate (13,14,35-37) and pancreatic (20) cancer, leukemia (38-41) and ovarian cancer (42-45). The functional mechanisms of G-Rh2 mainly include inducing apoptosis, arresting the cell cycle, inhibiting proliferation, angiogenesis and metastasis, and regulating the tumor microenvironment

to promote immunity. Additionally, combining G-Rh2 with chemotherapy drugs can reverse drug resistance and enhance drug sensitivity in various cancer types. These topics will be described later in the review. Therefore, this review provides a systematic summary of the anticancer effects of G-Rh2 in order to facilitate further studies involving G-Rh2.

2. Chemical properties of G-Rh2

The ginsenosides are classified as 20(S)-protopanaxadiol compounds (including ginsenosides Rb1, Rb2, Rc, Rh2 and Rg3, among others) or 20(S)-protopanaxatriol compounds (including ginsenosides Re, Rg1 and Rg2, among others). G-Rh2 has been found to inhibit the growth of various human cancer cell types, such as lung cancer cells (22-25), liver cancer cells (27,28) and colorectal cancer cells (17,30,31). The chemical formula for G-Rh2 is $C_{36}H_{62}O_8$, and the molecular weight is 622.87; it is a white crystal with strong biological activity. The antitumor activity of ginsenosides is related to factors such as the type of ginsenoside, the substituents, and the number and configuration of sugars. Due to the different spatial structures at the C20 position, there are two stereoisomeric forms of G-Rh2: 20(S)-G-Rh2 and 20(R)-G-Rh2 (Fig. 1). In comparison with 20(R)-G-Rh2, 20(S)-G-Rh2 shows more potent anticancer activity among different cancer cells (35,46). One study reported that the half maximal inhibitory concentration values of 20(S)-G-Rh2 and 20(R)-G-Rh2 in A549 cells were 45.7 and 53.6 μM, respectively (46). Another study compared the effect of 20(S)-G-Rh2 and 20(R)-G-Rh2 on LNCaP, PC3 and DU145 cells. The results showed that 25 μM 20(S)-G-Rh2 inhibited LNCaP proliferation by 70%, PC3 cell proliferation by 40% and DU145 cell proliferation by 20%, while 25 μM 20(R)-G-Rh2 did not affect the proliferation of these cells (35). In addition, cytotoxic potency is generally in the descending order of protopanaxadiol, 20(S)-G-Rh2 and then 20(R)-G-Rh2, indicating structure-related activities in which the compound with less polar chemical structures possesses higher cytotoxic activity towards cancer cells (47). As much of the literature does not indicate whether 20(S)-G-Rh2 or 20(R)-G-Rh2 is used, the term G-Rh2 in this review includes both of these configurations.

3. Anticancer effects and mechanisms of G-Rh2 in *in vitro* studies

Previous studies have demonstrated that G-Rh2 exerts significant anticancer activities through multiple molecular mechanisms. The mechanisms are mainly related to cell cycle arrest, apoptosis, proliferation, invasion, metastasis, angiogenesis, autophagy and immunity, which are summarized in Table I and Fig. 2. Although the anticancer activity of G-Rh2 has been widely investigated, the exact molecular mechanisms remain unclear. Based on existing research, the possible mechanisms of action of G-Rh2 are described in this review.

Induction of cell cycle arrest. The cell cycle is a controlled process involved in the growth, differentiation and proliferation of eukaryotic cells (48). Cells that undergo cell cycle arrest lose their ability to replicate and divide. Cyclin-dependent kinase (CDK) inhibitors are crucial for controlling the cell cycle and cell proliferation (49). The CDK inhibitor p21 plays a key role in the G_1 phase cell cycle checkpoint (50). G-Rh2 was reported to induce cell cycle G_1 phase arrest in MCF-7 cells by increasing p21 levels and decreasing CDK2 and cyclin E-dependent kinase activities (32). In human glioma A172 cells, G-Rh2 induced cell cycle G_1 phase arrest by downregulating CDK4 and cyclin E (21). In human lung cancer A549 cells, G-Rh2 induced cell cycle G_1 phase arrest by significantly reducing the expression of CDK4 and cyclin D1 (51). Furthermore, G-Rh2 induced cell cycle G_1 phase arrest in HL-60 and U937 cells by downregulating the expression of CDK4, CDK6, cyclin D1, cyclin D2, cyclin D3 and cyclin E. G-Rh2-mediated G_1 phase arrest and differentiation are closely linked to the regulation of TGF-β production in human leukemia cells (52).

Induction of apoptosis. Apoptosis (programmed cell death) plays an important role in animal development and adult life by eliminating damaged cells (53). The induction of apoptosis in cancer cells can inhibit tumor growth (54). Treatment of colorectal cancer cells with G-Rh2 has been proven to activate the p53 pathway, increasing the expression of the proapoptotic regulator Bax and decreasing the expression of the antiapoptotic regulator Bcl-2 (30). The extrinsic pathway and the intrinsic pathway are two core apoptotic pathways in mammalian cells (55). As shown in Table I, G-Rh2 can induce apoptosis via these two pathways. It was previously shown that G-Rh2 induced apoptosis by downregulating Bcl-2 and survivin and upregulating Bax, cleaved caspase-3 and cleaved caspase-9 in human pancreatic cancer Bxpc-3 cells (20). G-Rh2-triggered intrinsic apoptosis was related to the induced translocation of cytosolic Bak and Bax to the mitochondria, cytochrome *c* release and caspase-9 activation in HeLa and SW480 cells (56). G-Rh2 may induce apoptosis of Kasumi-1 and U937 leukemia cells via microRNA-21-modulated suppression of Bcl-2 (57). In addition, cotreatment with G-Rh2 and betulinic acid could induce apoptosis of HeLa, A549 and HepG2 cancer cells by enhancing caspase-8 expression, cytochrome *c* release and Bax translocation (58).

Inhibition of proliferation. Abnormal regulation of cell proliferation is the key cause of cancer development and progression (59). Both activation of oncogenes and the inactivation of tumor suppressor genes can promote the proliferation of cancer cells. Protein kinase B (Akt) is one of the well-known proto-oncogenes, and activated Akt promotes cancer cell proliferation and survival (60). G-Rh2 has been shown to inhibit HeLa and A172 cell proliferation by suppressing the Akt pathway (15,21). Another study revealed that G-Rh2 inhibited the proliferation of HCT116 colon cancer cells by targeting PDZ-binding kinase/T-LAK cell-originated protein kinase and downregulating the expression of p-ERK1/2 and p-histone H3 (17). G-Rh2 inhibits the proliferation of K562 and KG1-α cells by suppressing the expression and activity of HDAC1, HDAC2, and HDCA6, increasing histone H3 acetylation and regulating MAPK/JNK signaling pathways (61). Additionally, G-Rh2 could inhibit cancer cell proliferation by suppressing endoplasmic reticulum (ER) stress (22), by inhibiting the Src/ERK signaling pathway (62) and by targeting microRNA-128 (63).

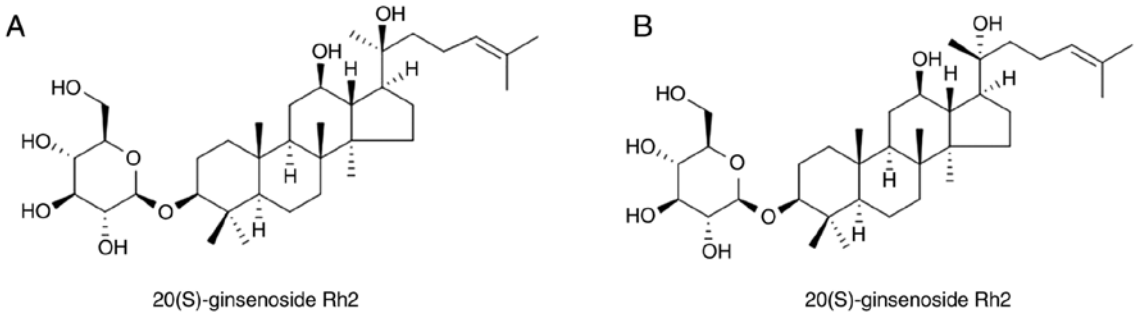

Figure 1. Chemical structures of the two forms of G-Rh2. (A) Chemical structure of 20(S)-G-Rh2; (B) Chemical structure of 20(R)-G-Rh2. G-Rh2, ginsenoside-Rh2.

Table I. Anticancer effects and mechanisms of ginsenoside-Rh2 in *in vitro* studies.

Cancer type	Cell type	Cellular effects	Mechanisms	(Refs.)
Lung cancer	H1299, A549, Lu-99, EBC-1, H460, CH27	Proliferation, apoptosis, cell cycle arrest	p21↓, CDK6↓, cyclin D1↓, cyclin E↓, ATF4↑, CHOP↑, caspase-4↑, mir-491↑, pRb2↑, DR4↑	(22,24-26)
Prostate cancer	PC3, DU145, LNCaP	Proliferation, apoptosis, invasion	p-STAT3↓, cyclin D1↓, cyclinB1↓, MMP2↓, MMP9↓, CDKN1A↑, PPAR-δ↑, miR-4295↓, pSmad2↑, p27↑	(14,35-37)
Colorectal cancer	HCT116, SW480, LoVo, SW620, HCT-8	Proliferation, apoptosis, invasion, cell cycle arrest	p-ERK↓, p-p90RSK↓, Bcl2↓, Bcl-xl↓, P-pg↓, cyclin D1↓, CDK2↓, p-Rb↓, Bcl-2↓, N-cadherin↓, vimentin↓, MMP9↓, Smad4↑, caspase-3↑, p-AMPK↑, NF-κB↑, ROS↑, Bad↑, Bax↑, cleaved caspase-3↑, p-IκB-α↑, E-cadherin↑	(17-19,30,31,78,80)
Breast cancer	MCF7, MDA-MB-231	Proliferation, apoptosis, cell cycle arrest	CASP1↓, INSL5↓, OR52A1↓, CDK2↓, CDK4↓, CDK6↓, cyclin A↓, cyclin D1↓, cyclin E↓, CLINT1↑, ST3GAL4↑, C1orf198↑, p21↑	(32-34)
Gastric cancer	SGC-7901	Proliferation, apoptosis, cell cycle arrest	Bcl2↓, Bax↑	(29)
Liver cancer	HepG2, Huh7, SMMC-7721, Hep3B	Proliferation, apoptosis, migration	MCL1↓, Nrf2↓, NF-κB↓, annexin A2↓, EZH2↓, H3K27me3↓, EGFR↓, cyclin D1↓, Bcl2↓, MMP3↓, p-AMPK↑, p-p38↑, p-JNK↑, p-ERK↑	(19,27,28,84,85)
Cervical cancer	HeLa	Proliferation, apoptosis	p-Akt↓, p-GSK-3β↓, N-cadherin↓, vimentin↓, ZEB1↓, Snail-1↓, E-cadherin↑, Fas↑, TNFR1↑, cleaved caspase-8↑, cleaved caspase-9↑, cleaved PARP↑, TNF-α↑, p53↑	(15,56)
Endometrial cancer	HEC1A, Ishikawa	Proliferation, apoptosis, invasion, migration	vimentin↓, TGF-β↓, Snail↓, cleaved PARP↑, cleaved caspase-3↑, E-cadherin↑	(65)
Skin squamous cell carcinoma	A431	Proliferation, autophagy	β-catenin↓, Beclin-1↑, Atg7↑, LC3-I↑, LC3-II↑	(76)
Pancreatic cancer	Bxpc-3	Proliferation, apoptosis, migration, cell cycle arrest	caspase-9↑, Bcl-2↓, survivin↓, cyclin D1↓, MMP-2↓, MMP-9↓, Bax↑, cleaved caspase-3↑	(20)

Table I. Continued.

Cancer type	Cell type	Cellular effects	Mechanisms	(Refs.)
Ovarian cancer	HRA, KK, KF, KFr, SKOV3, SKOv3ip, Hey	Proliferation, apoptosis, cell cycle arrest	Bcl-2↓, cleaved PARP↑, cleaved caspase-3↑	(42,44,45)
Leukemia	U937, K562, Jurkat, HL-60, Kasumi-1, KG1-α	Proliferation, apoptosis, autophagy, cell cycle arrest	β-catenin↓, TCF4↓, cyclin D1↓, NF-κB↓, Bcl-2↓, LC3-I↓, CDK4↓, HDAC1↓, HDAC2↓, HDAC6↓, p-ERK↓, caspase-3↑, PARP↓, LC3B↑, p62↓, cleaved caspase-3↑, cleaved caspase-9↑, cytochrome c↑, Bax↑, Beclin-1 ↑, LC3-II↑, p16↑, p21↑, p-p38↑	(38-41,57,61,74)
Glioma	A172, U87MG, U251	Proliferation, cell cycle arrest, apoptosis	p-Akt↓, Akt↓, p-EGFR↓, p-mTOR↓, CD31↓, MMP13↓, CDK4↓, cyclin E↓, cyclin D↓, CDK2↓, p27↑	(21,93,95)
Neuroblastoma	SK-N-BE(2)	Apoptosis	caspase-1↑, caspase-3↑, Bax↑	(91)
Retinoblastoma	Y79, RBL-13	Proliferation, apoptosis, autophagy	p-PI3K↓, p-Akt↓, p-mTOR↓, p62↓, Bcl2↓, cyclin D1↓, Beclin-1↑, ATG7↑, p53↑, Bax↑, cleaved caspase-3↑, cleaved caspase-9↑	(77)
Oral cancer	YD10B, Ca9-22, KB	Proliferation, apoptosis, cell cycle arrest, migration, invasion	cyclin D1↓, cyclin E1↓, vimentin↓, N-cadherin↓, MMP-2↓, VEGF↓, p-Src↓, p-B-Raf↓, p-ERK1/2↓, cleaved PARP↑, cleaved caspase-3↑, p53↑, E-cadherin↑	(62,67)

ATF4, activating transcription factor 4; CHOP, CCAAT/enhancer-binding protein homologous protein; CDK, cyclin-dependent kinase; DR4, death receptor 4; PPARδ, peroxisome proliferator-activated receptor δ; STAT3, signal transducer and activator of transcription 3; CDKN1A, cyclin-dependent kinase inhibitor 1A; MMP, matrix metallopeptidase; ERK, extracellular regulated protein kinases; NF-κB, nuclear factor-kB; ROS, reactive oxygen species; Bad, BCL2-associated agonist of cell death; Bax, BCL2-associated X protein; P-gp, permeability glycoprotein; AMPK, AMP-activated protein kinase; CASP1, caspase-1; INSL5, insulin-like peptide 5; OR52A1, olfactory receptor family 52 subfamily A member 1; CLINT1, clathrin interactor 1; ST3GAL4, ST3 β-galactoside α-2,3-sialyltransferase 4; C1orf198, chromosome 1 open reading frame 198; Nrf2, nuclear factor erythroid 2-related factor 2; JNK, c-Jun N-terminal kinase; ERK, extracellular-signal-regulated kinase; EZH2, enhancer of zeste homolog 2; EGFR, epidermal growth factor receptor; Akt, protein kinase B; GSK-3β, glycogen synthase kinase 3β; ZEB1, zinc finger E-box-binding homeobox 1; TNFR1, tumor necrosis factor receptor 1; PARP, poly (ADP-ribose) polymerase; Atg7, autophagy-related 7; LC3, microtubule-associated protein light chain 3; TCF-4, transcription factor 4; HDAC, histone deacetylases; mTOR, mammalian target of rapamycin; CD31, cluster of differentiation 31; PI3K, phosphoinositide 3-kinase; VEGF, vascular endothelial growth factor.

Inhibition of invasion and metastasis. Metastasis is the main cause of cancer treatment failure and recurrence. During metastasis, the cells become highly plastic or adaptive, similar to stem cells. This change is called epithelial-mesenchymal transition (EMT) (64). G-Rh2 could effectively inhibit tumor metastasis by suppressing EMT. G-Rh2 has been found to inhibit migration and invasion by increasing E-cadherin and suppressing vimentin expression in endometrial cancer cells (65) and oral cancer cells (62). In addition, matrix metalloproteinases (MMPs) play an important role in tumor metastasis, where they can degrade the extracellular matrix and basement membrane (66). G-Rh2 was previously found to effectively inhibit Bxpc-3 cell migration and invasion by downregulating MMP-2 and MMP-9 (20). Moreover, G-Rh2 was reported to significantly reduce the protein levels of VEGF, MMP-2 and MMP-9 in co-cultured lung cancer cells (23) and oral cancer cells (67). Therefore, G-Rh2 may play an inhibitory role in the process of cancer metastasis.

Anti-angiogenesis. Angiogenesis plays an important role in tumor growth and metastasis by providing the necessary nutrients and oxygen (68). TGF-β is a potent angiogenesis inducer *in vivo* (69). Anti-angiogenic treatment for tumors is considered a promising therapeutic strategy (70). G-Rh2 was reported to inhibit prostate cancer growth by impeding angiogenesis via decreasing the expression of CD31, VEGF, platelet-derived growth factor and CNNM1 in cancer cells (13). Furthermore, G-Rh2 could affect tumor angiogenesis by downregulating JAM expression in tumors (71).

Induction of autophagy. Autophagy is a catabolic process that degrades cytoplasmic constituents and organelles in lysosomes (72). There is increasing evidence that autophagy

Table II. Anticancer effects and mechanisms of ginsenoside-Rh2 in *in vivo* studies.

Animal model	Dose	Route	Effects	(Refs.)
Lung cancer xenograft mouse model (H1299 cells)	20 mg/kg/day	Intraperitoneally	Reduced tumor size and tumor weight; increased ATF4, CHOP, and caspase-4 levels	(22)
Prostate cancer xenograft mouse model (PC3 cells)	1 mg/kg, 2 times a week for 4 weeks	Tail-vein injection	Inhibited tumor growth	(36)
Colon cancer xenograft mouse model (HCT116 cells)	10,50 mg/kg; 3 times/week for 29 days	Intraperitoneally	Reduced tumor size and tumor weight; decreased p-ERK and p-H3 levels	(17)
Ovarian cancer xenograft mouse model (HRA cells)	0.4 to 1.6 mg/kg/day, 21 or 28 days	Gavage	Reduced tumor size and prolong survival time	(42)
Leukemia xenograft mouse model (K562)	20 mg/kg/day for 3 weeks	Gavage	Reduced tumor size and tumor weight; decreased HDAC1, HDAC2, and HDAC6 levels	(61)
Liver cancer xenograft mouse model (HepG2)	5 mg/kg for 5 weeks	Intraperitoneally	Reduced tumor size; decreased EZH2 and H3K27me3 levels	(84)
Liver cancer xenograft mouse model (HepG2)	20 mg/kg/day for 20 days	Gavage	Decreased tumor weight by downregulating β-catenin via activation of GSK-3β	(85)
Liver cancer xenograft mouse model (SMMC-7721)	1 mg/kg, 2 times a week for 1 month	Tail-vein injection	Decreased tumor volume and weight by targeting EGFR through upregulating miR-491	(28)
T-cell acute lymphoblastic leukemia (Jurkat cells)	40 mg/kg/day for 3 weeks	Gavage	Alleviated spleen infiltration by blocking the PI3K/Akt/mTOR signaling pathway, and enhanced immunity	(83)

ATF4, activating transcription factor 4; CHOP, CCAAT/enhancer-binding protein homologous protein; ERK, extracellular-signal-regulated kinase; p-H3, p-histone H3; HDAC, histone deacetylases; EZH2, enhancer of zeste homolog 2; GSK-3β, glycogen synthase kinase 3β; EGFR, epidermal growth factor receptor; PI3K, phosphoinositide 3-kinase; Akt, protein kinase B; mTOR, mammalian target of rapamycin.

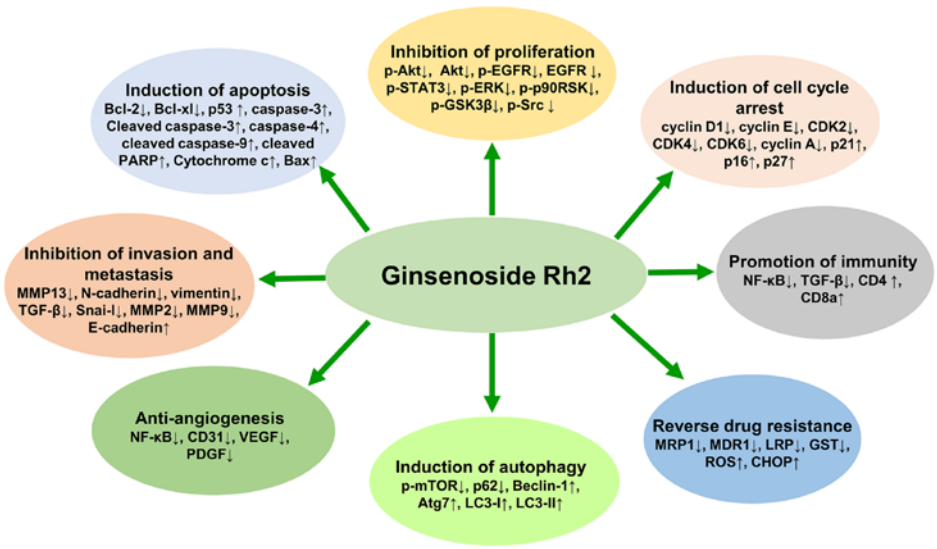

Figure 2. Possible anticancer mechanisms of G-Rh2. G-Rh2 exerts its anticancer activity by inducing apoptosis, autophagy, cell cycle arrest and immunity, and reversing drug resistance, as well as by inhibiting proliferation, invasion, metastasis and angiogenesis. ↑, indicates upregulation; ↓, indicates down-regulation. Bax, BCL2-associated X protein; MMP, matrix metallopeptidase; TGF-β, transforming growth factor β; NF-κB, nuclear factor-κB; CD31, cluster of differentiation 31; VEGF, vascular endothelial growth factor; PDGF, platelet-derived growth factor; Akt, protein kinase B; EGFR, epidermal growth factor receptor; STAT3, signal transducer and activator of transcription 3; ERK, extracellular signal-regulated kinase; GSK-3β, glycogen synthase kinase 3β; CDK, cyclin-dependent kinase; CD, cluster of differentiation; ROS, reactive oxygen species; CHOP, CCAAT/enhancer-binding protein homologous protein; MRP1, multidrug resistance-associated protein 1; MDR1, multidrug resistance protein 1; GST, glutathione S-transferase; Atg7, autophagy-related 7; LC3, microtubule-associated protein light chain 3; mTOR, mammalian target of rapamycin; p62, nucleoporin p62; G-Rh2, ginsenoside-Rh2.

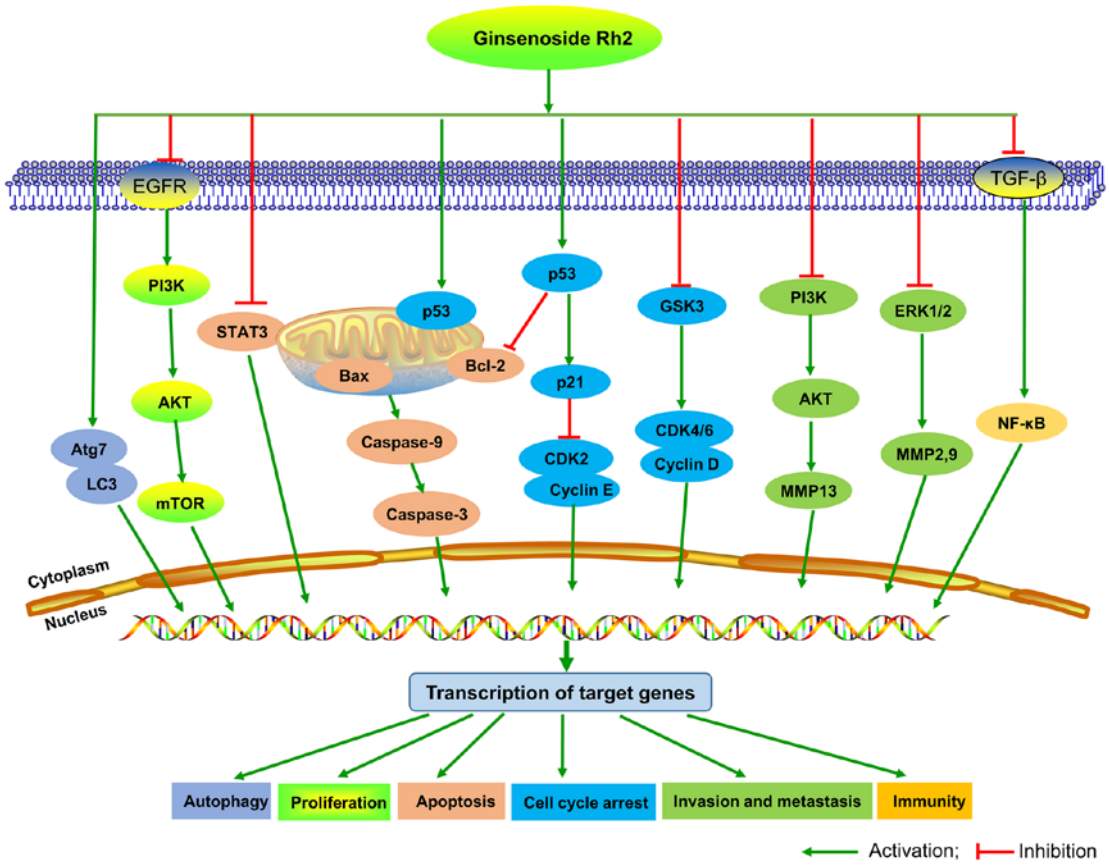

Figure 3. Potential anticancer signaling pathways of G-Rh2. G-Rh2 exhibits anticancer activity by modulating EGFR/PI3KAKT/mTOR, STAT3, p53, TGF-β/NF-κB and ERK/MMP signaling pathways. Atg7, autophagy-related 7; LC3, microtubule-associated protein light chain 3; EGFR, epidermal growth factor receptor; PI3K, phosphoinositide 3-kinase; Akt, protein kinase B; mTOR, mammalian target of rapamycin; STAT3, signal transducer and activator of transcription 3; GSK-3β, glycogen synthase kinase 3β; CDK, cyclin-dependent kinase; MMP, matrix metallopeptidase; ERK, extracellular-signal-regulated kinase; TGF-β, transforming growth factor β; NF-κB, nuclear factor-κB; G-Rh2, ginsenoside-Rh2.

signaling is closely related to oncogenic signaling. Autophagy may be one of the effective ways to prevent the formation and progression of tumors (72). Selective targeting of autophagy for cancer treatment has attracted considerable attention (73). The autophagy-related genes ATG5, ATG7, LC3B and beclin-1 were upregulated after treatment with G-Rh2 in U937 and K562 cells (74). The formation of autophagosomes involves the conversion from cytosolic LC3-I to the autophagosome-associating form of LC3-II (75). Treatment of U937 and K562 cells with G-Rh2 was found to induce the conversion from LC3-I to LC3-II and downregulate the protein level of p62 (74). G-Rh2 treatment increased autophagy through upregulating autophagy-related proteins Beclin, Atg7 and the ratio of LC3-II to LC3-I in A431 cells (76). Another study reported that G-Rh2 could promote cell autophagy in human retinoblastoma cell lines Y79 and RBL-13 by inactivating the phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) pathway (77).

Reverse drug resistance. Drug resistance is the leading cause of failure of cancer chemotherapy. Several studies have suggested that G-Rh2 has a role in reversing drug resistance and improving treatment efficacy. G-Rh2 has been found to reverse oxaliplatin resistance in colon cancer cells through decreasing the expression of P-glycoprotein (78), and to reverse drug resistance in Adriamycin-resistant human breast cancer MCF-7 cells (79). Another study reported that G-Rh2 effectively reversed 5-flurouracil resistance in colorectal cancer cells by regulating MDR1, MRP1, LRP and GST gene expression (80). In addition, G-Rh2 could enhance the antitumor effects of SMI-4a by increasing autophagy in melanoma cells (81) and could enhance the anticancer effects of cisplatin and prolong the survival time of nude mice (43). These reports suggest that G-Rh2 has an adjunctive effect in cancer chemotherapy and can delay the occurrence of drug resistance.

Promotion of immunity. It is well known that certain cancer treatments can temporarily weaken the immune system, and that enhancing the immune response could play an important role in cancer treatment. Ginseng has a long history of improving the immunity of patients in Asia. A growing number of studies indicate that G-Rh2 can improve immunity. One study reported that G-Rh2 enhanced the antitumor immunological response by triggering $CD4^+$ and $CD8a^+$ T-lymphocyte infiltration in B16-F10 melanoma cells derived from xenograft tumor tissues, as well as enhancing the cytotoxicity in spleen lymphocytes (82). Another study found that G-Rh2 could downregulate CASP1, INSL5 and OR52A1, and upregulate CLINT1, ST3GAL4 and C1orf198 expression in MCF-7 cells, indicating that G-Rh2 induces epigenetic methylation changes in genes involved in the immune response and tumorigenesis,

thereby contributing to enhanced immunogenicity and inhibiting the growth of cancer cells (33). G-Rh2 suppressed T-cell acute lymphoblastic leukemia (T-ALL) by blocking the PI3K/Akt/mTOR signaling pathway and enhanced immunity in the spleen by downregulating IL-4, IL-6, IL-10, CD3 and CD45, and upregulating IL-2 and INF-γ, and increased the number of natural killer cells (83). These studies indicate that G-Rh2 has the ability to enhance the immune response, which may play a role in the prevention and treatment of cancer.

4. Anticancer effects and mechanisms of G-Rh2 in *in vivo* studies

G-Rh2 exhibits anticancer effects in a number of animal models (17,22,36,43,61,83,84). Table II summarizes the anticancer effects of G-Rh2 in *in vivo* studies. In colon cancer xenograft mouse models, 10 and 50 mg/kg G-Rh2 three times a week via intraperitoneal injection significantly suppressed HCT116 xenograft tumor growth, and further research indicated that G-Rh2 could downregulate p-ERK1/2 and p-H3 expression by inhibiting TOPK activity *in vivo* (17). In lung cancer H1299 cell xenograft mouse models, G-Rh2 significantly inhibited lung cancer cell growth by inducing reactive oxygen species (ROS)-mediated ER stress *in vivo* (22). In leukemia K562 cell xenograft mouse models, treatment with G-Rh2 (20 mg/kg once a day for 3 weeks) significantly inhibited tumor growth *in vivo* (61). G-Rh2 was found to suppress HepG2 cell xenograft tumor growth (84,85), and the anticancer mechanism of G-Rh2 in HepG2 cells was related to downregulating β-catenin through the activation of glycogen synthase kinase-3β (85). In addition, G-Rh2 has poor oral absorption and low bioavailability. *In vivo* metabolism and pharmacokinetic studies indicated that oxygenation and deglycosylation were the major metabolic pathways of G-Rh2 (86); the deglycosylation of G-Rh2 led to formation of protopanaxadiol and the oxygenation of G-Rh2, and protopanaxadiol produced two monooxygenated metabolites (86). Another study reported that (24R)-pseudo-ginsenoside HQ and (24S)-pseudo-ginsenoside HQ are the main metabolites of 20(S)-G-Rh2 *in vivo*, with both of them showing antitumor activity through caspase and VEGF signaling pathways in H22-tumor bearing mice (87). After oral dosing, G-Rh2 was found to be distributed mainly to the liver and gastrointestinal tissues in rats. The bioavailability of G-Rh2 is ~5% in rats and 16% in dogs (88). Thus, modification of the chemical structure of G-Rh2 to increase bioavailability and enhance its pharmacological activity is also one of the current research directions.

5. Signaling pathway of G-Rh2 in cancer

According to current studies, the anticancer signaling pathway of G-Rh2 remains unclear. It was reported that G-Rh2 suppresses growth of oral squamous cell carcinoma cells by decreasing ROS, MMP-2 and VEGF (67). G-Rh2 inhibits glioma cell growth by targeting microRNA-128 (63) and inhibiting epidermal growth factor receptor (89). G-Rh2 induces leukemia cell differentiation and cell cycle arrest by upregulating TGF-β expression (52) and inducing apoptosis by inducing the release of mitochondrial cytochrome *c* and the activation of caspase-3 and -9 (90). In neuroblastoma cells, G-Rh2 induces apoptosis via activation of caspase-1 and -3 and upregulation of Bax (91). G-Rh2 suppresses cancer cell migration and invasion by downregulating the expression levels of MMP3 (92) and MMP13 (93), and by regulating CDKN2A-2B gene cluster transcription (84). G-Rh2 exerts anticancer activity in T-cell acute lymphoblastic leukemia cells (94), glioblastoma multiforme cells (95) and osteosarcoma cells (96) by suppressing the PI3K/Akt/mTOR signaling pathway. In addition, G-Rh2-induced DNA damage and autophagy in vestibular schwannoma is dependent on LAMP2 transcriptional suppression (97), and it improves the cisplatin effect in lung adenocarcinoma A549 cells by repressing superoxide generation and PD-L1 expression (98). Based on these reports, we hypothesize that PI3K/Akt/mTOR could be an important signaling pathway for G-Rh2 to exert its activity, which provides some context for further research on G-Rh2. The potential signaling pathways of G-Rh2 in cancer are demonstrated in Fig. 3.

6. Conclusions

As one of the main active components of ginseng, G-Rh2 has a wide range of pharmacological effects and plays a therapeutic role in numerous diseases. A number of studies have demonstrated that G-Rh2 exerts excellent anticancer activity *in vitro* and *in vivo*. G-Rh2 exerts its anticancer activity by inducing apoptosis, autophagy, cell cycle arrest and immunity, as well as by inhibiting proliferation, invasion, metastasis and angiogenesis. In addition, G-Rh2 in combination with specific anticancer drugs can overcome drug resistance and enhance the immune response. In summary, G-Rh2 exerts anticancer effects *in vitro* and *in vivo*, and is a promising agent for cancer prevention and treatment.

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Authors' contributions

HBZ, SP, HH, EK and JY collected the literature and designed the study. HBZ drafted the manuscript. SKC, ZYR and MK revised the manuscript. ZYR and MK confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

All authors declare hat they have no competing interests.

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