PN PHYTONUTRIENTS

Information available at: infor@phytonutrients.pk
PHYTONutrients
Journal home page: https://phytonutrients.pk/index.php/pn

Lyceum Publisher

MicroRNAs in the anti-cancer effects of Ginsenosides: A Systematic Review

Elham Hasheminasabgorji [1], Mohammad Amin Khazeei Tabari [2,3†], Abouzar Bagheri [4*]

Received: July 19, 2024
Accepted: May 03, 2024
Published: May 26, 2025
Volume: 2025
Article ID: pn.v4i1.32
https://doi.org/10.62368/pn.v4i1.32

[1] Arnie Charbonneau Cancer Institute, Department of Biochemistry and Molecular Biology, Cumming School of Medicine, University of Calgary, Calgary, AB, Canada; elham.hasheminasabgorji@gmail.com
[2] Student Research Committee, Mazandaran University of Medical Sciences, Sari, Iran; aminkhazeeitabari@gmail.com
[3] USERN Office, Mazandaran University of Medical Sciences, Sari, Iran
[4] Department of Clinical Biochemistry and Medical Genetics, Molecular and Cell Biology Research Center, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran; ab.bagherimg@gmail.com
*Correspondence: ab.bagherimg@gmail.com

ABSTRACT: This systematic review highlights the pivotal functions of ginsenosides in cancer treatment through miRNA regulation. Ginsenosides, bioactive herbal compounds derived from ginseng, exhibit significant anti-cancer properties through mechanisms including inhibition of cell proliferation, epithelial-to-mesenchymal transition (EMT), metastasis, invasion, and induction of autophagy and apoptosis. MicroRNAs (miRNAs), small non-coding RNAs, play critical roles in gene regulation and have emerged as potential diagnostic, prognostic, and therapeutic targets in various cancers. Ginsenosides influence miRNA expression, underexpressing oncogenic miRNAs and overexpressing tumor suppressor miRNAs, thereby exerting their anti-cancer effects. The literature review covered studies from 2011 to 2021 sourced from PubMed, Scopus, Cochrane Library, and Web of Science, adhering to the PRISMA guidelines. Eligible studies were screened, resulting in the selection of 26 preclinical studies. These studies demonstrate that ginsenosides modulate the expression of various miRNAs, contributing to anti-tumorigenic activities across different cancer types, including glioma, non-small cell lung cancer, breast cancer, acute leukemia, hepatocellular carcinoma, ovarian cancer, medulloblastoma, prostate cancer, liver cancer, oral squamous cell carcinoma, retinoblastoma, and gallbladder cancer. By influencing miRNA pathways, ginsenosides can inhibit tumor growth, migration, invasion, and induce apoptosis, highlighting their therapeutic potential in oncology.

Keywords: Ginsenosides; microRNAs; anti-cancer; epithelial-to-mesenchymal transition; apoptosis; autophagy; metastasis

1. Introduction

Although progress in the current treatment has reduced the mortality of different cancers but metastasis and toxicity issues remain the main reason for failure in cancer treatment. Therefore, an effective, low-toxicity agent is required to

improve the survival rate (Wang et al., 2016a; Zhang et al., 2008).

Ginsenosides are herbal compounds extracted from ginseng, with medicinal value, which is categorized into different groups, such as ginsenoside Rh2, ginsenoside Rb1, ginsenoside Rd, ginsenoside 20(S)-Rg3, and ginsenoside Rg3 (Sun et al., 2010) that have been reported to have anti-tumorigenesis activity either through inhibition of the cell migration, proliferation, and epithelial mesenchymal transition (EMT) or induction of autophagy and apoptosis in tumor cells (Chen & Qiu, 2015; Li et al., 2019; Liu et al., 2017; Wang et al., 2016a; Wen et al., 2015; Wu et al., 2011; Zhou et al., 2018). Numerous studies have reported the anti-tumor properties of ginsenosides in different cancers, including glioma (Wu et al., 2011), non-small cell lung cancer (NSCLC) (An et al., 2013), breast cancer (Wen et al., 2015), acute leukemia (Wang & Wang, 2015), hepatocellular carcinoma (HCC) (Chen & Qiu, 2015), ovarian cancer (Li et al., 2017), medulloblastoma (Y. Chen et al., 2018), prostate cancer (Gao & Zheng, 2018), liver cancer (W. Chen et al., 2018), oral squamous cell carcinoma (OSCC) (Cheng & Xing, 2019), retinoblastoma (Li et al., 2019), gallbladder cancer (Wu et al., 2019b). Expression of microRNAs (miRNAs, miRs) could be altered by ginsenosides in different types of cancer (An et al., 2013).

miRNAs are a group of small noncoding RNAs that regulate gene expression in a post-transcriptional manner. These regulators inhibit the expression of a large number of target genes and related biological processes by complementary binding to 3'-UTR, 5'-UTR, or coding regions (Mishan MA et al., 2021). miRNAs have been reported as potential diagnostic, prognostic, and therapeutic targets in a variety of diseases These noncoding RNAs, function as either oncogene or tumor suppressor contributing to induction, proliferation, migration, metastasis, and promote tumorigenesis (Akbari Korhkheyli V et al., 2021; Ghalehnoei H et al., 2020; Mishan et al., 2020). The aim of this study was to discuss research findings on the effects of ginsenosides on miRNAs to further elucidate their anti-tumor functions as a therapeutic of future.

2. MATERIALS AND METHODS

A literature review was done on the eligible papers published in PubMed, Scopus, Cochrane Library, and Web of Science (ISI) databases. The following keywords were used: (cancer OR malignancy OR neoplasm OR tumor OR malignant OR tumour OR neoplasia OR cancerous), (non-coding RNA OR noncoding RNA OR ncRNA OR miRNA OR miR- OR microRNA), and (ginsenoside OR panaxosides OR Panax). Subsequently, the PRISMA statement is used for describing the obtained data (Liberati et al., 2009).

Inclusion and Exclusion criteria

Two authors independently screened titles for duplicates. Afterward, reports that met the initial criteria were screened for the eligibility of titles and abstracts. In this study, inclusion criteria were in vivo and in vitro English studies which were published from 2011 to 2021. At the final stage, identified eligible items were compared and the unnecessary cases were excluded. For each eligible study, relevant information and data were extracted from full texts. Also, the exclusion criteria were unnecessary articles, book chapters, review articles, duplicative studies, non-English articles, letters, conference papers, editorials, short surveys, and meeting abstracts.

3. RESULTS AND DISCUSSION

Literature searching was performed according to the related keywords; 150 articles were identified. Eventually, out of all investigations, 26 relevant preclinical studies were chosen according to the study topic (Figure 1 and Table 1).

In six studies, the synergic inhibitory effect of ginsenoside as a potential anti-cancer chemical drug was reported as follows (Figure 2). In the first study, miR-31 was downregulated in medulloblastoma following the treatment by combining 30 μM ginsenoside Rh2 with miR-31 mimic (Y. Chen et al., 2018). In the second study, expression of miR-491 was increased by the treatment with 40 μg/ml ginsenoside Rh2 and miR-491 mimic combination in lung cancer (Chen et al., 2019). In the third study, a treatment by combining 75 μg/ml ginsenoside Rg3 with miR-221 mimic in OSCC was performed (Cheng & Xing, 2019). Moreover, a treatment by combining ginsenoside 20(S)-Rg3 at the dosage of 80 μg/mL and 40 μg/mL with 532-3p mimic (Zhou et al., 2018), and concentration of 100 μmol/L with miR-

4425 mimics in SKOV3 and 3AO ovarian cancer cell lines (Jiaojiao Lu et al., 2020). And finally, cell viability and migration were blocked in glioblastoma in vitro model treated with 100μM ginsenoside Rd with miR-144-5p mimic (G.-M. Liu et al., 2020).

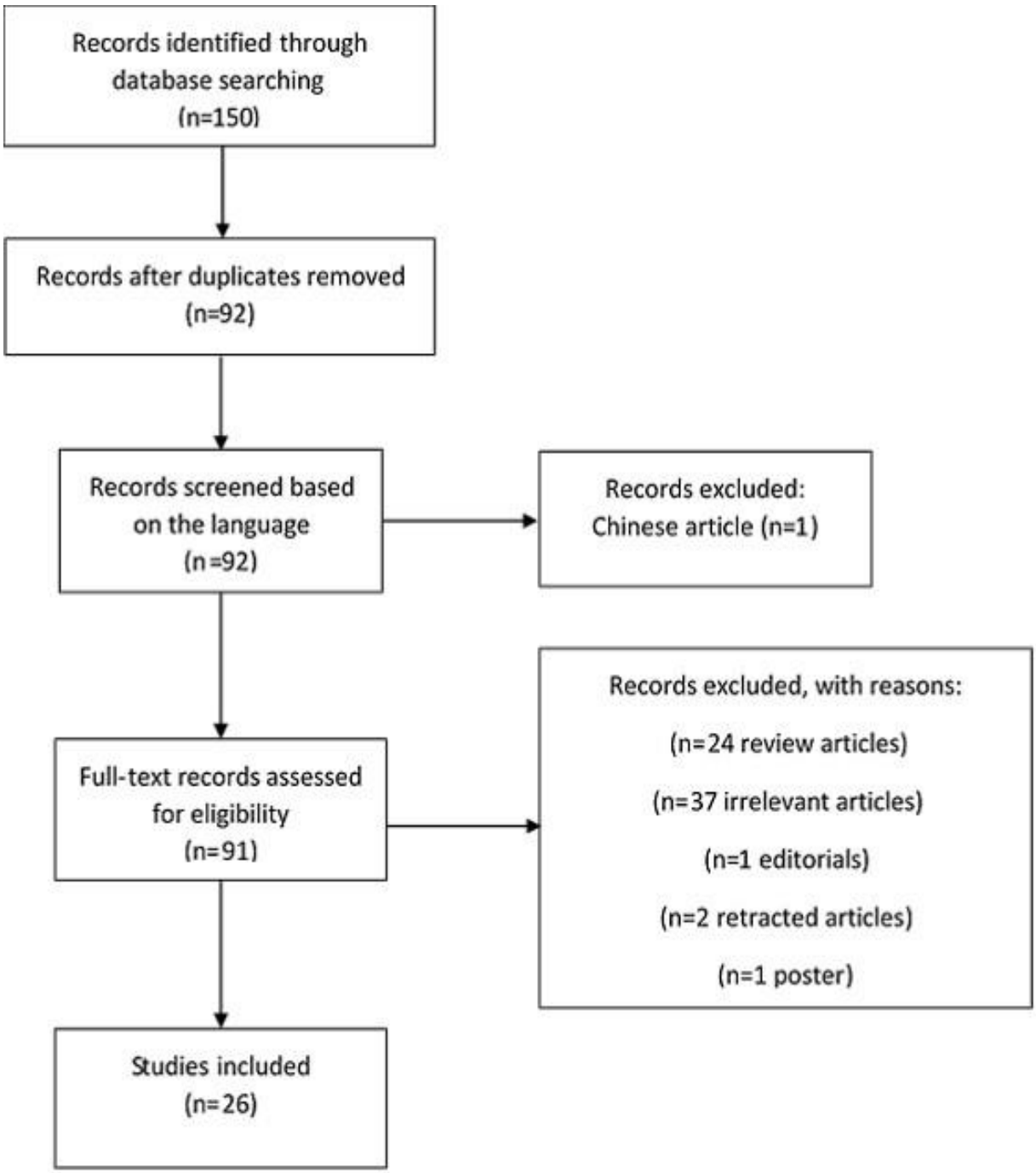

Figure 1. PRISMA Flow diagram of the study selection process

Table 1. Main features of the studies included in this review

Ref.	Year	Type of study	miRNA	Ginsenosides effect on miRNAs	Type of cancer	Sample	Mechanism of action	Treatment or Effective dose
(Wu et al., 2011)	2011	In vitro	miR-128	Upregulation	Glioma	Human U251 Cell line	Inhibition of cell proliferation, Induction of cell apoptosis	12μg/mL
(An et al., 2013)	2013	In vitro	ebv-miR-BHRF1-1, let-7d, miR-361-3p, let-7i, miR-3648, miR-1207-5p, miR-3651, miR-1225-5p, miR-3653, miR-1227, miR-3656, miR-1268, miR-3663-3p, miR-1290, miR-3665, miR-130b, miR-4270, miR-135a, miR-4281, miR-148a, miR-4284, miR-150*, miR-483-3p, miR-186, miR-574-5p miR-188-5p, miR-590-5p, miR-18b, miR-630,	Upregulation (ebv-miR-BHRF1-1, let-7d, miR-361-3p, let-7i, miR-3648, miR-1207-5p, miR-3651, miR-1225-5p, miR-3653, miR-1227, miR-3656, miR-1268, miR-3663-3p, miR-1290, miR-3665, miR-130b, miR-4270, miR-135a, miR-4281, miR-148a, miR-4284, miR-150*, miR-483-3p, miR-186, miR-574-5p, miR-188-5p, miR-590-5p, miR-18b, miR-630, miR-191*, miR-664, miR-1915, miR-767-3p, miR-196b, miR-939, miR-2116*, hsv1-miR-H18, miR-296-5p, hsv1-miR-H20,	Non-small cell lung cancer	A549 Cell line	Inhibition of cell proliferation,	40 μg/ml

			miR-191*, miR-664, miR-1915, miR-767-3p, miR-196b, miR-939, miR-2116*, hsv1-miR-H18, miR-296-5p, hsv1-miR-H20, miR-3180-5p, hsv1-miR-H6, miR-3195, hsv1-miR-K12-9*, let-7e, miR-27b, miR-100, miR-28-5p, miR-101, miR-30a, miR-125b, miR-31, miR-151-3p, miR-31*, miR-193a-3p, miR-3127, miR-193b, miR-365, miR-21, miR-424, miR-21*, miR-4252, miR-221, miR-486-5p, miR-224, miR-550a*, miR-23b, miR-98)	miR-3180-5p, hsv1-miR-H6, miR-3195, hsv1-miR-K12-9*), Downregulation (let-7e, miR-27b, miR-100, miR-28-5p, miR-101, miR-30a, miR-125b, miR-31, miR-151-3p, miR-31*, miR-193a-3p, miR-3127, miR-193b, miR-365, miR-21, miR-424, miR-21*, miR-4252, miR-221, miR-486-5p, miR-224, miR-550a*, miR-23b, miR-98)				

(Wen et al., 2015)	2015	In vitro	miR-29a, miR-222 and miR-34a	Downregulation (miR-29a, miR-222 and miR-34a)	Breast cancer	MCF-7, MCF-7/Adr, MCF-7/Doc	Inhibition of cell proliferation, Induction of cell apoptosis	MCF-7 cells with G-Rh2 (40 μM/l) treatment; MCF-7/Adr cells with G-Rh2 (80 μM/l) treatment and MCF-7/Doc cells with G-Rh2 (80 μM/l)
(Li et al., 2015)	2015	In vitro In vivo	miR-497	Upregulation	Glioblastoma	Human A172 Cell line	Inhibition of angiogenesi, cancer growth and invasion	0.01, 0.1, and 1 mg per ml (In vitro), 1 mg per kg body weight (In vivo)
(Wang & Wang, 2015)	2015	In vivo In vitro	miR-21	Upregulation	Acute leukemia	Kasumi-1 and U-937, NOD-SCID-gamma mouse	Induction of cell apoptosis, prolonged the survival of mice	0.01, 0.1, and 1 mg per ml (In vitro),1mg per kg body weight (In vivo)
(Chen & Qiu, 2015)	2015	In vivo In vitro	miR-491	Upregulation	Hepatocellular carcinoma	HepG2, SMMC-7721, NOD/SCID mice	Inhibition of cell proliferation and tumor growth	20 μM (In vitro) 1 mg per kg body weight (In vivo)
(Wang et al., 2016b)	2016	In vitro In vivo	miR-18a	Downregulation	Breast cancer	4T1 cell line and human MDA-MB-231 Cell line, BALB/c mice	Inhibition of cell migration and invasion	50, 100, 150 μM (In vitro) 50 mg per kg body weight (In vivo)

(Li et al., 2017)	2017	In vitro	miR-145	Upregulation	Ovarian cancer	Human SKOV3 and 3AO Cell line	Inhibition of EMT, cell migration, and invasion	80 μg/ ml (for SKOV3) or 160 μg/ml (for 3AO)
(Liu et al., 2017)	2017	In vitro	miR-25	Downregulation	Ovarian cancer	Human SKOV3 and 3AO Cell line	Inhibition of hypoxia-induced EMT	160 μg/ml
(Zhou et al., 2018)	2018	In vitro	miR-3163, miR-664a-5p, miR-6717-5p, miR-4329, miR-603, miR-324-5p, miR-1283, miR-532-3p, miR-33a-3p, miR-519a-5p,miR-486-3p, miR-4634, miR-1273e, miR-4532532-3p mimic, miR-7156-3p, miR-6730-3p, miR-2682-3p, miR-7843-3p, miR-195-3p, and miR-4425	Upregulation (miR-3163, miR-664a-5p, miR-6717-5p, miR-4329, miR-603, miR-324-5p, miR-1283, miR-532-3p, miR-33a-3p, miR-519a-5p, and miR-486-3p), Downregulation(miR-4634, miR-1273e, miR-4532, miR-7156-3p, miR- 6730-3p, miR-2682-3p, miR-7843-3p, miR- 195-3p, miR-4425)	Ovarian cancer	Human SKOV3 and A2780 Cell line	Inhibition of Warburg effect	80 μg/mL (for SKOV3) and 40 μg/mL (for A2780), 20(S)-Rg3+ miR-532-3p mimic
(Zheng et al., 2018)	2018	In vitro	miR-324-5p	Upregulation	Ovarian cancer	Human SKOV3 and A2780 Cell line	Inhibition of Warburg effect, Inhibition of cell proliferation	80 μg/mL (for SKOV3 cells) or 40 μg/mL (for A2780 cells)
(Y. Chen et al., 2018)	2018	In vitro	miR-31	Downregulation	Medulloblastoma	Daoy cell line	Inhibition of cell proliferation, migration.	30 μM + miR-31 mimic

							Induction of cell apoptosis	
(Gao & Zheng, 2018)	2018	In vitro	miR-4295	Downregulation	Prostate cancer	PC3 and DU145 PC cell lines	Inhibition of cell growth and cell proliferation	0.01, 0.1 and 1 mg/ mL.
(W. Chen et al., 2018)	2018	In vitro In vivo	miR-200b-5p, miR-224-3p, miR-146a-5p, miR-26b-3p and miR-29a-5p	Upregulation (miR-200b-5p, miR-224-3p and miR-146a-5p) Downregulation (miR-26b-3p and miR-29a-5p)	Liver cancer	HepG2 Huh7, and SMMC-7721, athymic nude mice	Inhibition of cell proliferation, colony formation , cell growth, Induction of cell apoptosis	20 μg/ml (In vitro) 1 mg per kg body weight (In vivo)
(Cheng & Xing, 2019)	2019	In vitro In vitro	miR-221	Downregulation	Oral squamous carcinoma	SCC-9 and HSC-5 cell line, BALB/c nude mice	Inhibition of cell proliferation, EMT and viability, promotion of cell apoptosis	75 μg/ml (In vitro), 10 mg per kg body weight (In vivo) Rg3+miR-221mimic
(Li et al., 2019)	2019	In vitro	miR-638	Downregulation	Retinoblastoma	Human RB Y79 and RBL-13 cell lines	Inhibition of cell proliferation, promotion of cell apoptosis and autophagy	30 μM
(Wu et al., 2019a)	2019	In vitro In vivo	miR-181b	Downregulation	Gallbladder cancer	GBC-SD cell line, BALB/c nude mice	Inhibition of cell proliferation autophagy	100 μM (In vitro) 20 mg per kg body weight(in vivo)
(Chen et al., 2019)	2019	In vitro	mir-491	Upregulation	Lung cancer	A549 and H1299	Inhibition of cell migration	40μ g/ml+ mir-491 mimic
Lu et al. (2019)	2019	In vitro	miR-603	Upregulation	Ovarian cancer	SKOV3 and A2780	Inhibition of cell growth, migration and invasion	80 μg/ml (for SKOV3) or 40 μg/ml (for A2780)

Lu et al. (2020)	2020	In vitro	miR-4425	Downregulation	Ovarian cancer	SKOV3 and 3AO	Inhibition of proliferation, migration and invasion	100 μmol/L + miR-4425 mimics
Liu et al. (2020)	2020	In vivo	miR-21	Downregulation	Gastric cancer	$Atp4a^{-/-}$ mouse	Inhibition of proliferation, and promotion of apoptosis	5 and 10 mg per kg body weight
Li et al. (2020)	2020	In vitro	miR-21	Downregulation	Hepatocellular carcinoma	MHCC97H and BEL7402	Inhibition of cell growth and invasion and promotion of apoptosis	20, 40, and 80 μM
Liu et al. (2020)	2020	In vitro In vivo	miR-144-5p	Upregulation	Glioblastoma	U251 and U87	Inhibition of proliferation and migration	100μM + miR-144-5p mimic (In vitro) 60 g per kg body weight (In vivo)
(W. Z. Zhou et al., 2020)	2020	In vitro	miR-192	Downregulation	Non-small cell lung cancer	H125 cell line	Inhibition of cell proliferation, invasion, and migration and promotion of cell apoptosis	hyaluronic acid HA100 μg/mL Rg3 nanoparticles
(J. Lu et al., 2020a)	2020	In vitro	miR-519a-5p	Upregulation	Ovarian cancer	SKOV3 and A2780 cell line	Inhibition of Warburg effect	80 μg/mL (for SKOV3 cells) or 40 μg/mL (for A2780 cells)
(J. Lu et al., 2020a)	2021	In vitro	miR-424-5p	Upregulation	Breast cancer	MCF-7cell line	promotion of cell apoptosis	20 and 50 μM

In two studies, effects of ginsenoside treatment in the microRNA expression profile of NSCLC investigated. In these studies, the expressions of ebv-miR-BHRF1-1, miR-2116*, miR-361-3p , let-7d, miR-3648, miR-1225-5p, miR-1207-5p, miR-130b, miR-3651, let-7i, miR-3653, miR-1268, miR-1227, miR-3656, miR-3665, miR-3663-3p, miR-1290, miR-135a, miR-4270, miR-4281, miR-4284, miR-148a, miR-186, miR-150*, miR-483-3p, miR-188-5p, miR-574-5p, miR-18b, miR-590-5p, miR-191*, miR-630, miR-767-3p, miR-664, miR-1915, miR-939, miR-196b, hsv1-miR-H18, hsv1-miR-H20, miR-296-5p, hsv1-miR-H6, miR-3180-5p, hsv1-miR-K12-9*, and miR-3195 (An et al., 2013) were increased following the treatment by 40 μg/ml ginsenoside Rh2 while let-7e, miR-27b, miR-28-5p, miR-100, miR-125b, miR-101, miR-30a, miR-151-3p, miR-31, miR-193a-3p, miR-31*, miR-193b, miR-3127, miR-365, miR-21*, miR-21, miR-424, miR-221, miR-4252, miR-224, miR-486-5p, miR-550a*, miR-98, and miR-23b were downregulated by the same amount of ginsenoside Rh2 treatment (An et al., 2013).

Moreover, gelatin and hyaluronic acid nanoparticles coated with the 100 μg/ml ginsenoside monomer were prepared to explore the miR-192 expression. The results revealed that miR-192 expression negatively influenced by ginsenoside Rg3 nanoparticles in NSCLC cells (W. Zhou et al., 2020).

Notably, miR-21 expression was decreased in Atp4a- /- mouse model of gastric cancer treated with 5 and 10 mg/kg body weight ginsenoside Rg3 (W. Liu et al., 2020). While, it was found that miR-21 expression was significantly increased in acute leukemia cells treated with 0.01, 0.1, and 1 mg/ml ginsenoside Rh2. Also, ginsenoside Rh2 may lead to apoptosis through suppression of anti-apoptotic protein Bcl-2. Besides, miR-21 upregulation prolonged the survival rate in the animal model of acute leukemia (Wang & Wang, 2015), while miR-21 was downregulated in HCC treated by 20, 40, and 80 μM of ginsenoside (Li et al., 2020). Similarly, miR-21 expression was decreased by 40 μg/ml ginsenoside in NSCLC (An et al., 2013). In an in vitro study, miR-638 downregulated following treatment with 30 μM ginsenoside Rh2 in RB Y79 and RBL-13 cell lines. Also, ginsenoside treatment in retinoblastoma cell lines was shown to promote autophagy and apoptosis while suppressed cell proliferation through miR-638 downregulation (Li et al., 2019). In an animal model of breast cancer, miR-18a downregulated in 4T1 cell-inoculated mice treated with ginsenoside Rd at the final concentration of 50 mg/kg body weight (Wang et al., 2016a). Furthermore, miR-18a significantly decreased in breast cancer cell lines treated with 50, 100, 150 μM ginsenoside Rd. Also, the miR-34a, miR-222, and miR-29a expression were significantly lower in MCF-7 cells treated with 40 μM/l ginsenoside (Wang et al., 2016a).

In addition, miR-29a, miR-222, and miR-34a expression in Adriacin (Adr) and Docetaxel (Doc) resistant cells treated with ginsenoside Rh2 were analyzed. Decreased expression of miR-34a, miR-222, and miR-29a has been observed in MCF-7/Adr and MCF-7/Doc cells after ginsenoside treatment at the dose of 80 μM/l (Wen et al., 2015). Also, miR-145, miR-324-5p, and miR-519a-5p found to be upregulated at a dose of 80 μg/mL and 40 μg/mL for SKOV3 and A2780 cell lines respectively in ovarian cancer (Li et al., 2017; J. Lu et al., 2020b; Zheng et al., 2018). Similarly, miR-603 found to be upregulated at the same dosage in ovarian cancer cell lines treated by ginsenoside Rg3 (Lu et al., 2019). While, miR-25 expression was decreased in ovarian cancer cell lines treated by 160 μg/ml ginsenoside Rb1 (Liu et al., 2017). Notably, miR-128 expression was increased after ginsenoside Rh2 intervention at the dose of 12μg/mL in human glioma U251 Cell line (Wu et al., 2011) while miR-497 was increased dose-dependently by 0.01, 0.1, 1 mg/ml ginsenoside Rh2 treatment in cultured A172 cells (Li et al., 2016).

Ginsenoside Rh2 has also resulted in an increased in miR-146a-5p, miR-224-3p, and miR-200b-5p expression while reduced miR-26b-3p and miR-29a-5p expression level in liver cancer in vitro and in vivo (W. Chen et al., 2018).To further study the effects of ginsenoside on the HCC, NOD/SCID mice and HCC cell lines were treated with 1 mg/kg body weight and 20 μM ginsenoside respectively. This study indicated that ginsenoside treatment led to upregulation of miR-491 and prevention of proliferation and tumor cell growth in HCC treated cells (Chen & Qiu, 2015). In in vitro, GBC-SD cell line, ginsenoside Rg3 (100 μM), induced the inhibition of autophagy and proliferation by downregulation of miR-181b. Similarly, in the xenograft model of the gallbladder cancer, ginsenoside Rg3 (20 mg/kg body weight) intervention has caused the same response (Wu et al., 2019b).

Moreover, ginsenoside Rh2 dose-dependently (0.01, 0.1, 1 mg/dl), in a prostate cancer cell line (PC3 and DU145), did not affect cell apoptosis but induced inhibition of cell proliferation and cell growth through downregulation of miR-4295 (Gao & Zheng, 2018).

Inhibition of autophagy and proliferation are important functions proposed for ginsenoside. It was demonstrated that miR-181b contributes to gallbladder carcinoma progression by downregulating CREB3 regulatory factor (CREBRF) and subsequently enhancing cAMP responsive element binding protein 3 (CREB3) levels. These results indicated that ginsenoside Rg3 decreased autophagy and cell proliferation in gallbladder carcinoma cells through the miR-181b/CREBRF/CREB3 signaling pathway (Wu et al., 2019b).

Also, ginsenoside Rg3 was shown to block proliferation, viability, and TGF-β1- EMT in OSCC. Moreover, ginsenoside Rg3 treatment induced apoptosis via decreasing the miR-221 expression and then increasing the expression of tumor inhibitor of metalloproteinases-3 (TIMP3) in OSCC cell lines. Also, it was demonstrated that phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) and MAPK/ERK signaling pathways could be suppressed by TIMP3 upregulation in OSCC both in vivo and in vitro (Cheng & Xing, 2019). Also, ginsenoside Rg3 treatment could suppress expression of miR-21, PI3K/AKT/mechanistic target of rapamycin (mTOR), Bcl-2, hexokinase-2 (HK2), and lactate dehydrogenase A (LDHA) in Atp4a- /- mouse model of gastric cancer which leads to inhibition of cell proliferation, and promotion of cell apoptosis (W. Liu et al., 2020).

Ginsenoside Rg3 was shown to supress the negative regulation of ATXN8OS long noncoding RNA (lncRNA) on miR-424-5p, leading to the suppression of DACH1, EYA1, and CHRM3 oncogenes and enhance apoptosis of breast cancer cell lines such as MCF-7 (Kim et al., 2021). Similarly, nanoparticles enveloped by Rg3 have been shown to promote apoptosis and inhibit cell proliferation, invasion, and migration via miR-192 downregulation, thus elevating the phosphatase and tensin homolog (PTEN) levels (W. Zhou et al., 2020).

Ginsenoside Rh2 has shown induction of apoptosis both in cell culture and animal model of glioma (Wu et al., 2011), breast cancer (Wen et al., 2015), acute Leukemia (Wang & Wang, 2015), medulloblastoma (Y. Chen et al., 2018), liver cancer (W. Chen et al., 2018), and retinoblastoma (Li et al., 2019). It was indicated that ginsenoside Rh2 could induce apoptosis in glioma cancer cells by caspase 3 activation as well as blocking transcription factor E2F3a through miR-128 overexpression (Wu et al., 2011). Also, ginsenoside Rh2 could trigger apoptosis and increase chemotherapy sensitivity in breast cancer through pro-apoptotic Bax overexpression and downregulation of Bax-targeting miRNAs including miR-29a, miR-222, and miR-34a (Wen et al., 2015). Further investigation revealed that ginsenoside Rh2 enhances miR-21 level which leads to a longer survival time and induces inhibition of apoptosis-associated protein Bcl-2 and induction of apoptosis in acute leukemia (Wang & Wang, 2015). Also, ginsenoside Rh2 treatment was demonstrated to decrease Bcl-2, MMP-2, MMP-9, and cyclin-dependent kinase 1 (CDK1) oncogene while overexpressing the cleaved caspase-9, caspase-3, and Bax expression, which results in the suppression of cell migration and proliferation. Furthermore, miR-31 is decreased in medulloblastoma cells treated with ginsenoside Rh2 and caused the induction of cell apoptosis and suppression of proliferation and migration by inactivating the Wnt/β-catein pathway (Y. Chen et al., 2018). In line with these results, upregulating miR-146a-5p meditated liver cancer colony formation, cell growth, and apoptosis through increasing the Bcl2 expression and decreasing the myeloid cell leukemia 1 (MCL1) and nuclear factor (erythroid-derived 2)-like 2 (Nrf2) expression in ginsenoside Rh2 treated cells (W. Chen et al., 2018). Also, ginsenoside Rh2 has led to the same responses in the retinoblastoma by targeting miR-638 which leads to increasing the p53 expression and negative regulation of the PI3K/AKT/mTOR pathway. Furthermore, caspase-9 and caspase-3 were activated via ginsenoside Rh2 through decreasing the Bcl2 and enhancing the Bax expression. Moreover, it was indicated that autophagy could be induced by ginsenoside Rh2 following the Beclin-1, ATG7, LC3-II/I, upregulating and downregulating of p62 expression, resulting in the inactivation of cell proliferation, promotion of cell apoptosis, and autophagy in retinoblastoma (Li et al., 2019).

Ginsenoside Rh2 has been shown to promote anti-tumor activity through inhibition of migration, invasion, angiogenesis, Warburg effect, proliferation and, tumor growth, rather than from the induction of programmed cell death in prostate cancer (Gao & Zheng, 2018), ovarian cancer (Li et al., 2017; Liu et al., 2017; J. Lu et al., 2020b; Zheng et al., 2018; Zhou et al., 2018), lung cancer (Chen et al., 2019), breast cancer (Wang et al., 2016a), hepatocellular carcinoma (Chen & Qiu, 2015), and glioblastoma (Li et al., 2016)

Based on the aforementioned studies, ginsenoside Rh2 negatively controlled the cyclin-

dependent kinase inhibitor 1A (CDKN1A), a miR-4295 target gene, at the post-transcriptional level, which results in the inhibition of prostate tumor growth (Gao & Zheng, 2018). Furthermore, ginsenoside Rh2 decreased MMP-9 via mir-491 and hence inhibited proliferation and tumor migration in lung cancer (Chen et al., 2019). In addition, it was shown that epidermal growth factor receptor (EGFR) signaling inactivation in ginsenoside Rh2 treated cells inhibits HCC cell proliferation and tumor growth by enhancing miR-491 expression (Chen & Qiu, 2015). In addition, suppression of the PI3K/Akt pathway via negative regulation of miR-21 leads to inhibition of cell growth and invasion as well as, induction of apoptosis in HCC cells treated by ginsenoside (Li et al., 2020). Similarly, it was demonstrated that treatment of glioblastoma in vitro and in vivo with ginsenoside Rh2 reduced vascularization, growth, and invasion via miR-497 upregulation which binds to the 3'UTR region of VEGF-A to suppress its expression (Li et al., 2016). In addition, ginsenoside Rd blocked migration and proliferation by miR-144-5p upregulation following the negative regulation of Toll-like receptor 2 (TLR2) in an in vitro and in vivo model of glioblastoma (G.-M. Liu et al., 2020). Consistent with the previous findings, treatment of breast cancer with ginsenoside Rd in-activated migration and cell invasion in mice inoculated with 4T1 cells, as well as, in vitro model, by downregulating miR-18a following the Smad2 and TGFβ targeting (Wang et al., 2016a). In addition, overexpression of miR-25 induced EMT in ovarian cancer, while treatment with ginsenoside Rb1 reduced miR-25 expression, which leads to overexpression of E-cadherin transcriptional activator EP300 and results in attenuation of the migration and hypoxia-induced EMT (Li et al., 2017). Furthermore, it was indicated that ginsenoside 20(S)-Rg3 could suppress migration, EMT, and invasion in ovarian cancer by negative regulation of DNMT3A (DNA Methyltransferase 3 Alpha) and thus increasing miR-145 expression following the FSCN1 (Fascin actin-bundling protein 1) inhibition (Li et al., 2017).

Other suggested anti-cancer mechanism for ovarian cancer cells treated with ginsenoside 20(S)-Rg3 include suppressing the Warburg effect through H19/miR-324-5p/PKM2 (pyruvate kinase M2) pathway (Zheng et al., 2018), DNMT3A/miR-532-3p/ HK2 pathway (Zhou et al., 2018), DNMT3A/miR-603/HK2 pathway (Lu et al., 2019), and DNMT3A/miR-519a-5p/HIF-1α (hypoxia-inducible factor-1α) pathway (J. Lu et al., 2020b). It was demonstrated that ginsenoside 20(S)-Rg3 negatively regulates DNA methylation mediated by DNMT3A in the miR-603 by targeting HK2 which result in the Warburg effect inactivation (Lu et al., 2019). Besides, the Warburg effect is inhibited via miR-519a-5p as well as, miR-532-3p overexpression. Also, it was shown that miR-532-3p could target HK2 while miR-519a-5p could decrease the HK2 expression via HIF-1α inhibition which leads to Warburg effect inactivation (J. Lu et al., 2020b; Zhou et al., 2018). Similarly, ginsenoside 20(S)-Rg3 has also resulted in decreased binding affinity of H19 to miR-324-5p, which increased inactivation of PKM2 by miR-324-5p and hence decreased the Warburg effect and ovarian cancer development (Zheng et al., 2018).

4. CONCLUSIONS

Taken together, numerous studies have shown the anti-neoplastic effects of ginsenosides in different types of cancer. Ginsenosides are able to induce cancer cell death either through suppression the cell proliferation, migration, angiogenesis, EMT, and invasion or promotion of cell apoptosis and autophagy, which miRNAs play a crucial function in the ginsenosides-mediated anti-cancer properties. Ginsenosides can inhibit tumorigenesis via the underexpression of oncogenic miRNAs, as well as overexpression of tumor suppressor miRNAs, which has been highlighted in the present review.

Abbreviations

Epithelial-to-Mesenchymal Transition (EMT)
MicroRNAs (miRNAs)
Hepatocellular Carcinoma (HCC)
Oral Squamous Cell Carcinoma (OSCC)
CREB3 Regulatory Factor (CREBRF)
cAMP Responsive Element Binding Protein 3 (CREB3)
Phosphatidylinositol 3-kinase (PI3K)/Protein kinase B (AKT)
Mechanistic Target of Rapamycin (mTOR)
Long Noncoding RNA (lncRNA)
Phosphatase and Tensin Homolog (PTEN)
Cyclin-Dependent Kinase 1 (CDK1)
Nuclear Factor (erythroid-derived 2)-like 2 (Nrf2)
Cyclin-Dependent Kinase Inhibitor 1A (CDKN1A)
Epidermal Growth Factor Receptor (EGFR)
Toll-like Receptor 2 (TLR2)
DNMT3A (DNA Methyltransferase 3 Alpha)
FSCN1 (Fascin Actin-Bundling Protein 1)

Author Contributions: Elham Hasheminasabgorji conceptualized, designed methodology and drafted the manuscript. Mohammad Amin Khazeei Tabari and Abouzar Bagheri were responsible for conceptualization, methodology, and supervision of the manuscript.

Funding: This research received no external funding.

Acknowledgements

Nil.

Conflicts of Interest: The author declare no conflict of interest.

References

Akbari Korhkheyli V, Mishan MA, Khonakdar Tarsi A, Mahrooz A, Rezaei Kanavi M, A, H.-M., & Bagheri, A. (2021). MicroRNAs may provide new strategies in the treatment and diagnosis of diabetic retinopathy: Importance of VEGF. *IJBMS*, *24*(3), 267-279. https://doi.org/10.22038/ijbms.2021.52164.11807

An, I. S., An, S., Kwon, K. J., Kim, Y. J., & Bae, S. (2013). Ginsenoside Rh2 mediates changes in the microRNA expression profile of human non-small cell lung cancer A549 cells. *Oncol Rep*, *29*(2), 523-528. https://doi.org/10.3892/or.2012.2136

Chen, W., Chu, S., Li, H., & Qiu, Y. (2018). MicroRNA-146a-5p enhances ginsenoside Rh2-induced anti-proliferation and the apoptosis of the human liver cancer cell line HepG2. *Oncol Lett*, *16*(4), 5367-5374. https://doi.org/10.3892/ol.2018.9235

Chen, W., & Qiu, Y. (2015). Ginsenoside Rh2 Targets EGFR by Up-Regulation of miR-491 to Enhance Anti-tumor Activity in Hepatitis B Virus-Related Hepatocellular Carcinoma. *Cell Biochem Biophys*, *72*(2), 325-331. https://doi.org/10.1007/s12013-014-0456-9

Chen, Y., Shang, H., Zhang, S. L., & Zhang, X. H. (2018). Ginsenoside Rh2 inhibits proliferation and migration of medulloblastoma Daoy by down-regulation of microRNA-31. *J. Cell. Biochem*, *119*(8), 6527-6534. https://doi.org/10.1002/jcb.26716

Chen, Y., Zhang, Y., Song, W., Zhang, Y., Dong, X., & Tan, M. (2019). Ginsenoside Rh2 Inhibits Migration of Lung Cancer Cells under Hypoxia via mir-491. *Anticancer Agents Med Chem*, *19*(13), 1633-1641. https://doi.org/10.2174/1871520619666190704165205

Cheng, Z., & Xing, D. (2019). Ginsenoside Rg3 inhibits growth and epithelial-mesenchymal transition of human oral squamous carcinoma cells by down-regulating miR-221. *Eur J Pharmacol*, *853*, 353-363. https://doi.org/10.1016/j.ejphar.2019.03.040

Gao, Q., & Zheng, J. (2018). Ginsenoside Rh2 inhibits prostate cancer cell growth through suppression of microRNA-4295 that activates CDKN1A. *Cell Prolif*, *51*(3), e12438. https://doi.org/10.1111/cpr.12438

Ghalehnoei H, Bagheri A, Fakhar M, & MA, M. (2020). Circulatory microRNAs: promising non-invasive prognostic and diagnostic biomarkers for parasitic infections. *Eur J Clin Microbiol Infect Dis*, *39*(3), 395-402. https://doi.org/https://doi.org/10.1007/s10096-019-03715-8

Kim, H., Ji, H. W., Kim, H. W., Yun, S. H., Park, J. E., & Kim, S. J. (2021). Ginsenoside Rg3 Prevents Oncogenic Long Noncoding RNA ATXN8OS from Inhibiting Tumor-Suppressive microRNA-424-5p in Breast Cancer Cells. *Biomolecules*, *11*(1), 118. https://doi.org/10.3390/biom11010118

Li, J., Lu, J. J., Ye, Z. X., Han, X., Zheng, X., Hou, H. L., Chen, W., Li, X., & Zhao, L. (2017). 20(S)-Rg3 blocked epithelial-mesenchymal transition through DNMT3A/miR-145/FSCN1 in ovarian cancer. *Oncotarget*, *8*(32), 53375-53386. https://doi.org/10.18632/oncotarget.18482

Li, M., Zhang, D., Cheng, J., Liang, J., & Yu, F. (2019). Ginsenoside Rh2 inhibits proliferation but promotes apoptosis and autophagy by down-regulating microRNA-638 in human retinoblastoma cells. *Exp Mol Pathol*, *108*, 17-23. https://doi.org/10.1016/j.yexmp.2019.03.004

Li, S., Gao, Y., Ma, W., Cheng, T., & Liu, Y. (2015). Ginsenoside Rh2 inhibits invasiveness of glioblastoma through modulation of VEGF-A. *Tumour Biol*. https://doi.org/10.1007/s13277-015-3759-6

Li, S., Gao, Y., Ma, W., Cheng, T., & Liu, Y. (2016). RETRACTED ARTICLE: Ginsenoside Rh2 inhibits invasiveness of glioblastoma through

modulation of VEGF-A. *Tumour Biol*, *37*(12), 15477-15482. https://doi.org/ 10.1007/s13277-015-3759-6

Li, Y., Li, Z., Jia, Y., Ding, B., & Yu, J. (2020). In vitro anti-hepatoma activities of notoginsenoside R1 through downregulation of tumor promoter miR-21. *Dig Dis Sci*, *65*(5), 1364-1375. https://doi.org/https://doi.org/10.1007/s10620-019-05856-4

Liberati, A., Altman, D. G., Tetzlaff, J., Mulrow, C., Gøtzsche, P. C., Ioannidis, J. P., Clarke, M., Devereaux, P. J., Kleijnen, J., & Moher, D. (2009). The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *Journal of clinical epidemiology*, *62*(10), e1-e34. https://doi.org/https://doi.org/10.1016/j.jclinepi.2009.06.006

Liu, D., Liu, T., Teng, Y., Chen, W., Zhao, L., & Li, X. (2017). Ginsenoside Rb1 inhibits hypoxia-induced epithelial-mesenchymal transition in ovarian cancer cells by regulating microRNA-25. *Exp Ther Med*, *14*(4), 2895-2902. https://doi.org/10.3892/etm.2017.4889

Liu, G.-M., Lu, T.-C., Sun, M.-L., Jia, W.-Y., Ji, X., & Luo, Y.-G. (2020). Ginsenoside Rd inhibits glioblastoma cell proliferation by up-regulating the expression of miR-144-5p. *Biol Pharm Bull*, *43*(10), 1534-1541. https://doi.org/https://doi.org/10.1248/bpb.b20-00338

Liu, W., Pan, H.-f., Yang, L.-j., Zhao, Z.-m., Yuan, D.-s., Liu, Y.-l., & Lin, L.-z. (2020). Panax ginseng CA Meyer (Rg3) ameliorates gastric precancerous lesions in Atp4a−/− mice via inhibition of glycolysis through PI3K/AKT/miRNA-21 pathway. *Evid Based Complement Alternat Med*, *2020*, 2672648. https://doi.org/https://doi.org/10.1155/2020/2672648

Lu, J., Chen, H., He, F., You, Y., Feng, Z., Chen, W., Li, X., & Zhao, L. (2020a). Ginsenoside 20(S)-Rg3 upregulates HIF-1α-targeting miR-519a-5p to inhibit the Warburg effect in ovarian cancer cells. *Clin Exp Pharmacol Physiol*. https://doi.org/10.1111/1440-1681.13321

Lu, J., Chen, H., He, F., You, Y., Feng, Z., Chen, W., Li, X., & Zhao, L. (2020b). Ginsenoside 20(S)-Rg3 upregulates HIF-1α-targeting miR-519a-5p to inhibit the Warburg effect in ovarian cancer cells. *Clin Exp Pharmacol Physiol*, 1455-1463. https://doi.org/10.1111/1440-1681.13321

Lu, J., Wang, L., Chen, W., Wang, Y., Zhen, S., Chen, H., Cheng, J., Zhou, Y., Li, X., & Zhao, L. (2019). miR-603 targeted hexokinase-2 to inhibit the malignancy of ovarian cancer cells. *Arch. Biochem. Biophys*, *661*, 1-9. https://doi.org/https://doi.org/10.1016/j.abb.2018.10.014

Lu, J., Zhou, Y., Zheng, X., Chen, L., Tuo, X., Chen, H., Xue, M., Chen, Q., Chen, W., & Li, X. (2020). 20 (S)-Rg3 upregulates FDFT1 via reducing miR-4425 to inhibit ovarian cancer progression. *Arch Biochem Biophys*, *693*, 108569. https://doi.org/https://doi.org/10.1016/j.abb.2020.108569

Mishan MA, Tabari K, Mahrooz A, & A, B. (2021). Role of microRNAs in the anticancer effects of the flavonoid luteolin: a systematic review. *Eur J Cancer Prev*, *30*(5), 413-421. https://doi.org/https://doi.org/10.1097/CEJ.0000000000000645

Mishan, M. A., Tabari, M. A. K., Zargari, M., & Bagheri, A. (2020). MicroRNAs in the anticancer effects of celecoxib: A systematic review. *Eur J Pharmacol*, 173325. https://doi.org/https://doi.org/10.1016/j.ejphar.2020.173325

Sun, S., Wang, C.-Z., Tong, R., Li, X.-L., Fishbein, A., Wang, Q., He, T.-C., Du, W., & Yuan, C.-S. (2010). Effects of steaming the root of Panax notoginseng on chemical composition and anticancer activities. *Food Chem*, *118*(2), 307-314. https://doi.org/https://doi.org/10.1016/j.foodchem.2009.04.122

Wang, P., Du, X., Xiong, M., Cui, J., Yang, Q., Wang, W., Chen, Y., & Zhang, T. (2016a). Ginsenoside Rd attenuates breast cancer metastasis implicating derepressing microRNA-18a-regulated Smad2 expression [Article]. *Sci. Rep*, *6*, Article 33709. https://doi.org/10.1038/srep33709

Wang, P., Du, X., Xiong, M., Cui, J., Yang, Q., Wang, W., Chen, Y., & Zhang, T. (2016b). Ginsenoside Rd attenuates breast cancer metastasis implicating derepressing microRNA-18a-regulated Smad2 expression [Article]. *Sci Rep*, *6*, Article 33709. https://doi.org/10.1038/srep33709

Wang, X., & Wang, Y. (2015). Ginsenoside Rh2 Mitigates Pediatric Leukemia Through Suppression of Bcl-2 in Leukemia Cells. *Cell Physiol Biochem*, *37*(2), 641-650. https://doi.org/10.1159/000430383

Wen, X., Zhang, H. D., Zhao, L., Yao, Y. F., Zhao, J. H., & Tang, J. H. (2015). Ginsenoside Rh2 differentially mediates microRNA expression to prevent chemoresistance of breast cancer. *Asian Pac J Cancer Prev*, *16*(3), 1105-1109. https://doi.org/10.7314/apjcp.2015.16.3.1105

Wu, K. R., Huang, J., Xu, T., Ye, Z. P., Jin, F., Li, N., & Lv, B. (2019a). MicroRNA-181b blocks gensenoside Rg3-mediated tumor suppression of gallbladder carcinoma by promoting autophagy flux via CREBRF/CREB3 pathway. *Am. J. Transl. Res*, *11*(9), 5776-+. <Go to ISI>://WOS:000488232800038

Wu, K. R., Huang, J., Xu, T., Ye, Z. P., Jin, F., Li, N., & Lv, B. (2019b). MicroRNA-181b blocks gensenoside Rg3-mediated tumor suppression of gallbladder carcinoma by promoting autophagy flux via CREBRF/CREB3 pathway. *Am. J. Transl. Res*, *11*(9), 5776-5787. <Go to ISI>://WOS:000488232800038

Wu, N., Wu, G. C., Hu, R., Li, M., & Feng, H. (2011). Ginsenoside Rh2 inhibits glioma cell proliferation by targeting microRNA-128. *Acta Pharmacol. Sin*, *32*(3), 345-353. https://doi.org/10.1038/aps.2010.220

Zhang, Q., Kang, X., Yang, B., Wang, J., & Yang, F. (2008). Antiangiogenic effect of capecitabine combined with ginsenoside Rg3 on breast cancer in mice. *Cancer Biother Radiopharm*, *23*(5), 647-654. https://doi.org/https://doi.org/10.1089/cbr.2008.0532

Zheng, X., Zhou, Y. Y., Chen, W., Chen, L. H., Lu, J. J., He, F., Li, X., & Zhao, L. (2018). Ginsenoside 20(S)-Rg3 Prevents PKM2-Targeting miR-324-5p from H19 Sponging to Antagonize the Warburg Effect in Ovarian Cancer Cells. *Cell Physiol Biochem*, *51*(3), 1340-1353. https://doi.org/10.1159/000495552

Zhou, W., Cai, C., Shi, H., Jin, H., & Wang, X. (2020). Effects of Ginsenoside Biopolymer Nanoparticles on the Malignant Behavior of Non-Small-Cell Lung Cancer. *Adv Polym Technol*, *2020*. https://doi.org/https://doi.org/10.1155/2020/1796701

Zhou, W. Z., Cai, C. L., Shi, H., Jin, H., & Wang, X. W. (2020). Effects of Ginsenoside Biopolymer Nanoparticles on the Malignant Behavior of Non-Small-Cell Lung Cancer. *Adv. Polym. Technol*, *2020*, Article 1796701. https://doi.org/10.1155/2020/1796701

Zhou, Y. Y., Zheng, X., Lu, J. J., Chen, W., Li, X., & Zhao, L. (2018). Ginsenoside 20(S)-Rg3 Inhibits the Warburg Effect Via Modulating DNMT3A/MiR-532-3p/HK2 Pathway in Ovarian Cancer Cells. *Cell Physiol Biochem*, *45*(6), 2548-2559. https://doi.org/10.1159/000488273