MicroRNAs as crucial mediators in the pharmacological activities of triptolide (Review)

KUN ZHOU[1], YINXIA CHANG[2], BO HAN[3], RUI LI[2] and YANMING WEI[2]

[1]Shanxi Institute of Energy, Taiyuan, Shanxi 030600; Colleges of [2]Chinese Medicine and Food Engineering and [3]Basic Medicine, Shanxi University of Chinese Medicine, Jinzhong, Shanxi 030619, P.R. China

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Abstract. Triptolide is the main bioactive constituent isolated from the Chinese herb *Tripterygium wilfordii* Hook F., which possesses a variety of pharmacological properties. MicroRNAs (miRNAs/miRs) are short non-coding RNAs that regulate gene expression post-transcriptionally. miRNAs are implicated in several intracellular processes, whereby their dysregulation contributes to pathogenesis of various diseases. Thus, miRNAs have great potential as biomarkers and therapeutic targets for diseases, and are implicated in drug treatment. Previous studies have reported that specific miRNAs are targeted, and their expression levels can be altered following exposure to triptolide. Thus, miRNAs are emerging as crucial mediators in the pharmacological activities of triptolide. The present review summarizes current literature on miRNAs as target molecules in the pharmacological activities of triptolide, including antitumor, anti-inflammatory, immunosuppressive, renal protective, cardioprotective, antiangiogenesis activities and multiorgan toxicity effects. In addition, the diverse signaling pathways involved are discussed to provide a comprehensive understanding of the underlying molecular mechanisms of triptolide in the regulation of target miRNAs.

Correspondence to: Dr Yanming Wei, College of Chinese Medicine and Food Engineering, Shanxi University of Chinese Medicine, No. 121, DaXue Street, Jinzhong, Shanxi 030619, P.R. China
E-mail: weiyanming2005@aliyun.com

Abbreviations: Hsp70, heat shock protein 70; VEGF, vascular endothelial growth factor; AKT, protein kinase B; AGO, argonaute protein; Mcl-1, myeloid cell leukemia-1; NF-κB, nuclear factor kappa-B; PI3K, phosphatidylinositol 3 kinase; ERK, extracellular regulated protein kinase; mTOR, mammalian target of rapamycin; Cav-1, caveolin-1; PTEN, phosphatase and tensin homolog; MAPK, mitogen-activated protein kinase; TLR, Toll-like receptor; LPS, lipopolysaccharide; SHIP-1, Src homology 2-containing inositol phosphatase-1; SCI, spinal cord injury; SLE, systemic lupus erythematosus; TGF-β, transforming growth factor-β; DKD, diabetic kidney disease; Treg, regulatory T cells; ITGA5, integrin subunit α 5; AhR, aryl hydrocarbon receptor; EZH2, enhancer of zeste homolog 2

Key words: triptolide, microRNAs, antitumor, anti-inflammatory, immunosuppressive, renal protective, cardioprotective, antiangiogenesis, multiorgan toxicity

Contents

1. Introduction

Traditional Chinese medicine is attracting great interest due to the high efficacy of its biological components for the treatment of several diseases. For example, *Astragalus membranaceus* is commonly used in various herbal formulations to cure inflammatory diseases and cancers (1), and *Forsythia suspense*-based treatments have provided notable protection against bacterial infections, allergies, neurodegeneration and cancers (2). Triptolide, a diterpenoid triepoxide, is one of the main active ingredients extracted from the traditional Chinese herb, *Tripterygium wilfordii* Hook F., which possesses several pharmacological activities. In preclinical *in vitro* and *in vivo* models, triptolide has exhibited a broad spectrum of potent antitumor activity (3,4). Triptolide can induce cell cycle arrest, interfere with tumor cell proliferation, suppress cell migration, invasion and metastasis, prevent angiogenesis, enhance caspase-dependent and -independent cell death, and produce a synergistic effect in combination with antitumor drugs (3,5). In addition, triptolide significantly inhibits the expression of pro-inflammatory cytokines and chemokines, and is considered a promising anti-inflammatory agent for the treatment of diseases, including rheumatoid arthritis (6). Triptolide exhibits profound immunosuppressive activity by regulating the proportion of immune cells and inhibiting the release of immune factors (7). Triptolide also alleviates renal and myocardial pathological injuries, and exerts protective roles in kidney and cardiovascular diseases (8,9). However, despite its desirable clinical applications, treatment with triptolide is

restricted due to its potential multiorgan toxicity, including hepatic, cardiac and reproductive toxicity (10).

Currently, the molecular mechanisms underlying the pharmacological activities of triptolide have been extensively investigated (11,12), which have revealed various cellular targets and the involvement of different signaling pathways, including the heat shock protein 70 (Hsp70), vascular endothelial growth factor (VEGF), c-Jun and protein kinase B (AKT) pathways (13). MicroRNAs (miRNAs/miRs) are highly conserved endogenous small non-coding RNA molecules that negatively regulate gene expression. The phylogenetic conservation of several miRNAs across mammals highlights the importance of the miRNAs regulatory network (14). Consistently, extensive studies of miRNA knockout and overexpression models have suggested that miRNAs participate in various essential cellular processes, including cell differentiation, organ development, metabolism and apoptosis (15,16). In addition, several diseases are associated with aberrant expression of miRNAs, and miRNAs exhibit potential as biomarkers and therapeutic targets for diseases, and are highly implicated in drug treatment (17). Increasing evidence suggest that miRNAs also serve as key regulatory elements for triptolide-mediated activity (18-22). Some studies assessing the effect of triptolide on miRNAs have demonstrated that the expression levels of specific miRNAs change several folds in different cell lines and tissues (23-25).

The present review summarizes miRNAs targeted by triptolide and discusses the underlying molecular mechanisms defining the association between the expression of miRNAs and triptolide to outline the critical roles these miRNAs play in the pharmacological activities of triptolide.

2. An overview of miRNAs

miRNAs are single-stranded RNA molecules that are 22 nucleotides in length, and their genes mostly reside in either introns or exons of non-coding transcripts, while others are located within introns of neighboring protein-coding pre-mRNAs and dispersed across the genome (26). miRNA genes are transcribed in the nucleus by RNA polymerase II or III into long primary transcripts called pri-miRNA, which are folded into double-stranded RNA hairpin. The pri-miRNA is cleaved by a microprocessor to release the miRNA precursor named pre-miRNA, containing a stem-loop of ~60 nucleotides, which is subsequently exported to the cytoplasm by exportin 5 and Ran-GTP (15,27). In the cytoplasm, the pre-miRNA is further processed by the endonuclease, Dicer, to generate the small double-stranded miRNA duplex (28). Once formed, the miRNA duplex recruits argonaute protein (AGO) for unwinding (29). A strand of the duplex, namely mature miRNA, associates with the RNA induced silencing complex and mediates mRNA degradation or translational repression by binding to the partially complementary sequences of the 3'-untranslated region of specific mRNAs (30).

Similar to protein-coding genes, miRNAs are subjected to stringent regulation, and transcriptional regulation is a major contributor to the tissue- or development-specific gene expression of miRNAs (31). Furthermore, during the maturation steps, specific RNA binding proteins intricately interact with the processing machineries of a range of miRNAs through functional interactions, which subsequently modulates the expression of their target mRNAs (32). In addition, single nucleotide polymorphisms, RNA editing and methylation are considered important mechanisms that control the expression levels and functions of miRNAs (33).

3. Triptolide modulates the expression of miRNAs

Recent studies have demonstrated that miRNAs are involved in antitumor, anti-inflammatory and immunosuppressive activities of triptolide (18,20,21,34-37). In addition, triptolide exerts renal protective, cardioprotective and antiangiogenesis functions by regulating the expression of miRNAs (38-42). Furthermore, miRNAs are closely associated with the multiorgan toxicity of triptolide (23,43). Tables I and II list evidence supporting miRNAs as pivotal mediators in the pharmacological properties of triptolide.

miRNAs involved in the antitumor activity of triptolide. Several miRNAs act as tumor-suppressor genes or oncogenes, and increasing evidence suggest that the suppressive function of triptolide on multiple tumors is mediated by regulating miRNAs (44,45). In human T-cell lymphocytic leukemia cells, triptolide significantly increases miR-16-1 expression and decreases miR-138-2 expression, and downregulation of miR-16-1 expression contributes to triptolide-induced apoptosis (46). Triptolide-induced hepatocellular carcinoma cell death is associated with the suppression of two oncogenic miRNA clusters, miR-17-92 and miR-106b-25 (24). In pancreatic cancer cells, treatment with triptolide increases miR-204 expression, which directly binds to myeloid cell leukemia-1 (Mcl-1), an antiapoptotic gene essential for cell survival (47), thereby inhibiting Mcl-1 protein expression and inducing cell-type dependent apoptosis or autophagic cell death (48). In addition, triptolide decreases miR-142-3p expression, which negatively regulates Hsp70 expression, a stress protein recognized as an apoptosis inhibitor (49), and suppresses pancreatic cancer cell proliferation (50). *In vivo* studies have confirmed that triptolide abrogates human pancreatic tumors xenografts by concurrent upregulation of miR-204 and miR-142-3p expression and downregulation of Mcl-1 or Hsp70 expression, respectively (48,50). In colon carcinoma cells, treatment with triptolide downregulates miR-191 expression, which in turn suppresses cell proliferation and migration, induces apoptosis and activates the nuclear factor kappa-B (NF-κB) and Wnt/β-catenin signaling pathways (34). Notably, these effects were reversed following transfection with miR-191 mimics, suggesting that the anti-colorectal cancer activities of triptolide are associated with the downregulation of miR-191 expression (34). Through upregulation of miR-146a expression, triptolide markedly decreases the expression levels of the *RhoA* and *Rac1* genes in breast cancer cells, which are both key members of the Rho GTPase family involved in tumor invasion and metastasis, and thus act as metastasis suppressors (18). Triptolide also induces S phase cell cycle arrest of human nasopharyngeal carcinoma cells by enhancing p85α-phosphatase and tensin homolog (PTEN) complex formation and inactivating AKT-mediated cyclin-dependent kinase 2 phosphorylation, which requires downregulation of miR-144 expression (35). Triptolide also

Table I. Triptolide modulates the expression of miRNAs *in vitro*.

miRNAs	Triptolide dosage and treatment time	Cell lines	miRNA status	Downstream targets	Related biological effects	Refs.
miR-16-1	80 nmol/l, 3, 6 or 12 h; 20, 40 or 80 nmol/l, 8 h	Human T-cell lymphocytic leukemia cell line, Molt-4	↓	Not mentioned	Apoptosis↑	(46)
miR-16-1	20, 40 or 80 nmol/l, 8 h	Human T-cell lymphocytic leukemia cell line, Jurkat	↓	Not mentioned	Not mentioned	(46)
miR-138-2	80 nmol/l, 3, 6 or 12 h; 20, 40 or 80 nmol/l, 8 h	Human T-cell lymphocytic leukemia cell line, Molt-4	↑	Not mentioned	Not mentioned	(46)
miR-138-2	20, 40 or 80 nmol/l, 8 h	Human T-cell lymphocytic leukemia cell line, Jurkat	↑	Not mentioned	Not mentioned	(46)
miR-17-92, miR-106b-25 clusters	200 nmol/l, 24 h	Human liver cancer cell line, HepG2	↓	PTEN and BIM	BIM and PTEN↑; apoptosis↑	(24)
miR-204	100 nmol/l, 24 h	Human pancreatic cancer cell line, MIA PaCa-2	↑	Mcl-1	Mcl-1↓; apoptosis↑	(48)
miR-204	100 nmol/l, 24 h	Human pancreatic cancer cell line, S2-VP10	↑	Mcl-1	Mcl-1↓; autophagic cell death↑	(48)
miR-142-3p	100 nmol/l, 24 h	Human pancreatic cancer cells lines, MIA PaCa-2, Capan-1 and S2-013	↑	Hsp70	Hsp70↓; cell proliferation↓	(50)
miR-191	50 or 100 nmol/l, 24 h	Human colorectal cancer cell lines, HT-29 and SW480	↓	Not mentioned	EMT↓; NF-κB and Wnt/β-catenin pathways↓; cell proliferation↓; migration↓; apoptosis↑	(34)
miR-146a	15 ng/ml, 24 h	Human breast cancer cell line, MDA-MB-231	↑	Rac1; RhoA	RhoA and Rac1↓; cell invasion and metastasis↓	(18)
miR-144	40 nmol/l, 36 h	Human nasopharyngeal carcinoma cell lines, NPC-TW039 and NPC-TW076	↓	PTEN	PTEN↑; p85α–PTEN complex↑; p-CDK2↓; S phase arrest	(35)
miR-193b-3p	25 nmol/l, 72 h	Human malignant rhabdoid kidney tumor cell line, G-401	↑	KLF4	KLF4↓; PI3K/AKT and ERK signaling pathways↓; cell viability↓; cell migration↓; apoptosis↑	(51)
miR-193b-3p	10, 25 or 50 nmol/l, 72 h	Human malignant rhabdoid kidney tumor cell line, WiT49	↑	Not mentioned	Not mentioned	(51)
miR-138	100 nmol/l, 24 h	Human medulloblastoma cell line, Daoy	↑	CDK6	CDK6↓; PI3K/AKT and Notch signaling pathways↓; cell proliferation↓; cell migration↓; apoptosis↑	(52)
miR-218	100 nmol/l, 24 h	Human benign prostatic hypertrophy epithelial cell line, BPH-1	↑	Survivin	Survivin↓; mTOR signaling pathway↓; apoptosis↑	(53)

Table I. Continued.

miRNAs	Triptolide dosage and treatment time	Cell lines	miRNA status	Downstream targets	Related biological effects	Refs.
miR-215, miR-146a, miR-199b, miR-449a, miR-190b	10 nmol/l, 48 h	Human non-small cell lung cancer cell line, H460	↑	Not mentioned	FAK↓; p-FAK, p-Src, p-p130Cas↓; p-ERK1/2, MMP14↑; cell migration, invasion and metastasis↓	(25)
miR-92a, miR-222, miR-23b, miR-27a, miR-25, miR-296	10 nmol/l, 48 h	Human non-small cell lung cancer cell line, H460	↓	Not mentioned	FAK↓; p-FAK, p-Src, p-p130Cas↓; p-ERK1/2, MMP14↑; cell migration, invasion and metastasis↓	(25)
miR-204-5p	50 or 100 nmol/l, 20 h	Human non-small cell lung cancer cell line, A549	↑	Sirt-1	Sirt-1↓, Cav-1↓; apoptosis↑	(58)
miR-21	25 or 50 nmol/l, 48 h	Human non-small cell lung cancer cell line, PC-9	↓	PTEN	PTEN↑; cell viability↓	(62)
miR-21	5 nmol/l, 72 h	Human multidrug-resistant chronic myeloid leukemia cell line, K562/A02	↓	PTEN	PTEN↑; adriamycin resistance↓	(63)
miR-6751	100 nmol/l, 24 h	Cisplatin-resistant human ovarian cancer cell line, A2780/CP70	↑	HK2	HK2↓; apoptosis↑; cisplatin resistance↓	(64)
miR-142-5p and miR-181a	10 ng/ml, 24 h	Dexamethasone-treated human multiple myeloma cell line, MM.1S	↓	GR	GR↑; dexamethasone resistance↓	(65)
miR-181a	150 nmol/l, 24 h	Human osteosarcoma cell lines, SAOS2 and U2OS	↓	PTEN	PTEN↑; cell proliferation↓; apoptosis↑; cell invasion↓	(66)
miR-181a	20 nmol/l, 24 h	Human neuroblastoma cell line, SH-SY5Y	↑	Not mentioned	MAPK and NF-kB signaling pathways↑; cell proliferation↓; apoptosis↑; cell migration↓	(67)
miR-155	0.05, 0.1 or 0.5 μmol/l, 0.5 h	LPS-stimulated murine macrophage cell line, RAW264.7	↓	Not mentioned	Proinflammatory cytokines (TNF-α, IL1β, and IL-6) ↓	(70)
miR-155	15 nmol/l, 24 h	LPS-stimulated monocytes of patients with rheumatoid arthritis	↓	SHIP-1	SHIP-1↑; proinflammatory cytokines (TNF-α, IL-6)↓	(19)
miR-155	40 nmol/l, 12 h	Human wild-type αSyn preformed fibrils-treated mouse primary microglia	↓	SHIP-1	SHIP-1↑; PI3K/AKT signaling pathway↓; NF-κB activity↓; proinflammatory cytokines (TNFα and IL-1β)↓	(20)
miR-16-1	20 ng/ml, 24 h	Human primary intestinal fibroblasts from strictured anastomosis tissue	↓	Hsp70	Hsp70↑; cell migration↓; cell proliferation↓; extracellular matrix-associated proteins (Col-I, Col-III and α-SMA)↓	(36)
miR-20b	20 ng/ml, 1 h	LPS- and ATP-treated human monocytic cell line, THP-1	↓	NLRP3	NLRP3↑; cleaved caspase-1↓; proinflammatory cytokines (IL-1β and TNF-α)↓	(37)

Table I. Continued.

miRNAs	Triptolide dosage and treatment time	Cell lines	miRNA status	Downstream targets	Related biological effects	Refs.
miR-96	12.5 nmol/l, 24 h	LPS-treated murine microglial cell line, BV2	↑	IKKβ	IKKβ↓; Iba-1↓; proinflammatory cytokines (TNF-α and IL-1β)↓	(100)
miR-96	10 nmol/l, 0.5 h	LPS-treated rat primary microglia	↑	Not mentioned	Iba-1↓; NF-κB signaling pathway↓; proinflammatory cytokines (TNF-α and IL-1β)↓	(100)
miR-125a-5p	10 nmol/l, 3 days	Splenocytes of B6 mice	↑	Not mentioned	Foxp3↑; Treg proportion↑	(21)
miR-125a-5p	0.2 mg/kg/day, 91 days	Splenocytes of MRL/lpr mice	↑	Not mentioned	Foxp3↑; Treg proportion↑	(21)
miR-30	10 ng/ml, 24 h	TGF-β-treated immortalized human podocyte cell line	↑	Not mentioned	Cell injury mediators (MAPK, NF-κB and NFATC3)↓	(22)
miR-188-5p	5 ng/ml, 48 h	High glucose-treated human proximal tubular epithelial cell line, HK-2	↓	PTEN	PTEN↑; PI3K/AKT signaling pathway↓; renal EMT↓	(38)
miR-141-3p	10 μmol/l, 48 h	High glucose-treated human mesangial cell	↓	PTEN	PTEN↓; autophagy↑; AKT/mTOR signaling pathway↓; diabetic renal fibrosis↓	(75)
miR-137	10 μg/l, 48 h	High glucose-treated human renal mesangial cell	↑	Notch1	Notch1 signaling pathway↓; extracellular matrix proteins (Col IV and FN)↓	(39)
miR-21	10 ng/ml, 24 h	Rat myocardial cell line, H9C2	↓	TLR4	TLR4↓; MAPK/NF-κB signaling pathway↓; proinflammatory cytokines (TNF-α, IL-6, and IL-17)↓	(40)
miR-92a	3 μmol/l, 12 h	Human dermal microvascular endothelial cell line, HMEC-1	↑	Integrin subunit alpha 5 (ITGA5)	Angiogenic mediators (eNOS, VEGFR2 and VEGF)↓; ITGA5↓; ERK and PI3K/AKT signaling pathways↓	(42)
miR-26a	120 nmol/l, 24 h	Mouse Leydig cell line MLTC-1	↑	GSK3β	GSK3β↓; apoptosis↑	(43)

miRNA/miR, microRNA; ↑, upregulation; ↓, downregulation; PTEN, phosphatase and tensin homolog; Mcl-1, myeloid cell leukemia-1; Hsp70, heat shock protein 70; EMT, epithelial-to-mesenchymal transition; NF-κB, nuclear factor kappa-B; CDK2, cyclin-dependent kinase 2; KLF4, Kruppel-like factor; PI3K, phosphatidylinositol 3 kinase; AKT, protein kinase B; ERK, extracellular regulated protein kinase; mTOR, mammalian target of rapamycin; FAK, focal adhesion kinase; MMP14, matrix metalloprotease 14; Cav-1, caveolin-1; HK2, hexokinase 2; GR, glucocorticoid receptor; MAPK, mitogen-activated protein kinase; SHIP-1, Src homology 2-containing inositol phosphatase-1; Col I, collagen I; α-SMA, α-smooth muscle actin; LPS, lipopolysaccharide; NLRP3, nucleotide-binding oligomerization domain-like receptor family pyrin domain-containing protein 3; IKKβ, inhibitor of nuclear factor kappa B kinase subunit β; Treg, regulatory T cells; FN, fibronectin; TLR4, Toll-like receptor 4; eNOS, endothelial nitric oxide synthase; VEGFR2, vascular endothelial growth factor receptor-2; ITGA5, integrin subunit α 5; GSK3β, glycogen synthase kinase-3β.

Table II. Triptolide modulates the expression of miRNAs *in vivo*.

miRNAs	Triptolide dosage and treatment time	Tissue type	miRNA status	Downstream targets	Related biological effects	Refs.
miR-17-92; miR-106b-25 clusters	0.2 mg/kg/day, 14 days	Xenografted hepatocellular carcinoma from BALB/c nude mice	↓	Not mentioned	Tumor volume↓; apoptosis↑	(24)
miR-204	0.42 mg/kg/day, 7 days	Xenografted human pancreatic ductal adenocarcinoma from SCID mice	↑	Mcl-1	Mcl-1↓; tumor volume↓	(48)
miR-142-3p	0.42 mg/kg/day, 7 days	Xenografted human pancreatic ductal adenocarcinoma from SCID mice	↑	Hsp70	Hsp70↓	(50)
miR-191	0.1, 0.3 or 1 mg/kg/day, 28 days	Xenografted human colon carcinoma from BALB/c nude mice	↓	Not mentioned	Tumor volume and weight↓	(34)
miR-155	0.07 mg/kg every 2 days, 56 days	Small intestinal of IL-10 deficient mice performed ileocecal resection	↓	SHIP-1	SHIP-1↑; anastomosis inflammation score↓; MPO and calprotectin↓; inflammatory cytokines TGF-β ↑, IFN-γ and IL-4, IL-17 ↓	(72)
miR-16-1	0.07 mg/kg every 2 days, 56 days	Small intestinal of IL-10 deficient mice performed ileocecal resection	↓	Hsp70	Hsp70↑; anastomosis inflammation score↓; CD4+ cell infiltration area↓; fibrosis score↓; extracellular matrix-associated proteins (collagen, procollagen I and III)↓; inflammatory cytokines (TGF-β1, IL-6 and TNF-α)↓	(73)
miR-96	0.1 mg/kg/day, 10 days	Spinal cord of spinal cord injury rat	↑	Not mentioned	Iba-1↓; NF-κB pathway↓; inflammatory cytokines (TNF-α and IL-1β)↓	(100)
miR-344b-3p; miR-30b-3p	200 μg/kg/day, 56 days	Rat renal cortex with adriamycin-induced nephropathy	↓	Not mentioned	Nephrin↑; proteinuria↓; renal pathological lesions↓	(76)
miR-30a	10 ng/ml, 24 h	TGF-β1-treated isolated glomeruli of mouse or rats	↑	Not mentioned	Not mentioned	(22)
miR-188-5p	200 μg/kg/day, 84 days	Diabetic rat kidney	↓	PTEN	PTEN↑; renal EMT↓; PI3K/AKT signaling pathway↓	(38)
miR-137	100 μg/kg/day, 84 days	Diabetic rat kidney	↑	Not mentioned	Extracellular matrix proteins (Col IV and FN)↓; Notch1 signaling pathway↓	(39)
miR-21	0.4 mg/kg every 7 days, 28 days	Adjuvant arthritis rat cardiac tissue	↓	TLR4	TLR4↓; apoptosis↓; proinflammatory cytokines (TNF-a, IL-6, and IL-17)↓; MAPK/NF-κB signaling pathway↓	(41)
miR-546, miR-343, 108 miRNAs	0.1 mg/kg, single oral dose, 14 days	Left ventricular tissue from rats	↑	Not mentioned	Regulation of cell adhesion, cell cycling, action potential, cell-cell communication, and DNA binding	(23)

Table II. Continued.

miRNAs	Triptolide dosage and treatment time	Tissue type	miRNA status	Downstream targets	Related biological effects	Refs.
miR-384-3p, miR-384-5p, 8 miRNAs	0.1 mg/kg, single oral dose, 14 days	Left ventricular tissue from rats	↓	Not mentioned	Regulation of calmodulin activity, heterodimerization activity, and signal transduction	(23)
miR-483-3p	0.1 mg/kg, single oral dose, 14 days	Left ventricular tissue from rats	↑	AhR	AhR↓; CYP1A1↓	(23)
miR-15a-3p, miR-615, miR-4833p, miR-127-5p	0.1 mg/kg, single oral dose, 14 days	Plasma from rats	↑	Not mentioned	Not mentioned	(23)
miR-122	0.2, 0.4 or 0.8 μmol/l, 48 h	Zebrafish larvae	↑	Not mentioned	Histology score of hepatocyte vacuolation, hepatocyte disarray and oncotic necrosis↑; liver volume↓	(83)

miRNA/miR, microRNA; ↑, upregulation; ↓, downregulation; Mcl-1, myeloid cell leukemia-1; Hsp70, heat shock protein 70; SHIP-1, Src homology 2-containing inositol phosphatase-1; MPO, myeloperoxidase; NF-κB, nuclear factor kappa-B; PTEN, phosphatase and tensin homolog; EMT, epithelial-to-mesenchymal transition; Col IV, collagen IV; FN, fibronectin; TLR4, Toll-like receptor 4; MAPK, mitogen-activated protein kinase; AhR, aryl hydrocarbon receptor.

inhibits the proliferation of nephroblastoma and medulloblastoma cells, and benign prostatic epithelial cells. In addition, triptolide inhibits the migratory ability of these cells but promotes apoptosis by upregulating the expression levels of miR-193b-3p, miR-138 and miR-218 (51-53). The latter activities involving miRNAs corresponded to inactivation of the phosphatidylinositol 3 kinase (PI3K)/AKT and extracellular regulated protein kinase (ERK) signaling pathways by downregulating Kruppel-like factor, inactivating the Notch signaling pathway, as well as suppressing CDK6 expression, or negatively regulating survivin and inactivating the mammalian target of rapamycin (mTOR) signaling pathway, respectively (51-53).

Sequencing data indicate that triptolide markedly alters the expression profiles of miRNAs in human non-small cell lung cancer cells. miRNAs associated with cell motility, such as miR-146a, miR-23b and miR-199b (54-56), are significantly upregulated or downregulated, and subsequent studies have validated that triptolide notably decreases lung cancer cell migration, invasion and metastasis by suppressing focal adhesion kinase expression and disrupting its downstream signaling pathways (25). Furthermore, treatment with triptolide causes a dose-dependent upregulation of miR-204-5p expression, along with decreased expression of its target, SIRT-1, a member of the class III histone deacetylase family required for caveolin-1 (Cav-1) expression (57), thereby coupling Cav-1 downregulation with activation of classical AKT/Bax-mediated apoptosis in non-small cell lung cancer cells (58).

Drug resistance is one of the main reasons for therapy failure in cancer treatment (59). miRNAs are critical regulators of molecular pathways implicated in cancer drug resistance (60). miR-21 is highly expressed and associated with disease progression and multidrug-resistance in different types of cancer (61). Triptolide notably decreases miR-21 expression in non-small cell lung cancer cells, and increases PTEN protein expression, which acts as a tumor suppressor and participates in tumor occurrence and development, and decreases cell proliferation and enhances apoptosis (62). However, ectopic miR-21 expression rescues the effect of triptolide on PTEN protein expression and cell viability, suggesting that triptolide mediates the decrease in miR-21 expression, which in turn promotes cell death by upregulating PTEN expression (62). Furthermore, triptolide significantly enhances adriamycin-induced cytotoxicity by decreasing miR-21 expression and increasing PTEN expression, and reverses drug resistance to human chronic myeloid leukemia cells (63).

In cisplatin-resistant human ovarian cancer cells, upregulation of miR-6751 expression via triptolide decreases hexokinase 2 protein expression, which confers resistance to cisplatin by enhancing autophagic activity, and sensitizes cells to the proapoptotic effect of cisplatin (64). A similar synergistic proapoptotic effect was detected following combined treatment with triptolide and dexamethasone in human multiple myeloma cells, which has the ability to overcome the glucocorticoid resistance of these cells by enhancing glucocorticoid receptor expression by inhibiting miR-142-5p and miR-181a expression (65). Furthermore, miR-181a expression is notably attenuated in osteosarcoma cells following treatment with triptolide, which directly upregulates PTEN expression, whereas transfection with miR-181a mimics restores PTEN expression, and decreases the inhibitory effect of triptolide on osteosarcoma cell proliferation and invasion, suggesting the anti-osteosarcoma properties of triptolide depended on the regulation of miR-181a and its targeting of the *PTEN* gene (66). Conversely, upregulated miR-181a expression participates in the suppressive effect of triptolide against human neuroblastoma cell proliferation and migration, and via activation of the mitogen-activated protein kinase (MAPK) and NF-κB signaling pathways (67). Thus, triptolide can upregulate or downregulate the expression of specific miRNAs, influence downstream targeting signaling pathways, and thus exert antitumor activities (Fig. 1).

miRNAs involved in the anti-inflammatory and immunosuppressive activities of triptolide. Several miRNAs are substantially activated by inflammatory stimuli, such as the Toll-like receptor (TLR), ligand lipopolysaccharide (LPS) and components of the inflammatory processes by post-transcriptional regulation of either signal transduction proteins of inflammatory pathways or specific inflammatory cytokines (68). For example, miR-155, which is implicated in the pathogenesis of inflammatory diseases (69), has prompted investigation on the association between triptolide and miR-155 in these inflammatory diseases. cDNA array and northern blot analysis have demonstrated that the expression of inflammatory cytokine, as well as miR-155 are markedly upregulated in macrophages following LPS stimulation, whereas triptolide attenuates the induction of these genes in a dose-dependent manner (70).

Similar observations were obtained in monocytes from patients with rheumatoid arthritis (19), whereby overexpression of miR-155 reverses the inhibitory effect of triptolide on LPS-induced interleukin (IL)-6 and tumor necrosis factor-α production, and antagonizes the effect of triptolide on Src homology 2-containing inositol phosphatase-1 (SHIP-1), which is a target of miR-155 and functions as a potent inhibitor of several inflammatory pathways (71). Thus, it has been proposed that triptolide inhibits miR-155 expression, which negatively affects its downstream target, SHIP-1, and suppresses the inflammatory response stimulated by LPS.

The miR-155/SHIP-1 axis plays a critical role in triptolide-induced improvement on the symptoms of other inflammatory diseases. Inhibiting the miR-155/SHIP-1 axis via triptolide suppresses NF-κB activity via the PI3K/AKT pathway, which significantly inhibits microglial activation and stimulates inflammatory cytokines stimulated by prion-like preformed fibril (20).

Triptolide has also been reported to suppress miR-155 expression and simultaneously promote SHIP-1 expression in the small intestine, particularly in the anastomosis of IL-10 deficient mice subjected to ileocecal resection (72). These effects are also associated with decreased levels of inflammatory cytokines, and eventually exert therapeutic effects on Crohn's disease, an inflammatory bowel disease (72). Studies on Crohn's disease have reported that triptolide effectively reverses upregulated miR-16-1 expression and downregulated Hsp70 expression at the anastomosis sites in IL-10 deficient mice, as well as in patients with Crohn's disease, which represents a protective mechanism against postsurgical inflammation and anastomotic fibrosis (37,73).

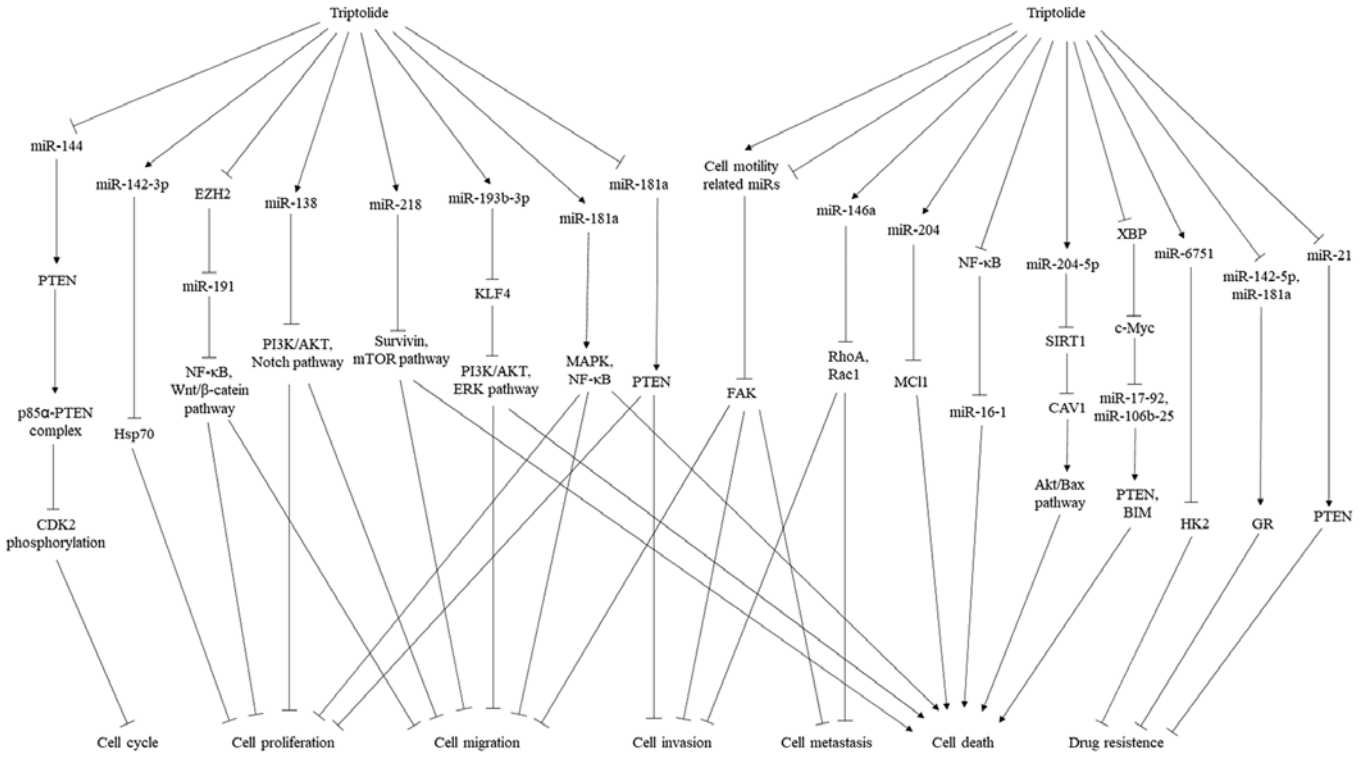

Figure 1. miRNAs involved in the antitumor activity of triptolide. Through upregulation and downregulation of specific miRNAs, triptolide affects downstream signaling pathways, which induces tumor cell cycle arrest, interferes with cell proliferation, suppresses cell migration, invasion and metastasis, enhances cell death and reverses drug resistance. miRNA, microRNA; PTEN, phosphatase and tensin homolog; CDK, cyclin-dependent kinase; Hsp, heat shock protein; EZH2, enhancer of zeste homolog 2; NF-κB, nuclear factor kappa-B; PI3K, phosphatidylinositol 3 kinase; AKT, protein kinase B; mTOR, mammalian target of rapamycin; KLF4, Kruppel-like factor; ERK, extracellular regulated protein kinase; Cav-1, caveolin-1; HK2, hexokinase 2; GR, glucocorticoid receptor.

With regards to the effects on osteoarthritis, downregulation of miR-20b expression following treatment with triptolide notably upregulates its target inflammasome-related gene, nucleotide-binding oligomerization domain-like receptor family pyrin domain-containing protein 3, which limits the activation of caspase-1 and subsequently inhibits the maturation of inflammatory cytokines, thus preventing the development of osteoarthritis (41). In the treatment of spinal cord injury (SCI), the anti-inflammatory activity of triptolide is mediated by upregulated miR-96 expression, which inactivates NF-κB by negatively regulating the inhibitor of NF-κB kinase subunit β expression, and decreases inflammatory cytokine levels in LPS-induced primary microglia and spinal cord tissues of SCI rats (42).

Triptolide is frequently used as an immunosuppressive compound in the treatment of chronic autoimmune diseases, including systemic lupus erythematosus (SLE) (12). Recently, a novel miRNA target was identified following treatment of SLE by triptolide. Triptolide was demonstrated to markedly increase miR-125a-5p expression, as well as the percentage of regulatory T cells (Treg), which are important in modulating self-tolerance and autoimmunity (74). Conversely, miR-125a-5p inhibitor significantly abrogates the effects of triptolide on Treg, suggesting that triptolide stimulates Treg activation via miR-125a-5p, and thereby alleviates the clinical and histological symptoms observed in the SLE mouse model (21). Taken together, these findings suggest that changes in the expression levels of specific miRNAs contribute to the anti-inflammatory and immunosuppressive properties of triptolide.

miRNAs involved in the renal protective and cardioprotective activities of triptolide. Increasing evidence suggest that triptolide exhibits renal protective effects by regulating the expression of miRNAs (22,38,39,75,76). In adriamycin-induced nephropathy rat models, elevated expression levels of miR-344b-3p and miR-30b-3p were observed, the effects of which were reversed following treatment with triptolide. Triptolide also increases the levels of nephrin protein, which is involved in the maintenance of the glomerular filtration barrier structure in the kidney cortex, thereby attenuating podocyte damage and partially improving renal function (76). Thus, it has been speculated that miR-344b-3p and miR-30b-3p are involved in the protective effect of triptolide towards podocytes from adriamycin-induced nephropathy (76). In the transforming growth factor-β (TGF-β)-induced podocyte injury model, the presence of triptolide completely reversed TGF-β-induced miR-30 downregulation, suppressed TGF-β-stimulated activation of downstream damaging pathways, including MAPK, NF-κB and calcineurin/NFATC3, and alleviated podocyte injury *in vitro* and *ex vivo* (22).

During the development of diabetic kidney disease (DKD), miRNAs are closely associated with multiple pathological modifications (77). Triptolide notably mitigates the impaired renal function by exerting a therapeutic effect on DKD through the regulation of miRNA-mediated signaling pathways (38,75). For example, triptolide significantly reverses the increase in miR-188-5p expression induced by high glucose in human proximal tubular epithelial cells and diabetic kidneys, which enhances the expression of action target PTEN, and

inactivates the downstream PI3K/AKT signaling pathway and inhibits renal epithelial-to-mesenchymal transition in DKD (38). Similarly, downregulation of miR-141-3p expression via triptolide affects the target PTEN protein in human renal mesangial cells, under high glucose, with an opposite tendency. In addition, triptolide-induced autophagic activation and fibrosis alleviation in association with the activation of the PTEN/AKT/mTOR pathway is blocked following overexpression of miR-141-3p (75).

The miR-137/Notch1 pathway prevents glomerulosclerosis under diabetic conditions (39). Unlike miR-188-5p and miR-141-3p, miR-137 expression significantly decreases in high glucose-incubated renal mesangial cells and in diabetic rat kidney tissues, but returns to normal levels following treatment with triptolide (39). Triptolide inactivates the Notch1 pathway reliance on miR-137, which suppresses extracellular matrix protein accumulation of collagen IV and fibronectin, thus improving DKD by protecting against glomerulosclerosis (39).

The cardioprotective activity of triptolide has been attributed to the changes in miR-21 expression. Treatment with triptolide markedly decreases miR-21 expression, inactivates the TLR4/MAPK/NF-κB signaling pathway and prevents cell apoptosis in LPS-treated myocardial cells, as well as in cardiac tissues from adjuvant arthritis rat models (40,41). Collectively, these findings confirm the regulatory role of miRNAs in the renal protective and cardioprotective activities of triptolide.

miRNAs involved in the antiangiogenesis activity of triptolide. A key feature of atherosclerosis is the dysregulated progression of endothelial cell angiogenesis within the plaques, a process that is regulated by several miRNAs, particularly miR-92a (78). miR-92a blocks angiogenesis both *in vitro* and *in vivo* (79), and participates in the inhibitory effect of triptolide in atherosclerosis. In human dermal microvascular endothelial cells, triptolide effectively induces miR-92a expression in a dose-dependent manner, and impedes the production of integrin subunit α 5 (ITGA5), which is a direct target of miR-92a (42). Overexpression of ITGA5 inactivates the ERK and PI3K/AKT signaling pathways, as well as the accumulation of a number of angiogenesis-related factors, such as endothelial nitric oxide synthase, VEGF receptor-2 and VEGF, which inhibits angiogenesis (42). These effects are reversed following ITGA5 knockdown (42). Thus, miR-92a acts as a crucial mediator in the antiangiogenesis activity of triptolide by inactivating the ERK and PI3K/AKT signaling pathways following downregulation of ITGA5 expression.

miRNAs involved in the multiorgan toxicity of triptolide. Several miRNAs, such as miR-122 and miR-26a, are involved in the multiorgan toxicity observed following treatment with triptolide, and are considered sensitive early warning indicators (80). Oral administration of triptolide in male rats led to cardiac dysfunction and myocardial cell death. These effects were associated with at least a 2-fold increase in the expression levels of 108 miRNAs, as well as a 2-fold decrease in the expression levels of eight miRNAs in heart tissue samples. Furthermore, changes in plasma miRNAs were also detected (81). Among these, 28 miRNAs were predicted to simultaneously regulate the expression of aryl hydrocarbon receptor (AhR), which is a transcription factor that is closely associated with cardiac pathophysiology (81). Another study confirmed that triptolide significantly decreases myocardial and plasma AhR levels, as well as its downstream gene, *CYP1A1* (23).

miR-122 expression is significantly upregulated in adult zebrafish or mice livers following treatment with liver toxicants, including acetaminophen or tamoxifen (82). A similar trend has been observed in zebrafish larvae following treatment with triptolide, which causes hepatic injury (83), which is considered to be a crucial event resulting from deregulated hepatic function caused by altered miR-122 expression (84). Taken together, these findings suggest that miR-122 may be used as a diagnostic predictor and an attractive therapeutic target for the hepatotoxicity activity of triptolide.

miR-26a expression is upregulated in triptolide-induced reproductive toxicity in mouse Leydig cells (43). This results in cytotoxicity via inhibition of its downstream target, glycogen synthase kinase-3β, which possesses antiapoptotic effects (43). Collectively, these findings indicate the association between miRNAs and the multiorgan toxicity of triptolide.

4. Molecular mechanisms underlying regulation of miRNAs by triptolide

The regulation of the expression of miRNAs by triptolide is partly mediated by specific transcription factors. Triptolide has been reported to interfere with TGF-β-induced Smad2/3 phosphorylation and activation, and prevents phosphorylated protein binding to Smad4 and their subsequent translocation to nuclei where they are known to regulate gene expression as transcription factors, and thereby completely restore miR-30 downregulation in podocytes (22). As a multifunctional transcription factor, NF-κB positively and negatively regulates the expression of miRNAs (85). Treatment with triptolide decreases the expression and nuclear accumulation of NF-κB in a dose-dependent manner, accompanied by the differential expression of 23 miRNA genes in lymphocytic leukemic cell lines, suggesting that triptolide-induced cytotoxic effects may occur by inhibiting NF-κB transcriptional activity, and consequently influencing the expression of miRNAs (46). c-Myc also plays a critical role in repressing two oncogenic miRNA clusters by triptolide in hepatocellular carcinoma cells (24). Through direct binding to the E-box element in the promoter region of MCM7, c-Myc transactivates both miR-7-92 and miR-106b-25, which function as oncogenes in cancer initiation and progression (86). Triptolide significantly antagonizes the transcription of these two miRNA clusters by targeting c-Myc, and XPB (also known as ERCC3) is involved in this process (24). XPB is a subunit of general transcription factor TFIIH, which is essential for RNA polymerase II-dependent transcription initiation and nucleotide excision repair (87). It has been reported that triptolide covalently binds to the Cys342 residue of XPB, the largest subunit of the general transcription factor TFIIH (88), and impedes its ATPase activity, inhibiting RNA polymerase II mediated transcription (89). Triptolide also acts as an inhibitor of RNA polymerase II by selectively activating CDK7, and subsequently by triggering proteasome-dependent degradation of hyperphosphorylated Rpb1, which is the largest RNA polymerase II subunit (90). On the other hand, triptolide directly suppresses the recruitment

of B-related factor 1 to TFIIIB complex, an essential transcription initiation factor of RNA polymerase III (91), and significantly inhibits RNA polymerase III transcription (92). Based on these observations and the fact that miRNA genes are transcribed by RNA polymerase II or III, it is reasonable to speculate that the inhibition of RNA polymerase II and III mediated general transcription underlies miRNAs regulation by triptolide.

Triptolide may also regulate the expression of miRNAs via an enhancer of zeste homolog 2 (EZH2)-involved mechanism. EZH2, a well-known histone methyltransferase (93), is capable of binding to the promoter regions of miRNAs genes and catalyzing histone H3 trimethylation, resulting in the transcriptional silencing of miRNAs genes (94). It has been proven that EZH2 is a target for triptolide in prostate cancer and multiple myeloma cells (95), and a recent study demonstrated that treatment with triptolide significantly downregulates miR-191 expression in colon carcinoma cells in an EZH2-dependent manner, suggesting a modulatory activity of triptolide on the expression of miRNAs at the epigenetic level (34).

The regulation of miRNAs by triptolide may be ascribed to the autophagy pathway. Autophagy can be modulated by triptolide through multiple machineries and signaling pathways, such as oxidative stress, cytoplasmic calcium and the AKT/mTOR/p70S6K pathway (13). miRNAs also play vital roles in autophagy as they can target autophagy-related genes or other related regulators, and participate in regulating the dynamic process of autophagy (96). Triptolide has been reported to significantly decrease miR-141-3p expression, which directly acts on PTEN, and thereby reverses the induced autophagy in high glucose treated human mesangial cells and in DKD rats (75). Conversely, autophagy selectively regulates miRNA homeostasis, which is activated by promoting autophagy receptor-mediated degradation of miRNA pathway components, including DICER and AGO2, further proving an association between autophagy and miRNAs (97). Although the molecular mechanisms involved in the role of autophagy in triptolide regulation of miRNAs remain unclear, it is possible that autophagy may play a functional role, and the precise molecular targets and regulatory networks remain to be elucidated. Collectively, these findings suggest that the expression of triptolide-regulated miRNAs is mediated by multiple intracellular components and molecular mechanisms.

5. Conclusions and future perspective

Traditional Chinese medicines have been effectively used to cure various diseases. Thus, investigating the molecular mechanisms underlying their activities is highly warranted. As a biologically active ingredient derived from the Chinese herb, *Tripterygium wilfordii* Hook F., recent studies have demonstrated that triptolide exerts effects on the expression of a series of endogenous miRNAs, which in turn employ several downstream cellular factors to achieve its multiple pharmacological activities, highlighting miRNAs as critical mediators for triptolide-induced effects (18,20,21,23,34-43). However, determining the exact role of these miRNAs is limited by the *in vitro* models offered by these studies. Haploid cells are effective tools to study gene function due to having one set of chromosomes (98,99). Thus, prospective studies will aim to use haploid cells to screen novel miRNAs for specific functions following treatment with triptolide, and additional evidence from *in vivo* experiments will validate the conclusions. In addition, regulation of miRNA expression is usually cell- or tissue-specific, and as precise base pairing between miRNA and their corresponding mRNA targets is not required. A single miRNA may simultaneously target several mRNAs, whereas one mRNA can be regulated by different miRNAs (28,30,33). Triptolide may affect PTEN expression by regulating the expression of several miRNAs, including miR-21 (62,63), miR-181a (66), miR-188-5p (38), miR-144 (35), miR-141-3p (75), miR-17-92 and miR-106b-25 clusters (24). miR-21 is a common target when triptolide controls the expression of both TLR4 and PTEN (40,41,62,63). Thus, the intricate complexity and integration of the activity of miRNAs present challenges in identifying their function and regulatory pathways. Supplementary bioinformatics tools may provide integrated analyses of these complicated networks that participate in the pharmacological activities of triptolide. Prospective studies are required to investigate whether miRNAs play roles in other pharmacological activities of triptolide.

In conclusion, specific miRNAs have been identified as primary targets when triptolide displays multiple pharmacological effects. These findings also suggest that triptolide can serve as a novel molecular probe for studying miRNAs. A comprehensive understanding of the regulatory pathways and specific functions of these miRNAs will help determine the underlying molecular mechanisms of triptolide and provide more effective strategies for drug development and disease treatment.

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Authors' contributions

YW conceived and supervised the present study. KZ drafted the initial manuscript. YC, BH, RL and YW reviewed the manuscript for important intellectual content. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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