Pristimerin Exerts Pharmacological Effects Through Multiple Signaling Pathways: A Comprehensive Review

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Abstract: Pristimerin, a natural triterpenoid isolated from the plants of southern snake vine and Maidenwood in the family Weseraceae, is anti-inflammatory, insecticidal, antibacterial, and antiviral substance and has been used for its cardioprotective and antitumor effects and in osteoporosis treatment. These qualities explain Pristimerin's therapeutic effects on different types of tumors and other diseases. More and more studies have shown that pristimerin acts in a wide range of biological activities and has shown great potential in various fields of modern and Chinese medicine. While Pristimerin's wide range of pharmacological effects have been widely studied by others, our comprehensive review suggests that its mechanism of action may be through affecting fundamental cellular events, including blocking the cell cycle, inducing apoptosis and autophagy, and inhibiting cell migration and invasion, or through activating or inhibiting certain key molecules in several cell signaling pathways, including nuclear factor κB (NF-κB), phosphatidylinositol 3-kinase/protein kinase B/mammalian-targeted macromycin (PI3K/Akt/mTOR), mitogen-activated protein kinases (MAPKs), extracellular signal-regulated protein kinase 1/2 (ERK1/2), Jun amino-terminal kinase (JNK1/2/3), reactive oxygen species (ROS), wingless/integrin1 (Wnt)/β-catenin, and other signaling pathways. This paper reviews the research progress of Pristimerin's pharmacological mechanism of action in recent years to provide a theoretical basis for the molecular targeting therapy and further development and utilization of Pristimerin. It also provides insights into improved treatments and therapies for clinical patients and the need to explore pristimerin as a potential facet of treatment.

Keywords: pristimerin, signaling pathway, pharmacological effects, review

Introduction

In recent years, natural compounds have attracted much attention in the treatment of human diseases due to their long history of use, diverse pharmacological effects and better safety than synthetic compounds. Pristimerin is a natural triterpenoid with orange-yellow needle-like crystals, a density of 1.16 g/cm3, a melting point of 214~217 °C, a boiling point of 607.7 °C, a refractive index of 1.582, a molecular weight of 464.64, and a molecular formula of $C_{30}H_{40}O_4$. Pristimerin can be dissolved in methanol, ethanol, dimethylsulfoxide, and other organic solvents (Figure 1).[1] It is widely found in a variety of Weigeliaceae plants, such as southern snake vine (Celastrus orbiculatus Thunb.) and Maidenwood (Gymnosporia acuminata Hook. f.), and can be used in traditional Chinese medicine to treat a variety of diseases. There is considerable evidence that many natural products from traditional Chinese medicine, such as resveratrol, dioscin, berberine and curcumin, have antitumor effects. In contrast, Pristimerin has a broader range of biological effects,
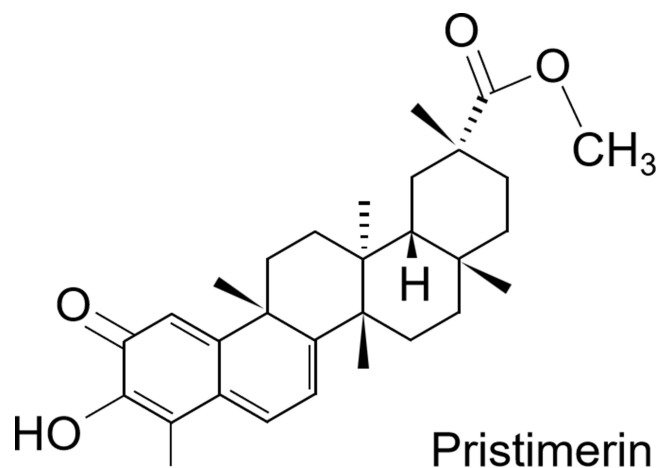
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Figure 1 Chemical structure of pristimerin. The structure of pristimerin includes (20a)-3-hydroxy-2-oxo-24-nor-friedela-1(10),3,5,7tetraen-carboxylic acid-(29)-methylester, molecular formula: $C_{30}H_{40}O_4$.

including anti-inflammatory, insecticidal, antibacterial, and antiviral effects and has been used for the treatment of osteoporosis and for cardioprotection, anti-tumor, and other biological effects. In addition, Pristimerin has significant potential for clinical application than other triterpenoids because it is a methyl ester of tretinoin, which not only acts similarly to tretinoin, but is also structurally stable, soluble, simple to obtain, has fewer toxic side effects, and inhibits tumor cell proliferation in a time- and dose-dependent manner. According to existing research, the pharmacological effects of pristimerin involve a variety of important signaling pathways such as NF-κB, PI3K/Akt/mTOR, MAPKs, ROS, and Wnt/β-catenin and are closely related to the cell cycle, apoptosis and autophagy, and cell metastasis. However, there is still a lack of systematic summarization of the molecular mechanisms by which pristimerin acts in different pathways. In this paper, we will comprehensively describe the pharmacological effects of pristimerin and the molecular mechanisms through which various signaling pathways exert their pharmacological effects so as to provide valuable references for further expanding research into the potential functions and clinical applications of pristimerin.

Pristimerin Exerts Pharmacological Effects by Blocking the Cell Cycle

The cell cycle refers to the process of cell growth and division and consists of four main phases: the G_1 phase, S phase, G_2 phase, and M phase. Among them, the G_1 phase is the preliminary stage of DNA synthesis and determines whether or not the cell can enter into the S phase. If the cell stops in the G_1 phase, ie, when it is in a dormant state, it is called the G_0 phase. The S phase is the synthesis of DNA, where the cell carries out DNA replication. The G_2 phase is the preparatory stage when the cell is ready to enter into the dividing phase.[2] The M phase is also known as mitosis, wherein the cell carries out nuclear and cytoplasmic division.[3]

In tumor cells, the cell cycle is disturbed. Some tumor cells may enter into a state of endless proliferation, leading to the continuous enlargement of the tumor. Therefore, many anticancer drugs inhibit the proliferation of tumor cells by interfering with the tumor cell cycle.[4] Pristimerin has been reported to exert antiproliferative effects by inducing tumor cells to arrest in the G_1 phase. Pristimerin inhibits the proliferation of breast cancer cells,[5] CML cells,[6] cholangiocarcinoma cells,[7] and colorectal cancer (CRC) cells,[8] mainly by inhibiting the expression levels of cyclinD1 and cyclin-dependent kinase (CDK) 4 and 6. Goel et al found that pristimerin induced cell cycle arrest by reducing CDK4/6 activity through inhibition of retinoblastoma (Rb) protein phosphorylation.[9] Yousef et al. Pristimerin (0.5, 1, 2, and 4 μM) reduced p-Rb expression levels in a dose-dependent manner, while total Rb protein expression remained unchanged under time-constant (48 h) conditions.[8] Coqueret et al found that p21 and p27 could interact with the cell cycle and could be associated with cell cycle arrest. p21 and p27 can bind to the cyclin-CDK complex, inhibit its catalytic activity, and induce cell cycle block.[10] p21 is an important member of the cell cycle protein-dependent kinase inhibitor family,[11] and pristimerin promotes the expression of p21 by activating p53, blocking the cell cycle, and inhibiting the growth of tumor cells.[12] Pristimerin can act as another cell cycle protein-dependent kinase inhibitor. Pristimerin inhibits tumor cell

proliferation in oral squamous cell carcinoma (OSCC),[13] prostate cancer,[14] and pancreatic cancer by up-regulating the expression levels of p53, p21, and p27.[15] In other words, pristimerin exerts its pharmacological effects on the cell cycle mainly by inhibiting the expression of cyclinD1 and CDK2/4/6, upregulating the expression of p53, p21, and p27, and inducing cell cycle arrest to inhibit tumor growth (Table 1 and Figure 2).

Pristimerin Exerts Pharmacological Effects by Inducing Apoptosis

Apoptosis, also known as programmed cell death, refers to the autonomous and orderly death of cells through genetic control in order to maintain the stability of the internal environment. Inducing apoptosis is an important method of inhibiting tumor growth, proliferation, and migration. Studies have shown that pristimerin can act in combination with other drugs to induce apoptosis in tumor cells, such as enhancing the chemosensitivity of gemcitabine to induce apoptosis in pancreatic cancer cells or working with paclitaxel to promote apoptosis in cervical cancer cells.[15,16] Costa et al found that pristimerin inhibited DNA synthesis in a dose-dependent manner to promote acute promyelocytic leukemia cell (HL-60) apoptosis.[18] Apoptosis involves two important pathways: the endogenous and exogenous pathways. The endogenous pathway (ie, DNA damage) leads to the release of apoptotic factor cytochrome C from the mitochondria, which binds to APAF-1 and caspase-9 to form an apoptotic complex, activates caspase-9, and heterogeneously activates caspase-3 zymogen, which cleaves substrates. Pristimerin induces apoptosis through the endogenous pathway in breast cancer cells,[19] cervical cancer cells,[17] pancreatic cancer cells,[20] and prostate cancer cells.[21] The exogenous pathway (ie, death receptor-mediated apoptosis) is the binding of a death ligand to the receptor. Tumor necrosis factor apoptosis-associated ligand (FASL), which is a member of the TNF family, binds to FAS on the cell membrane, recruits the connexin protein FADD through the death structural domain DD, and binds to caspase-8 to form a death-inducing signaling complex, which, upon activation of the caspase-8 zymogen, cleaves caspase-3 and further causes apoptosis. Related results showed that pristimerin promoted apoptosis in breast cancer cells and pancreatic cancer cells through an exogenous pathway.[19,20] In addition, Bcl family proteins play an important role in apoptosis,[50] including pro-apoptotic proteins (eg, Bax, Bak, and Noxa) and anti-apoptotic proteins (eg, BCL-2, Bcl-xL, and Mcl-1).[51,52] Pristimerin up-regulated the expression level of pro-apoptotic protein Bax in cervical cancer cells and down-regulated the expression levels of Bcl-2 and Bcl-xL in pancreatic cancer cells,[17,20] ovarian cancer cells,[23] prostate cancer cells,[22] and fibrosarcoma cells to promote cell apoptosis.[24] Zhao et al found that pristimerin increased apoptosis in CRC cells by activating the interaction between Noxa and Mcl-1.[25] Overall, pristimerin induces apoptosis by mediating the mitochondrial pathway and death receptor pathway and by regulating Bcl family proteins, enhancing the expression of pro-apoptotic proteins, and down-regulating the expression of anti-apoptotic proteins (Table 1 and Figure 2).

Pristimerin Exerts Pharmacological Effects by Inducing Cellular Autophagy

Cellular autophagy is part of the process of cellular self-renewal, which maintains cell morphology and function and promotes cell metabolism for normal cell growth. Induction of cellular autophagy plays a key role in promoting cell death.[53] It was shown that pristimerin activated autophagy in breast cancer cells MDA-MB-231 and MCF-7 by enhancing LC3-II expression and increasing the LC3-II/LC3-I ratio.[26] The experimental results also showed that autophagy inhibitors can inhibit Pristimerin-induced cell viability and apoptosis, ie, they can exert anti-tumor effects through autophagy.[26] In addition, pristimerin can synergize with paclitaxel to induce autophagy by inhibiting the ERK1/2 signaling pathway, increasing p62 degradation and beclin1 expression.[27] Huang et al demonstrated that, in esophageal cancer Eca109 and Ec9706 cells, pristimerin increased the ratio of LC3-II/LC3-I and induced cellular autophagy.[28] Jiang et al found that pristimerin alleviated tendinopathy by promoting autophagy and regulating the stability of AIM2-PYCARD/ASC.[29] However, the opposite situation also exists; pristimerin inhibited autophagy, down-regulated the expression levels of LC3-II and beclin1, and enhanced the sensitivity of A549 and NCI-H446 cells to cisplatin (Table 1 and Figure 2).[30]

Pristimerin Exerts Pharmacological Effects by Inhibiting Cell Migration, Invasion, and Angiogenesis

Migration and invasion are the main processes of tumor cell metastasis. It has been reported that after transfection of RGS4 with specific siRNA, the level of RGS4 was significantly reduced and the inhibitory effect of pristimerin on

Table 1 Anti-Cancer Activities of Pristimerin in Various Cancer Cell Lines and Models

Action Pathway	Disease	Cells	Animal Model	Action Mechanism	Reference
Cell cycle	Breast cancer	MDA-MB-231 and MDA-MB-468	MDA-MB-231 tumor xenografts in nude mice	Induced apoptosis and autophagy via activation of ROS/ASK1/JNK pathway	[5]
	Leukemia	K562 (CML)	——	Induced autophagy-mediated cell death through the ROS/JNK signaling pathway	[6]
	Cholangiocarcinoma	QBC and RBE	Tumor xenograft model	Lowered the expression of apoptosis related proteins (Bcl-2, Bcl-xL, and procaspase-3) but increased the Bax expression; Resulted in G0/G1 cell cycle arrest, reducing the expression of cell-cycle-related proteins (cyclinE, CDK2, and CDK4), and increased the expression of autophagy related proteins (LC3)	[7]
	Colorectal cancer	HCT-116	Human colorectal cancer xenograft model	Downregulated PI3K/Akt/mTOR pathway and its subsequent downstream p70S6K and E4-BP1 proteins	[8]
	Oral squamous cell carcinoma	CAL-27 and SCC-25	——	Induced apoptosis via G1 phase arrest and MAPK/Erk1/2 and Akt signaling inhibition	[13]
	Prostate cancer	LNCaP and PC-3	——	Induced apoptosis through ubiquitin-proteasomal degradation of antiapoptotic survivin	[14]
	Pancreatic cancer	PANC-1, BxPC-3, and AsPC-1	——	Caused G_1 arrest, induced apoptosis, and enhanced the chemosensitivity to Gemcitabine; Inhibited the translocation and DNA-binding activity of NF-κB	[15]

Apoptosis	Pancreatic cancer	PANC-1, BxPC-3, and AsPC-1	——	Caused G_1 arrest, induced apoptosis, and enhanced the chemosensitivity to Gemcitabine; Inhibited the translocation and DNA-binding activity of NF-κB	[15]
	Cervical Cancer	HeLa	Tumor xenografts in nude mice	Synergized with taxol to induce cell death by increasing intracellular ROS levels, upregulating DR5, activating Bax, and dissipating mitochondrial membrane potential	[16]
		HeLa, CasKi, and SiHa	——	Induced mitochondrial cell death by ROS-dependent activation of Bax and poly(ADP-ribose) polymerase-1	[17]
	Leukemia	HL-60	——	Inhibited DNA synthesis	[18]
	Breast cancer	MDA-MB-231	——	Resulted in a rapid release of cytochrome c from mitochondria, which preceded caspase activation, and the decrease of mitochondrial membrane potential but did not significantly alter the protein level of Bcl-2 family members or induce Bax translocation	[19]
	Pancreatic cancer	MiaPaCa-2 and Panc-1 (PDA)	——	Induced apoptosis through the inhibition of pro-survival Akt/NF-κB/mTOR signaling proteins and anti-apoptotic Bcl-2	[20]
	Prostate cancer	PC-3, LNCaP, and C4-2B	——	Induced apoptosis by targeting the proteasome and inhibited proteasomal chymotrypsin-like activity	[21]
		LNCaP and PC-3	——	Induced apoptosis in prostate cancer cells by down-regulating Bcl-2 through the ROS-dependent ubiquitin-proteasomal degradation pathway	[22]
	Ovarian carcinoma	OVCAR-5, MDAH-2774, OVCAR-3, and SK-OV-3	——	Inhibited Akt/NF-κ B/mTOR signaling pathway; Inhibited the expression of NF-kB-regulated anti-apoptotic Bcl-2, Bcl-xL, C-IAP1, and survivin	[23]
	Fibrosarcoma	HT1080	Mice with subcutaneous grafts comprising human fibrosarcoma cells	Inhibited cell and tumor proliferation by inhibiting Akt and MAPK signaling	[24]
	Colorectal cancer	HCT-116	Tumor xenografts in nude mice	Induced apoptosis through activation of ROS/ER stress-mediated noxa and elevated the expression of ER stress-related proteins, resulting in the activation of the IRE1a and JNK signal pathway through the formation of the IRE1a-TRAF2-ASK1 complex	[25]

(*Continued*)

Table 1 (Continued).

Action Pathway	Disease	Cells	Animal Model	Action Mechanism	Reference
Autophagy	Breast cancer	MCF-7 and MDA-MB-231	Human breast cancer xenograft model	Induced apoptosis and an incomplete autophagy	[26]
		MDA-MB-231	——	Combination of pristimerin and paclitaxel additively induced autophagy in human breast cancer cells via ERK1/2 regulation	[27]
	Esophageal cancer	Eca109 and Ec9706	Tumor xenograft mode	Decreased the protein expression of CDK2, CDK4, cyclin E, and Bcl-2 and increased the expression of CDKN1B; Elevated the ratio of LC3-II/LC3-I	[28]
	Tendinopathy	——	——	Degraded SQSTM1/p62-mediated selective autophagy by modulating AIM2-PYCARD/ASC stability	[29]
	Lung cancer	A549 and NCI−H446	Human lung tumors xenograft model	Enhanced the effect of cisplatin by inhibiting the miR−23a/Akt/GSK3β signaling pathway and suppressing autophagy	[30]
Cell migration, invasion, and angiogenesis	Breast cancer	MDA-MB-231	Human breast cancer xenograft model	Inhibited tumor migration and invasion by inhibiting proteasomal activity and increasing the levels of RGS4	[31]
			——	Inhibited cancer progression and EMT reversion by suppression of integrin β3	[32]
		SKBR3	——	Decreased HER2 expression, decrease fatty acid synthase and inhibited the Akt, MAPK, and mTOR signaling pathways to affect metastasis and apoptosis	[33]
	Prostate cancer	PC-3	——	Inhibited the hypoxia-induced proliferation, invasion, spheroid formation, colony formation, stem cell characteristics, and EMT protein expression	[34]
			Intra-tibial injection mouse model	Inhibited bone metastasis by targeting PC-3 stem cell characteristics and VEGF-induced angiogenesis; Inhibited the bone destruction by the invasion of the tumor and reduced the tumorigenic potential of bone metastasis	[35]
			——	Inhibited HIF-1a through the SPHK-1 pathway	[36]
	Lung cancer	NCI-H1299 (NSCLC)	——	Inhibited angiogenesis targeting the Shh/Gli1 signaling pathway	[37]

NF-κB signaling pathway	Pancreatic cancer	AsPC-1, BxPC-3, and PANC-1	——	Caused G_1 arrest, induced apoptosis, and enhanced the chemosensitivity to Gemcitabine; Inhibited the translocation and DNA-binding activity of NF-κB	[15]
	Leukemia	KBM5 and KBM5-T315I	Imatinib-resistant Bcr-Abl-T315I xenografts in nude mice	Blocked the TNF-α-induced IkBa phosphorylation, translocation of p65, and expression of NF-κB regulated genes; Inhibited the expression of Bcr-Abl	[38]
	Esophageal squamous cell carcinoma	EC9706, EC109, and KYSE30	Human ESCC xenograft model	Inhibited proliferation, migration, and invasion via suppressing the NF-κB pathway	[39]
	Colorectal cancer	HCT-116	Human colorectal cancer xenograft model	Inhibited the NF-κB signaling pathway	[40]
	Glioma	U373	——	Targeted AGO2 and PTPN1 expression via miR-542-5p	[41]
	Chronic obstructive pulmonary disease	——	CS-induced COPD mice model and cell model	Alleviated CS-induced COPD by inhibiting the NF-κB pathway	[42]
PI3K/Akt/mTOR signaling pathway	Colorectal cancer	HCT-116	Human colorectal cancer xenograft model	Downregulated the PI3K/Akt/mTOR pathway and its subsequent downstream p70S6K and E4-BP1 proteins; Inhibited tumor growth and induced apoptosis	[8]
	Breast cancer	SKBR3	——	Decreased HER2 expression, decrease fatty acid synthase and inhibited the Akt, MAPK, and mTOR signaling pathways to affect metastasis and apoptosis	[33]
		HUVECs	Human breast cancer xenograft model	Reduced tumor volume and weight and inhibited tumor growth and angiogenesis associated with downregulation of VEGF	[43]
	Osteosarcoma	MNNG (CRL1547) and 43B (CRL1427)	Human osteosarcoma xenograft model	Downregulated the levels of Akt, mTOR, and NF-κB	[44]
	Uveal melanoma	RGC-5 and D407	——	Induced apoptotic cell death through inhibition of PI3K/Akt/FoxO3a pathway	[45]
	Sepsis	——	C57BL/6 J mice model with sepsis-induced brain injuries	Ameliorated neuronal injury by regulating PI3K/Akt signaling in mice with sepsis-induced brain injuries	[46]

(*Continued*)

Table 1 (Continued).

| Action Pathway	Disease	Cells	Animal Model	Action Mechanism	Reference
MAPKs signaling pathway	Oral squamous cell carcinoma	CAL-27 and SCC-25	——	Induced apoptosis via G1 phase arrest and MAPK/Erk1/2 and Akt signaling inhibition	[13]
	Breast cancer	MDA-MB-231	——	Combination of pristimerin and paclitaxel additively induced autophagy in human breast cancer cells via ERK1/2 regulation	[27]
		HUVECs	Human breast cancer xenograft model	Reduced tumor volume and weight and inhibited tumor growth and angiogenesis associated with downregulation of VEGF	[43]
	Cutaneous squamous cell carcinoma	A431 and A388	——	Inhibited growth and proliferation of cSCC through JNK activation	[47]
ROS	Breast cancer	MDA-MB-231 and MDA-MB-468	MDA-MB-231 tumor xenografts in nude mice	Induced apoptosis and autophagy via activation of the ROS/ASK1/JNK pathway	[5]
	Colorectal cancer	HCT-116	Human colorectal cancer xenograft model	Downregulated the PI3K/Akt/mTOR pathway and its subsequent downstream p70S6K and E4-BP1 proteins; Inhibited tumor growth and induced apoptosis	[8]
			Tumor xenograft in nude mice	Induced apoptosis through activation of ROS/ER stress-mediated noxa and elevated the expression of ER stress-related proteins, resulting in activation of the IRE1α and JNK signaling pathway through the formation of the IRE1a-TRAF2-ASK1 complex	[25]
	Cervical Cancer	HeLa, CasKi, and SiHa	——	Induced mitochondrial cell death by ROS-dependent activation of Bax and poly(ADP-ribose) polymerase-1	[17]
	Uveal melanoma	RGC-5 and D407	——	Induced apoptotic cell death through inhibition of the PI3K/Akt/FoxO3a pathway	[45]
	Glioma	U87	Human glioma xenograft model	Triggered AIF-dependent programmed necrosis in glioma cells via activation of JNK	[48]
	Hepatocellular carcinoma	HepG2	——	Generated ROS, induced release of cytochrome c, and down-regulated EGFR protein	[49]
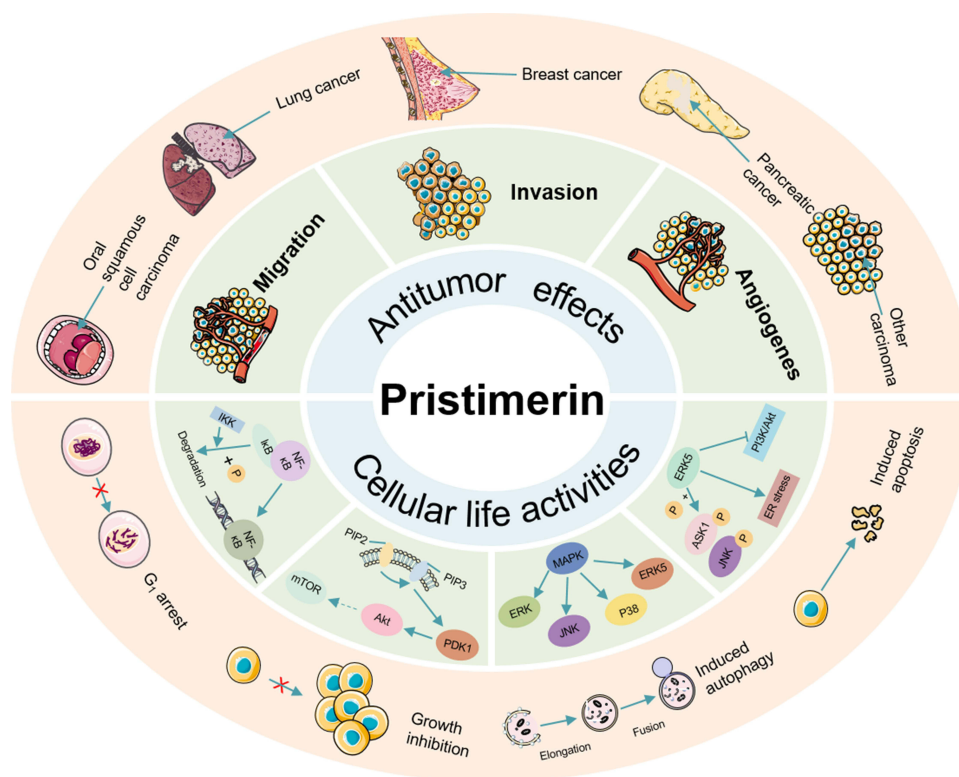
Figure 2 Antitumor effects of Pristimerin and effects on cellular life activities.Pristimerin has antitumor effects, such as oral squamous cell carcinoma, lung cancer, breast cancer, pancreatic cancer, and other carcinoma.Pristimerin affects cellular life activities by blocking the cell cycle, inducing apoptosis, inducing cellular autophagy, and inhibiting cell migration, invasion, and angiogenesis. Pristimerin can mediate NF-κB signaling pathway, PI3K/Akt/mTOR signaling pathway and MAPKs' signaling pathway to exert antitumor effects.

Abbreviations: Iκκ, inhibitor of kappa B kinase; IκB, inhibitor of kappa B; NF-κB, nuclear factor kappa-B; Akt, protein kinase B; mTOR, mammalian target of rapamycin; PDK1, pyruvate dehydrogenase kinase 1; PIP2, phosphatidylinositol-4,5-bisphosphate; PIP3, phosphatidylinositol-3,4,5-triphosphate; MAPK, mitogen activated protein kinase; ERK, extracellular signal-regulated kinase; JNK, c-JUN N-terminal kinase; ASK1, recombinant apoptosis signal regulating kinase 1; p38, p38 MAP kinase.

migration and invasion of breast cancer cells was significantly attenuated. Therefore, it is important for pristimerin to increase the expression of RGS4. Pristimerin inhibits the migration and invasion of breast cancer cells MDA-MB-231 by inhibiting proteasomal activity and up-regulating the expression level of regulator of G protein signaling 4 (RGS4) and to inhibit the migration and invasion of SKBR3 in breast cancer cells by targeting epidermal growth factor receptor 2 (HER2).[31,33] In prostate cancer (PCa) PC-3 cells, pristimerin reversed the epithelial–mesenchymal transition (EMT) in stem cells and cancer cells and inhibited tumor cell metastasis and invasion under hypoxia, as evidenced by changes in the expression of EMT-associated markers including N-calmodulin, fibronectin, waveform protein, and ZEB1.[34] Furthermore, knockdown of integrinβ3 using siRNA significantly increased the expression of E-calmodulin and decreased the expression of N-calmodulin, confirming that integrinβ3 is involved in the EMT of MDA-MB-231. Pristimerin reversed EMT and inhibited metastasis of MDA-MB-231 in triple-negative breast cancer cells by down-regulating integrinβ3.[32] The role of angiogenesis in tumor growth is equally important. Lei et al demonstrated that pristimerin inhibits angiogenesis in non-small cell lung cancers (NSCLCs) by targeting the Shh/Gli1 signaling pathway.[37] They also found that pristimerin inhibited Shh-induced endothelial cell proliferation, migration, invasion, and pericyte recruitment to endothelial tubules, inhibited chorioallantoic membrane (CAM) formation in chick embryos, and reduced microvessel density (MVD) and pericyte coverage in NCI-H1299 xenografts. Pristimerin inhibited the growth of NSCLC cells by targeting PC-3 stem cell properties and vascular endothelial growth factor (VEGF)-induced angiogenesis in bone marrow-derived endothelial pre-germ cells (BM-EPCs) to inhibit prostate cancer bone metastasis.[35] In hypoxic prostate cancer PC-3 cells, based on the experimental results Pristimerin inhibited the phosphorylation of Akt and GSK-3β, the SPHK-1 inhibitor SKI blocked the expression of HIF-1α and the phosphorylation of Akt and GSK-3β, pristimerin and SKI inhibited the activity of SPHK-1, and the use of siRNA transfection of SPHK-1 inhibited pristimerin-mediated

inhibition of SPHK-1 after transfection with siRNA, all of which confirmed the involvement of SPHK-1 in pristimerin-mediated inhibition of HIF-1α in hypoxia.Pristimerin reduces VEGF production through inhibition of the sphingosine kinase 1 (SPHK-1) pathway and suppression of hypoxia-inducible factor 1 alpha (HIF-1α) expression (Table 1 and Figure 2).[36]

Pristimerin Exerts Pharmacological Effects Through the NF-κB Signaling Pathway

The NF-κB family of transcription factors plays a key role in cell survival and inflammation,[54] including C-REL, RELB, RelA/p65, NF-κB1 (p50/p105), and NF-κB2 (p52/p100). The NF-κB signaling pathway is involved in a variety of biological processes and is closely related to apoptosis and tumorigenesis.[55] Unactivated NF-κB binds to its inhibitor IκB, forming a complex in the form of a homo- or heterodimer and is inactivated in the cytoplasm.[56] When the cell is stimulated or stressed, IκB kinase (IKK) is activated, promoting the phosphorylation of IκB with its own ubiquitination, rapid degradation, and release by proteolytic enzymes, thus releasing the inhibitory effect of IκB. Mediated by nuclear localization signals, NF-κB binds to specific DNA, promotes transcription and protein synthesis of downstream target genes, and participates in various biological processes.[55,56] In pancreatic cancer cells, a pristimerin-induced G1 phase block was also associated with the NF-κB pathway, while inhibition of the NF-κB pathway enhanced its chemosensitivity to gemcitabine.[15] In chronic granulocytic leukemia (CML), after pristimerin treatment, imatinib pretreatment inactivated Bcr-Abl without eliminating TNFα-induced NF-κB activation, and transfection of p65 using specific siRNA did not affect the level of Bcr-Abl, which means that NF-κB inactivation and Bcr-Abl inhibition may be a parallel mechanism of pristimerin-induced CML cell activity.[38] Pristimerin significantly inhibited TNFα-induced downstream targets of the NF-κB pathway, including MMP9, cytokinin D1, and c-myc, inhibiting ESCC cell growth. It was also found that BIM played an important role in pristimerin-induced apoptosis in ESCC cells, and ESCC cells transfected with BIM were more sensitive to pristimerin, whereas knockdown of BIM using siRNA resulted in ESCC cells that were much less sensitive to pristimerin.[39] In CRC, pristimerin reversed IKK activation, phosphorylation, and dephosphorylation of IκBα and p65, inhibited the activation of the NF-κB pathway, and exhibited anticancer activity.[40] In gliomas, it was found that transfection of miR-542-5p inhibitor followed by siRNA resulted in higher expression levels of AGO2 and lower PTPN1; treatment with pristimerin transfected with miR-542-5p inhibitor significantly enhanced the expression of AGO2 and decreased the expression of PTPN1, implying that pristimerin and miR-542-5p silencing had a synergistic effect in glioma cells. That is, pristimerin targets the expression of protein tyrosine phosphatase non-receptor type 1 (PTPN1) via miR-542-5p, a gene associated with the NF-κB pathway and encoding PTP1B, the activation of which mediates the expression of PTP1B.[41] Furthermore, pristimerin attenuates smoking (CS)-induced chronic obstructive pulmonary disease (COPD) by inactivating the NF-κB pathway (Table 1).[42]

Pristimerin Exerts Pharmacological Effects Through the PI3K/Akt/mTOR Signaling Pathway

PI3K is a heterodimeric lipid kinase consisting of a catalytic subunit (p110α, p110β, or p110δ, encoded by the PIK3CA, PIK3CB, and PIK3CD genes, respectively) and a regulatory subunit (p85).[57] Akt is a key mediator of downstream signaling of PI3K and plays a central role in a wide range of cellular processes essential for cell growth, metabolism, and survival.[58] mTOR is a key kinase downstream of PI3K/Akt, is a member of the PI3K-associated kinase (PIKK) protein family, and includes two complexes, mTORC1 and mTORC2. mTOR has important functions in the regulation of cell growth, proliferation, motility, survival, protein synthesis, and transcription.[59] Phosphorylation of PI3K activates Akt, which regulates several downstream molecules, including mTOR.[60] The PI3K/Akt/mTOR pathway plays an important role in many aspects of cell growth and survival, is frequently over-activated or altered in many types of human cancers, and may be a valuable target for disease treatment.[61] In colorectal cancer, the inhibitory effect of pristimerin on P70S6K/4EBP1 inhibited the metastasis and invasion of cancer cells.[8] Moreover, related studies confirmed that in SKBR3 breast cancer cells, pristimerin inhibited the phosphorylation of mTOR, phosphoprotein 70 ribosomal protein S6 kinase (p70S6K), and eukaryotic initiation factor 4E-binding protein 1 (4EBP1) and also inhibited the expression of FASN through the PI3K/Akt/mTOR pathway.[33] Mori et al found that pristimerin exerts an antiproliferative effect by inducing

apoptosis in osteosarcoma cells through the PI3K/AKT/mTOR pathway.[44] Yan et al found that pristimerin induced apoptotic Uveal melanoma cell death by inhibiting the PI3K/Akt/FoxO3a pathway, enhancing the expression of pro-apoptotic molecules BIM, p27Kip1, cleaved cystatinase-3, PARP, and Bax, and inhibiting anti-apoptotic proteins cytosolic protein D1 and Bcl-2.The experimental results also showed that knockdown of FoxO3a using the siRNA approach resulted in partial elimination of the Pristimerin effect in FoxO3a-knockdown UM cells, demonstrating the partial attenuation of Pristimerin-induced apoptosis in UM cells after knockdown of FoxO3a.[45] Mu et al found that pristimerin affected VEGF-induced angiogenesis by inhibiting PI3K, Akt, mTOR, and ERK1/2.[43] In addition to these results, pristimerin, also through the PI3K/Akt signaling pathway, ameliorated neuronal inflammation and protected cognitive function in mice with sepsis-induced brain damage (Table 1).[46]

Pristimerin Exerts Pharmacological Effects Through MAPKs' Signaling Pathway

MAPKs mediate important signaling pathway cascades from extracellular signals to intracellular responses in eukaryotic cells, connecting with cell surface receptors and extracellular signals to regulate biological processes such as cell proliferation, differentiation, and apoptosis.[62] There are four distinct cascades: the extracellular signal-regulated protein kinase 1/2 (ERK1/2), Jun amino-terminal kinase (JNK1/2/3), p38 MAPK, and ERK5 signaling pathways.[63] Wu et al demonstrated that pristimerin induced apoptosis in OSCC cells via MAPK and ERK1/2.[13] It was shown that pristimerin synergizes with paclitaxel to induce autophagy in breast cancer cells by regulating ERK1/2 and inhibits VEGF-induced capillary angiogenesis in human umbilical vascular endothelial cells (HUVECs) via ERK1/2.[27,43] Moreover, it was recently found that in cutaneous squamous cell carcinoma (cSCC), pristimerin mediates reactive oxygen species (ROS) activation of the JNK signaling pathway, enhances the sensitivity of skin cancer cells to conventional anticancer drugs, and induces cell death (Table 1).[47]

Pristimerin Exerts Its Pharmacological Effects by Affecting ROS Generation

Elevated intracellular ROS have been reported to induce cancer cell cycle arrest and apoptosis.[64] In breast cancer, inhibition of Trx-1 by pristimerin resulted in ROS accumulation, which then induced phosphorylation of ASK1 and JNK, leading to cell death.[5] Zhao et al found that pristimerin treatment generated excessive ROS, leading to increased mitochondrial permeability and decreased mitochondrial membrane potential, while triggering ER stress, resulting in the accumulation of unfolded proteins and inducing apoptosis in CRC cells.[25] Yan et al demonstrated that pristimerin promoted the accumulation of cells in the G_0/G_1 phase of the cell cycle by increasing ROS and decreasing mitochondrial membrane potential, inducing UM cell death.[45] Furthermore, the reduced glioma cell viability and increased necrosis induced by pristimerin were mostly reversed after AIF knockdown using siRNA, confirming that pristimerin-induced glioma cell necrosis is AIF-dependent. Thus, In addition, pristimerin activates JNK through overproduction of ROS and induces mitochondrial dysfunction, which promotes apoptosis in cervical cancer cells and triggers AIF-dependent necrosis in glioma cells.[17,48] Similarly, pristimerin can mediate ROS and induce apoptosis in HCT-116 cells and HepG2 cells (Table 1).[8,49]

Pristimerin Exerts Its Pharmacological Effects Through Other Pathways

In breast cancer MCF-7 cells, pristimerin induced apoptosis by inhibiting the Wnt/β-catenin signaling pathway through degradation of LRP6.[26] In NSCLC, pristimerin inhibited angiogenesis by inhibiting Shh/Gli1 and its downstream pathway, while eliminating Shh-induced pericyte recruitment to neovessels, thus attenuating the level of VEGFR2 phosphorylation and inhibiting tumor angiogenesis.[37] In HCT116 and HT-29 CRC cells, pristimerin down-regulated the expression of Wnt target genes, including c-Myc, cyclinD1, and cox-2, for an antiproliferative effect on tumor cells.[65] In addition, pristimerin can also regulate the levels of EMT-related proteins and miRNAs to inhibit the survival of NCI-H1299 in NSCLC cells.[66] Xie et al proposed that pristimerin exerts antiproliferative effects on UM cells through the IGF-1R/Akt/mTOR and ERK1/2 pathways. They also found that pristimerin could stimulate the expression of p21 and inhibit the expression of cyclin D1, inducing G_1 phase arrest.[67] Research also showed that pristimerin could be used in combination with nanoparticles to down-regulate MTDH to inhibit the FA pathway and enhance tumor sensitivity to platinum-based chemotherapeutic agents (Table 2).[68]

Table 2 Other Anti-Cancer Mechanisms of Pristimerin in Different Cell Lines

Disease	Cells	Action Mechanism	Reference
Breast cancer	MCF-7	Inhibited Wnt signaling and triggered incomplete autophagy in MCF-7s breast cancer cells	[26]
		Overcame Adriamycin resistance in breast cancer cells through suppressing Akt signaling	[69]
Colorectal cancer	HCT116 and HT-29	Inhibited inflammatory responses and Wnt/b-catenin signaling	[65]
Lung cancer	NSCLC	Inhibited angiogenesis targeting the Shh/Gli1 signaling pathway	[37]
		Decreased migration and invasion of H1299, which correlated with EMT-related proteins and mRNA	[66]
	Human lung cancer CRCs	Regulated the expression level of Notch signaling pathway-related proteins	[70]
	Lung tissue samples from patients	Exerted anti-cancer activities by aggravating mitochondrial impairment and ER stress through EphB4/CDC42/N-WASP signaling	[71]
Uveal melanoma	Human uveal melanoma cell lines	Inhibited cell proliferation through downregulating phosphorylated IGF-1R and its downstream signaling	[67]
Endometrial cancer	Patient-derived xenograft model of endometrial cancer	Increased sensitivity to platinum-based agents in tumors with MTDH overexpression by inhibiting the FA pathway	[68]
Prostate cancer	LNCaP and PC-3	Inhibited hTERT mRNA expression and native and phosphorylated hTERT protein and hTERT telomerase activity attributed to the inhibition of transcription factors SP1, c-Myc, and STAT3 and protein kinase B/Akt	[72]
Pancreatic cancer	MiaPaCa-2 and Panc-1 (PDA)	Inhibited hTERT expression by suppressing the transcription factors Sp1, c-Myc, and NF-kB, which control hTERT gene expression	[73]

In summary, pristimerin exerts its pharmacological effects by blocking the cell cycle, inducing apoptosis and autophagy, inhibiting cell migration, invasion and angiogenesis, and mediating multiple signaling pathways. Considering that pristimerin induces biological effects in a time- and concentration-dependent manner, the dose varies in different cell types and there are differences in in vivo and in vitro utilization. Therefore, we summarized the concentrations at which pristimerin induced biological effects in the above pathways (Table 3). In addition, different or common mechanisms exist in different cell types, eg, pristimerin can act in most cells through the NF-κB signaling pathway, Akt signaling pathway. However, there are still many unanswered questions about whether there are underlying mechanisms as well as specific mechanisms for pristimerin that are not yet known.

Antitumor Effects of Pristimerin

According to the above, pristimerin treats breast cancer by inducing cell cycle arrest at apoptosis and autophagy,[5,16,19] inhibiting metastasis,[31,33] reversing EMT,[32] and modulating signaling pathways.[5,26,27,33] However, the challenge of breast cancer treatment is still the resistance to chemotherapeutic drugs. Pristimerin can kill ADR-resistant MCF-7 cells by inhibiting Akt signaling, inhibiting BAD phosphorylation, and down-regulating the expression of the anti-apoptotic protein Bcl-xL.[69] It can also achieve therapeutic effects by killing breast cancer tumor stem cells and affecting the vitality of the tumor population.[26] In prostate cancer, in addition to interfering with the cell cycle,[14] mediating the mitochondrial pathway,[21] regulating Bcl family proteins,[22] and inhibiting angiogenesis,[35,36] it can also target telomerases. Pristimerin inhibited telomerase activity in LNCaP and PC-3 cells by inhibiting the expression of human telomerase reverse transcriptase (hTERT) and its mRNA encoding the catalytic subunit of the telomerase. Moreover, the expression of hTERT-regulated proteins c-Myc, Sp1, p-STAT3, and p-Akt was also inhibited.[72] The same effect is also reflected in pancreatic

Table 3 The Dosage of Pristimerin to Exert Antitumor Effects in vivo and in vitro

Cancer type	Time	Dose and administration (IC50 value or inhibition rate)	Mechanisms	References
Breast cancer	24h	0.6μM significantly caused MDA-MB-231 and MDA-MB-468 cells death	Induced apoptosis and autophagy via activation of ROS/ASK1/JNK pathway	[5]
	72h	0.42–0.61 μM IC50 against MDA-MB-231	Resulted in a rapid release of cytochrome c from mitochondria, which preceded caspase activation and the decrease of mitochondrial membrane potential but not significantly altered the protein level of Bcl-2 family members, nor did it induce Bax translocation	[19]
	2 days	1 mg/kg, i.p.	Induced apoptosis and an incomplete autophagy	[26]
	24h	10μM caused 40–60% MDA-MB-231 cell each	Combination of pristimerin and paclitaxel additively induced autophagy in human breast cancer cells via ERK1/2 regulation	[27]
	2h	0.125–1μM significantly reduced the relative proteasome activity in MDA-MB-231 cells	Inhibited tumor migration and invasion by inhibiting proteasomal activity and increasing the levels of RGS4	[31]
	24h	2.4 μM IC50 against SKBR3	Decreased HER2 expression, fatty acid synthase and inhibited the Akt, MAPK, and mTOR signaling pathways to affect metastasis and apoptosis	[33]
	48h	0.5 μM wholly decreased the MDA-MB-231 cell viability	Inhibited cancer progression and EMT reversion by suppression of integrin β3	[32]
	2 days	3 mg/kg, s.c.	Reduced tumor volume and weight, inhibited tumor growth and angiogenesis associated with downregulation of VEGF	[43]
Colorectal cancer	72h	1.11 μM IC50 against HCT-116	Downregulated PI3K/AKT/mTOR pathway and its subsequent downstream p70S6K and E4-BP1 proteins.Inhibited tumor growth and induced apoptosis	[8]
	24h	0.98 μM IC50 against HCT-116	Induced apoptosis through activation of ROS/ER stress-mediated noxa and elevated the expression of ER stress-related proteins, resulting in activation of the IRE1a and JNK signal pathway through the formation of the IRE1a-TRAF2-ASK1 complex	[25]
	48h	0.83 μM IC50 against HCT-116		
	2 days	1 mg/kg, i.p.	Inhibited NF-κB signaling pathway	[40]

(*Continued*)

Table 3 (Continued).

Cancer type	Time	Dose and administration (IC50 value or inhibition rate)	Mechanisms	References
Prostate cancer	72h	1.25–5μM caused 47–73% LNCaP and PC-3 cell death	Induced apoptosis through ubiquitin-proteasomal degradation of antiapoptotic survivin	[14]
	24h	24h 0.5–5μM caused 30–85% PC-3 cell death	Induced apoptosis by targeting the proteasome and inhibited the proteasomal chymotrypsin-like activity	[21]
	24h	5μM caused 40% C4-2B cell death		
	8h	5μM gradually decreased LNCaP cell to complete ablation		
	72h	1.25 μM caused 55% LNCaP cell death	Induced apoptosis in prostate cancer cells by down-regulating Bcl-2 through ROS-dependent ubiquitin-proteasomal degradation pathway.	[22]
		1.25 μM caused 47% PC-3 cell death		
	36h	0.4 μM can inhibit the proliferation of PC-3 cells and the effect was more marked at 0.8 μM	Inhibited the hypoxia-induced proliferation, invasion, spheroid formation, colony formation, stem cell characteristics and EMT protein expression	[34]
	24h	7.5×10^3 cells/μL 1.6 μM pre-treated PC-3 cells	Inhibited bone metastasis by targeting PC-3 stem cell characteristics and VEGF-induced angiogenesis. Inhibited the bone destruction by the invasion of the tumor and reduced the tumorigenic potential of bone metastasis.	[35]
	4h	1μM caused 55% SPHK-1 cell death	Inhibited HIF-1a through the SPHK-1 pathway.	[36]
Cholangiocarcinoma	——	The cell viability was lowest at 20μM	Lowered the expression of apoptosis related proteins (Bcl-2, Bcl-xL, and procaspase-3), but increased the Bax expression. Resulted in the G0/G1 cell cycle arrest, reducing the expression of cell cycle related proteins (cyclinE, CDK2, and CDK4), and increased the expression of autophagy related proteins (LC3)	[7]
OSCC	72h	0.70 μM IC50 against CAL-27	Induced apoptosis via G1 phase arrest and MAPK/Erk1/2 and Akt signaling inhibition	[13]
		0.73 μM IC50 against SCC-25		
Hepatocellular carcinoma	72h	1.44 μM IC50 against HepG2	Generated ROS, induced release of cytochrome c, and down-regulated EGFR protein	[49]

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Pancreatic cancer	24h	0.65 μM, 0.97 μM, 1.26 μM, IC50 against BxPC-3, PANC-1, and AsPC-1, respectively	Caused G1 arrest, induces apoptosis, and enhances the chemosensitivity to Gemcitabine.Inhibited translocation and DNA-binding activity of NF-kB	[15]
	48h	0.28 μM, 0.34 μM, and 0.38 μM IC50 against BxPC-3, PANC-1, and AsPC-1, respectively		
	72h	0.19 μM, 0.26 μM and 0.30 μM IC50 against BxPC-3, PANC-1, and AsPC-1, respectively		
	72h	0.625~5 μM caused 52~85% MiaPaCa-2 cell death	Induced apoptosis through the inhibition of pro-survival Akt/NF-kB/mTOR signaling proteins and anti-apoptotic Bcl-2	[20]
	72h	0.625~5 μM caused 13~81% Panc-1 cell death		
Cervical Cancer	72h	0.85–1.7 μM IC50 against HeLa, CasKi, and SiHa	Induced Mitochondrial Cell Death by ROS-dependent activation of Bax and Poly (ADP-ribose) Polymerase-1	[17]
Ovarian carcinoma	72h	1.25 μM caused 44% OVCAR-5 cell death 1.25 μM caused 28% MDAH-2774 cell death 2.5 μM caused 27% OVCAR-3 cell death 2.5 μM caused 36% SK-OV-3 cell death	Inhibited AKT/NF-k B/mTOR signaling pathway.Inhibited the expression of NF-kB-regulated antiapoptotic Bcl-2, Bcl-xL, C-IAP1 and survivin.	[23]
Leukemia	72h	1.49 μM IC50 against K562	Induced autophagy-mediated cell death through the ROS/JNK signaling pathway	[6]
	72h	0.61 μM IC50 against HL-60	Inhibited DNA synthesis.	[18]
ESCC	2 days	1 mg/kg, i.t.	Inhibited proliferation, migration, and invasion via suppressing NF-kB pathway	[39]
Esophageal cancer	72h	1.98 μM IC50 against EC9706	Decreased the protein expression of CDK2, CDK4, cyclin E, and Bcl-2 and increased the expression of CDKN1B Elevated the ratio of LC3-II/LC3-I	[28]
	72h	1.76 μM IC50 against EC109		
Lung cancer	——	0.8 mg/kg pristimerin and 2 mg/kg cisplatin, s.c.	Enhanced the effect of cisplatin by inhibiting the miR−23a/Akt/GSK3β signaling pathway and suppressing autophagy	[30]
	16 days	0.2mg/kg pristimerin and 0.4mg/kg pristimerin, s.c.	Inhibited angiogenesis targeting Shh/Gli1 signaling pathway	[37]
Glioma	——	2μM remarkably inhibited U373	Targeting AGO2 and PTPN1 expression via miR-542-5p	[41]
	6h	5.0 μM IC50 against U87	Triggered AIF-dependent programmed necrosis in glioma cells via activation of JNK.	[48]
		4.5 μM IC50 against U251		
	2 days	1 and 3 mg/kg, s.c.		
Uveal melanoma	24h	30μM, only 20% toxicity	Induced apoptotic cell death through inhibition of PI3K/Akt/FoxO3a pathway.	[45]

cancer.[73] The treatment of pancreatic cancer involves cell cycle arrest and apoptosis and the NF-κB signaling pathway.[15,20] In glioma, in addition to mediating the NF-κB pathway,[41] pristimerin can also reduce the expression of miR-542-5p, increase the expression of AGO2, inhibit the expression of PTPN1, and realize its anti-proliferative effect.[41] In NSCLC, pristimerin not only promotes apoptosis by regulating the expression levels of Notch signaling pathway-related proteins Notch1, HES1, and CyclinD3,[70] but also promotes apoptosis through the tyrosine protein kinase receptor B4 (EphB4)/ cellular visual cycling protein (CDC42)/Neural Wiskott-Aldrich Syndrome Protein (N-WASP) signaling pathway, which exacerbates cellular damage in lung adenocarcinoma of human origin through mitochondrial damage and endoplasmic reticulum stress. The experimental results show that siRNA knockout of EphB4 enhanced ROS production and MMP loss in pristimerin-induced CRLCs, suggesting that EphB4 is involved in pristimerin-induced mitochondrial dysfunction and endoplasmic reticulum (ER) stress.[71] In ovarian cancer, pristimerin exerts potent anti-proliferative and apoptosis-inducing effects by regulating the expression of anti-regulatory proteins and inhibiting the Akt/NF-κB/mTOR signaling pathway.[23] In CRC, pristimerin exerts anti-tumor effects by blocking the cell cycle, mediating the NF-κB/ROS pathway, activating Noxa, and inhibiting P70S6K/4EBP1.[8,25,40] In melanoma, pristimerin inhibits tumor growth via PI3K/Akt/mTOR/ROS/IGF-1R.[45,67] In addition, pristimerin has been shown to be effective in treating cholangiocarcinoma,[7] Esophageal cancer,[28,39] OSCC,[13] and chronic granulocytic leukemia (Table 1 and Table 2, Figure 2).[6,38]

Other Pharmacological Effects of Pristimerin

Pristimerin has anti-inflammatory effects, reducing DSS-induced colitis in mice by inhibiting the expression of miRNA-155,[74] regulating the ratio of Th17/Treg cells in peripheral blood of AA model rats, and reducing the secretion of pro-inflammatory cytokines interleukin (IL)-6, IL-17, IL-18 and IL-23. Pristimerin has also been shown to upregulate the expression of inhibitors IL-10 and INF-γ, inhibiting RA synovial inflammation and the TNF-α-induced endothelial adhesion molecule ICAM-1. This inhibits RA synovial inflammation and secretion and up-regulates the expression of the anti-inflammatory factors IL-10 and INF-γ to inhibit RA synovial inflammation.[75] Pristimerin also inhibits the expression of endothelial adhesion molecules ICAM-1 and VCAM-1 and pro-inflammatory cytokines IL-6, IL-8, and MCP-1 induced by TNF-α-induced inhibition and acts in conjunction with the PD-L1+ exosomes to alleviate the iron-toxicity-related changes in psoriatic skin, thereby inhibiting excessive inflammation.[76,77]

Pristimerin has insecticidal properties, killing Leishmania protozoa and Trypanosoma cruzi and acting as an effective drug for Leishmaniasis and Chagas disease.[78] Pristimerin has antimicrobial properties as well, acting by altering the permeability of the plasma membranes of Staphylococcus epidermidis and Staphylococcus aureus.[79,80] Pristimerin also has an antiviral effect and can inhibit the synthesis of human cytomegalovirus DNA, but it has no virucidal effect on cell-free HCMV.[81] Pristimerin has a therapeutic effect on osteoporosis as well; it can inhibit the activation of the RANKL-induced NF-κB, MAPK, ERK, JNK, and PI3K-Akt signaling pathways, and it blocks the expression of the downstream transcription factors c-Fos and NFATc1 and the expression of NFATc1 and NFATc1. NFATc1 expression inhibited osteoclastogenesis and ameliorated ovariectomy (OVX)-induced bone loss.[82–84] It also restored the normal level of TRAF-6, reduced osteoclast activity, and improved osteoporosis.[85] Pristimerin has a cardioprotective effect, effectively attenuating adriamycin (DOX)-induced cardiotoxicity by enhancing the Nrf2 signaling pathway and inhibiting MAPK and NF-κB signaling.[86] Lu et al demonstrated that Pristimerin improves the expression of the PPARα/PGC1 pathway and exerts a protective effect on pathological cardiac hypertrophy.[87] In addition, pristimerin can act as a monoacylglycerol lipase (MAGL) inhibitor to prevent paclitaxel-induced neuropathic pain (CINP) and oxidative stress;[88] it can also inhibit epithelial-mesenchymal transition of trophoblast cells through the miR-5425p/EGFR axis, blocking embryo implantation and treating ectopic pregnancy (EP) (Table 4 and Figure 3).[89] Although pristimerin has a wide range of actions, it is unable to inhibit the activation of CatSper in human spermatozoa to achieve contraception.[90] It also has potential toxic side effects such as bone marrow suppression.[38] However, there are still no clinical trials on pristimerin, and other side effects have not been explored.

Summary and Outlook

Natural compounds and herbal medicines have been increasingly emphasized in clinical drug development, and pristimerin extracted from a variety of weigela plants exhibits a wide range of bioactivities and pharmacological effects

Table 4 Other Pharmacological Effects of Pristimerin

Effect	Animal Model	Action Mechanism	Reference
Anti-inflammatory	Model of DSS-induced colitis in mice	Inhibited the increase in microRNA-155 expression induced by DSS-induced colitis; Further inhibition of the inflammatory response and oxidative stress occurred	[74]
	Lewis (LEW/SsNHsd) (RT.1L) rats	Exhibited a reduction in the pro-inflammatory cytokines (IL-6, IL-17, IL-18, and IL-23) and the IL-6/IL-17-associated transcription factors (pSTAT3 and ROR-γt), coupled with an increase in the immunomodulatory cytokine IL-10; Increased IFN-γ, which can inhibit IL-17 response	[75]
	Acute lung inflammation model	Inhibited the expression of TNF-α-induced endothelial adhesion molecules (intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1)) and the pro-inflammatory cytokine (IL-6, IL-8, and monocyte chemoattractant protein-1 (MCP-1))	[76]
	In vitro and in vivo models of psoriasis	Increased pristimerin uptake with CD4+ T cells and keratinocytes, significantly inhibited the proliferation of Th17 cells, and promoted Treg differentiation in a psoriasis-like model	[77]
insecticidal	——	Exhibited marked in vitro leishmanicidal activity against promastigotes	[78]
Antibiosis	——	Caused functional alterations on the cytoplasmic membrane of S. epidermidis cells and altered the membrane permeability of S. aureus	[79,80]
Antiviral	Human embryonic fibroblast cell line	Inhibited the cytopathic effects in HCMV-infected cells and inhibited the synthesis of viral DNA but had no virucidal effect on cell-free HCMV	[81]
Anti-osteoporosis	Ovariectomy model	Inhibited the differentiation and activation of osteoclasts in vitro and in vivo	[82–84]
	Ovariectomized rats	Caused TRAF-6-deficient transgenic mice to suffer from osteoporosis and exhibit increased osteoclastogenesis	[85]
Cardioprotection	DOX-injected rats	Enhanced the activation of the nuclear factor-erythroid 2 related factor 2 (Nrf2) signaling pathway; Inhibited MAPK/NF-kB signaling and subsequently inhibited inflammatory mediators.	[86]
	Mouse model of pathological cardiac hypertrophy	Exerted protective activity against pathological cardiac hypertrophy through improvement of the PPARα/PGC1 pathway	[87]
Relieve neuropathic pain	Rodent models	Inhibited both mouse brain and paw skin MAGL activity	[88]
Prevention of ectopic pregnancy	HTR-8/SVneo	Inhibited trophoblast cell proliferation, migration, and EMT, while inducing trophoblast cell apoptosis	[89]

for the treatment of a variety of diseases. In this paper, we reviewed the pathways and molecular mechanisms involved in the treatment of different diseases by pristimerin, including mediating various signaling pathways such as NF-κB, PI3K/Akt/mTOR, MAPKs, ROS, and other pathways, regulating cell cycle progression, promoting apoptosis, inducing autophagy, inhibiting tumor invasion and metastasis and angiogenesis, and exerting anti-tumor, anti-inflammatory, insecticidal, anti-bacterial, anti-viral, anti-osteoporosis, and cardioprotective.Moreover, pristimerin can also combine with other chemotherapeutic drugs to play a synergistic effect and improve the sensitivity of tumor cells to chemotherapy, such as combining with gemcitabine in the treatment of pancreatic cancer, paclitaxel in the treatment of breast cancer and cervical cancer, cisplatin in the treatment of lung cancer and 5-FU in the treatment of ESCC. This indicates that the
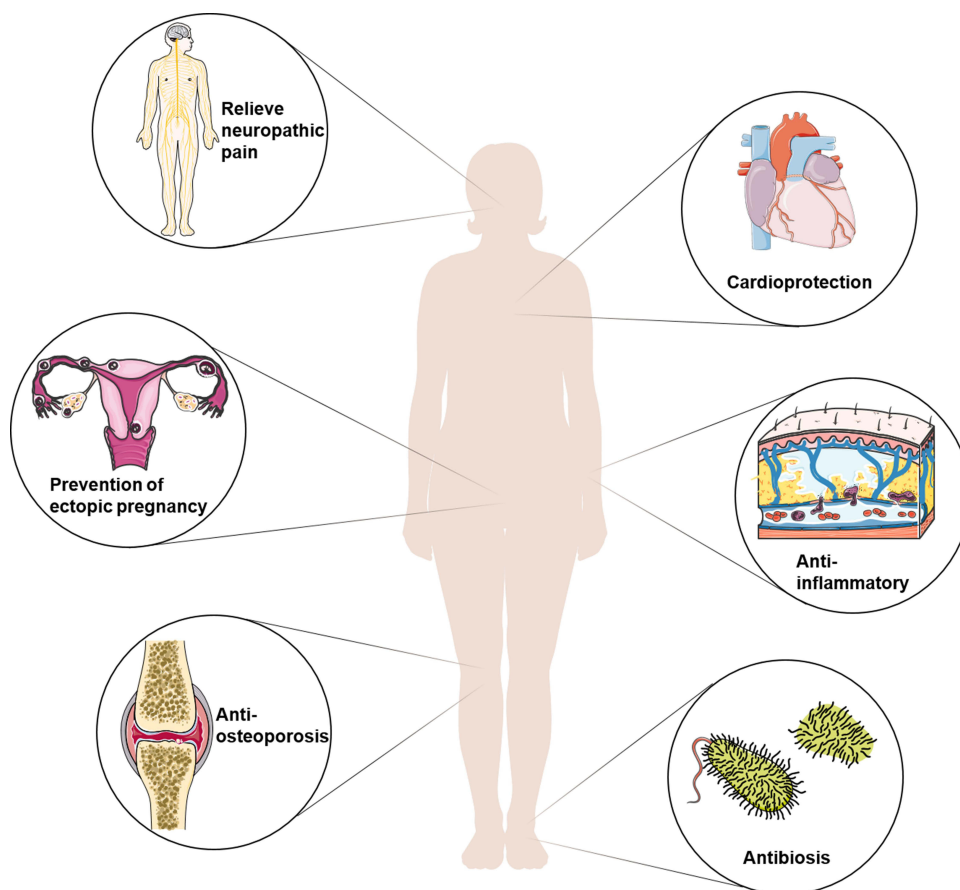
Figure 3 Other pharmacological effects of Pristimerin expected in humans. Pristimerin will exert other pharmacological effects on the human body in the future, including relief of neuropathic pain, prevention of ectopic pregnancy, anti-osteoporosis, cardioprotective, anti-inflammatory, antibacterial, and antibiotic properties.

mechanism of action of pristimerin is multi-pathway, multi-target and multi-system, which also precisely reflects the advantages of systematization, wide angle and multi-targets of Chinese medicinal preparations in disease treatment.

However, there are still many unanswered questions about pristimerin, such as the specific mechanism and target are not clear, the more detailed mechanism is not explored, the common tumor types are not comprehensive, the toxic and side effects are not clear, the clinical application is seriously lacking and the bioavailability is different.In order to achieve success, future studies should focus on the following aspects: (1) to further exploring the specific mechanisms and targets of pristimerin and more detailed mechanisms through network pharmacology, molecular docking.; (2) broadening the studies on other common tumors, such as gastric, renal, and lung cancers, enriching the therapeutic spectrum of pristimerin, and attempting to explore whether pristimerin can induce apoptosis of tumor cells by way of iron metabolism and iron death; (3) to explore the pharmacokinetics and safety of pristimerin after administration, and to clarify the toxicity characteristics of pristimerin through more toxicological studies, a process that may need to be further confirmed with more rational clinical trials and more patient participation; (4) to explore whether the structure of pristimerin can be altered to reduce toxicity; (5) due to the difference between in vitro and ex vivo experiments, which involve direct interaction with cells, and in vivo experiments, in which animals need to absorb the drug through the bloodstream to function, the specific mechanism of the difference in bioavailability between pristimerin in vivo and ex vivo is still unclear and needs to be further explored; (6) using different cell types and changing the concentration of pristimerin to further explore its unknown effects. In conclusion, pristimerin has a strong potential for use in medicine, and further research will lead to the development of new pristimerin-based drugs to promote the progress and development of medicine.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accounF for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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