Molecular Mechanisms of Anticancer Activities of Puerarin

Abstract: Medicinal plants are a vital source of natural products (NPs) that can cure cancer through modulation of different pathways, including oxidative stress, extrinsic and intrinsic apoptosis, cell cycle, inflammation, NF-kB, PI3K/AKT/mTOR, AMPK (JNK), MEK/ERK (Raf)-MEK-ERK and autophagy. Puerarin (Pue), an important NP belonging to the isoflavone glycoside group, is derived from Pueraria lobata (Willd.) Ohwi, Pueraria thomsonii Benth, and Pueraria tuberosa (Willd.). This NP was approved by the Chinese Ministry of Health for the treatment of different diseases in 1993, but it was also later reported to exhibit anticancer activity. Pue causes cancer cells death through modulation of different mechanisms including oxidative stress, intrinsic and extrinsic, Survivin and XIAP, PI3K/AKT/mTOR, Ras-Raf-MEK-ERK, JNK, cell cycle, AMPK, NF-kB, inflammation and autophagy pathways. Therefore, this review compiles for the first time the studies about the anticancer mechanism of Pue and provides comprehensive information about the anticancer effects of Pue. This review may serve as a basis for future research and clinical treatment.

Keywords: medicinal plants, natural products, Puerarin, Pueraria lobata, Pueraria thomsonii, Pueraria tuberosa

Introduction
Cancer is a major cause of mortality worldwide; therefore, the development of cancer treatment is highly important. Natural products (NPs) are widely used in cancer treatment because of their low toxicity and high level of success. Medicinal plants proven to get active compounds contain NPs. In the recent era, medicinal plants have been explored for the treatment of a wide spectrum of physiological diseases. Common medicinal plant-derived NPs are isoflavones with a 3-phenylchroman skeleton. Legumes of the Leguminosae and Fabaceae families, including lupine, kudzu, barley, cauliflower, soy, and fava beans, are a major source of plant-based isoflavones. Puerarin (Pue), depicted in Figure 1, is an important bioactive isoflavone glycoside. Pue has been isolated from several leguminous plants of the genus Pueraria, including Pueraria tuberosa (Willd.), Pueraria lobata (Willd.) Ohwi (Gegen in Chinese), and Pueraria thomsonii Benth. Pueraria plants have been interlinked with Asian culture because of their use in decoration, cooking, and disease treatment. Systematic biology is an emerging approach that focuses on molecular interactions with biological systems. Pue has molecular weight 416, possesses several pharmacological activities against osteoporosis, cardiovascular diseases, fever, neurological dysfunction, liver injury, and hangover, and they have been used in clinical treatments and experimental research. Pue injections are used extensively in China, but their effects on human health remain unclear to
Pue was approved for clinical treatment in 1993 by the Chinese Ministry of Health, and it was initially used for the treatment of cardiovascular diseases; however, Pue was later reported to have anticancer activity. Furthermore, Li et al (2006) reported the tissue distribution and pharmacokinetics after oral administration of high dose of Pue and a complex of Pue and phospholipids (400 mg/kg) through high-performance liquid chromatography (HPLC). Although Pue in high dose has effects of saturation and change metabolism, but have no evidence for the presence in tissue. The excretion of Pue also remains unclear, whether they excrete with bile, following enterohepatic circulation or excreted through urine. Recently, it is reported that the Pue eliminated very rapidly from the body. Although the level of Pue was very low after 6 h of administration, the excretion in feces and urine remained unchanged till 24 h (45.33%). These studies also clear that the Pue excrete through urine and feces. Pue is an NP against cancer, and a number of reviews about this topic are available. However, a review of its anticancer activity remains lacking. Therefore, this review summarizes the available studies on the anticancer activity of Pue to encourage future research and clinical trials on Pue. The following are the mainly reported mechanisms through which Pue inhibits cancer cell proliferation and induces death.

**Pue Anticancer Molecular Mechanisms**

Pue possesses anticancer effect through different molecular mechanisms which are listed below.

### Oxidative Stress

Multiple studies show that the high oxidative stress in cancer cells increases cell proliferation, survival, metastasis, and angiogenesis; disrupts cell death signaling; and promotes drug resistance. Increased generation of reactive oxygen species (ROS) and inhibition of mitochondrial membrane potential (MMP) lead to oxidative stress. ROS play a vital role in different types of cellular processes, including gene expression, cell survival, proliferation, differentiation, enzyme regulation, and eliminating foreign particles and pathogens. ROS promote tumor growth, but recent studies suggest that this property of ROS can be beneficial for cancer therapy. Various in vitro and in vivo experiments showed that phytochemicals induce exogenous ROS generation above a threshold level in cancer cells and decrease the MMP that selectively kills these cancer cells. Pue is a potential NP that inhibits the proliferation and induces the apoptosis of SMMC7721 cells through oxidative stress via ROS generation and MMP dissipation. Oxidative stress activates the intrinsic apoptosis pathway as shown in Table 1 and Figure 2.

### Intrinsic Apoptosis Pathway

Mitochondrial-dependent apoptosis is an important pathway for the induction of apoptosis, and disturbance in this pathway can inhibit apoptosis. The intrinsic pathway is controlled by the B-cell lymphoma 2 (Bcl-2) family proteins, which either increase or decrease the mitochondrial membrane permeability for the release of cytochrome-c and other apoptotic proteins. Furthermore, caspase activation and DNA fragmentation are the main features of induced apoptosis. Plant-derived NPs induce apoptosis in cancer cells through the mitochondrial-dependent pathway.

Pue significantly induces apoptosis in HT-29 colon cancer cells, human mental cell lymphoma (MCL) Z138, SMMC7721, MDA-MB-231, MCF-7, NB4, A549 cells and HS578T cell lines through the intrinsic apoptosis pathway. In this pathway, Pue downregulates Bcl-2, which causes Bax upregulation and increases the permeability of the mitochondria to apoptosis-inducing factor (AIF), which is then released from the mitochondria to the cytosol. In the cytosol, AIF activates caspase-3, caspase-7, and caspase-9, which trigger DNA fragmentation and ultimately induce apoptosis, as illustrated in Table 1 and Figure 2.
Survivin and X-Linked Inhibitor of Apoptosis Protein (XIAP) Pathway

Survivin, which belongs to the inhibitor of apoptosis protein (IAP) family, is one of the top 5 cancer-associated genes. In different types of cancer, survivin is upregulated and is associated with resistance to radiotherapy and chemotherapy along with poor prognosis. Overexpression of survivin in tumors decreases apoptosis and increases cell division. XIAP also belongs to the family of IAP, and it is upregulated in different cancers, including lung, breast, bladder, and renal carcinoma. A variety of NPs reduce the expression levels of survivin and XIAP in different cancers.
inhibits the expression of survivin in NB4 cells\textsuperscript{54} and XIAP\textsuperscript{51} in Z138\textsuperscript{51} cells, which further activate caspase-3 and −9, as shown in Table 1 and Figure 2.

Extrinsic Apoptosis Pathway

The extrinsic pathway is activated through the tumor necrosis factor (TNF) family proteins, including Fas or TNF receptor-1 (TNFR1).\textsuperscript{63} Fas or TNFR1 activates caspase-8 via Fas-associated death domain protein, creating a death-inducing signaling complex that triggers caspase-3 activation and cell death.\textsuperscript{64,65} The NPs also modulate the extrinsic apoptosis pathway in different cancers.\textsuperscript{66,67}

Pue induces the extrinsic apoptosis pathway in NB4 and SMMC7721\textsuperscript{41} cells through activation of Fasl,\textsuperscript{54} which further activates caspase-8;\textsuperscript{53,54} activated caspase-8 then activates caspase-3\textsuperscript{38,54} and causes apoptotic cell death.\textsuperscript{43,54} This pathway is further summarized in Table 1 and Figure 2.
Phosphatidylinositol-3-Kinase/Protein Kinase B/Mammalian Target of Rapamycin (PI3K/AKT/mTOR) Pathway

The PI3K/AKT/mTOR signaling pathway increases cell growth and survival through various mechanisms.68,69 The PI3K/AKT/mTOR pathway is activated in a number of human cancers through different mechanisms.70–73 For example, phosphorylation of the two residues of AKT, including threonine (Thr 308) and serine 473 (Ser 473), leads to AKT activation.74 Following activation, AKT enters the nucleus, where they affect the activity of transcription-regulating factors. PI3K/AKT signaling increases the expression of mTOR, and this expression of mTOR is associated with poor prognosis. NPs reportedly inhibit the PI3K/AKT/mTOR pathway in cancer cells.75

A natural Pue induces apoptosis in bladder cancer T24, EJ cells and human mental lymphoma Z13851 cells through the PI3K/AKT/mTOR51,76 pathway by downregulating PI3K,75 Akt,76 p-mTOR,76 and p-p70S6K51,76 This PI3K/AKT/mTOR pathway is further summarized in Table 1 and Figure 2.

MAPK/ERK (Ras-Raf-MEK-ERK) Pathway

The mitogen-activated protein kinase/extracellular signal-regulated kinases (MAPK/ERK) pathway are also known as Rat Sarcoma (Ras)-Rapidly Accelerated Fibrosarcoma (Raf)-MEK-ERK pathway.77 The MAPK/ERK pathway possesses different cascades and is mostly deregulated in human cancers.77 It regulates several cellular processes, such as cell growth, proliferation, differentiation, migration, senescence, and apoptosis.78 The molecules of the MAPK/ERK pathway become activated through its phosphorylation. The activated ERK enters the nucleus, where it activates transcription factors. The activated transcription factors then bind to various genes, including growth factors and cytokines. Such genes are responsible for the increase in cell proliferation and decrease in apoptosis.79 Disturbance of the normal signaling of this pathway causes senescence, drug resistance, and tumorigenesis.78,80,81 Failure of this pathway has been detected in various cancers.82,83 NPs trigger the death of cancer cells through the MAPK/ERK pathway.84 Pue induces apoptosis in SMMC772143,85 cells through ROS43-mediated p-38 MAPK85 and ERK143 activation.43,85 In NSCLC cells, Pue inhibits the IL-4-induced activation of MEK and ERK1/2 and its nuclear translocation.86 In addition, Pue reverses the oxidized low-density lipoprotein (ox-LDL)-induced increase in VSM cell proliferation through ERK1/2 and proliferating cell nuclear antigen activation,87 as depicted in Table 1 and Figure 2.

c-Jun NH2 Terminal Kinase (JNK) Pathway

The JNK pathway controls different physiological processes, such as cell survival, death, differentiation, proliferation, inflammation, and protein expression. Dysregulation of the JNK pathway is linked with different diseases, including auto-immune disease, cardiac hyper therapy, asthma, diabetes, and cancer.88 JNK is involved in oncogenic changes. The JNK signaling pathway is involved in apoptosis elimination through suppression of Ras transformation.89 Aromatase P450 is an enzyme that is expressed in different parts of the body, and changes in its level in the body cause different diseases.90 Different NPs induce apoptosis in cancer cells through the regulation of the JNK pathway.91–93

The oxidative stress generated in SMMC7721 cells with Pue treatment due to ROS causes JNK activation, which causes c-jun inhibition and cell apoptosis.43 Another study revealed that Pue alone or in combination with arsenic trioxide induces apoptosis in NB4 cells through JNK activation.54 In endometriosis, uterine fibroblast and endometrial cancer Aromatase P450 (P450 (arom)) is overexpressed, which is downregulated by Pue in RL95-2 and Ishikawa cell lines at both protein and mRNA levels.94 Furthermore, with Pue treatment, c-jun regulates the P450 (arom) expression and activity, which was confirmed by c-jun knockdown through siRNA. Therefore, inhibition of P450 (arom) activity and expression with Pue may be linked with transcription factor AP-1 or c-jun down-regulation,94 as shown in Table 1 and Figure 2.

Cell Cycle

Cell growth is controlled by the cell cycle. Cells are regulated at different checkpoints by the interactions of various cyclins with their exact cyclin-dependent kinases (CDKs) to make active complexes. The process of each checkpoint completes accurately before the progression to the next phase of the cell cycle.95 Among CDKs, p21 regulates cell cycle at different checkpoints.96,97 In cell cycle regulation, p53 plays a key component role. It is activated in a wide range of damage and stresses.98,99 Recently, NPs have attracted the attention of researchers because of their potential to reverse cancer through the cell cycle.100
Pue induces cell cycle arrest in T24, EJ, NB4, Kasumi-1, U937, HL-60, MDA-MB-231, MCF-7, HS578T, vascular smooth muscle cells (VSMCs) and bladder cancer cells at the G1/G0, and negates adhesion, which further increases p21 expression and triggers cell apoptosis, as illustrated in Table 1 and Figure 2.

AMP-Activated Protein Kinase (AMPK) Pathway
AMPK plays a role as a key sensor for cellular energy because it phosphorylates and activates enzymes, including acetyl-CoA carboxylase (ACC). The glycogen synthase kinase-3beta (GSK-3b) causes the phosphorylation of cAMP-responsive element-binding protein (CREB), and inhibition of GSK-3b increases multi-drug resistance 1 (MDR1) gene expression. The overexpression of MDR1, which has been reported in several cancers, lowers drug efficacy. A variety of anticancer natural compounds derived from vegetables, fruits, herbs, and oilseed regulate MDR1 activity.

In the human breast cancer cell line MCF-7/adriamycin (MCF-7/adr), plant-derived Pue activates AMPK, ACC, and GSK-3b, which lead to the inhibition of CREB and MDR1. Pue-induced suppression of MDR1 can be reversed by inhibitor of AMPK (compound C). Furthermore, both protein kinase A/CRE inhibitor (H89) and Pue inhibit the transcriptional activities of both cAMP-responsive element (CRE) and MDR1 protein. These results show that Pue inhibits the expression of MDR1 through CRE transcriptional activity-dependent upregulation of AMPK in MCF-7/adr cells, as depicted in Table 1 and Figure 3A.

Nuclear Factor kB (NF-kB) Pathway
NF-kB is a transcription factor complex consisting of hetero- and homodimers of five members of a Reticuloendotheliosis oncogene cellular homolog (Rel) family, including RelA (p65), RelB, c-Rel, NF-kB1 (p50/p105), and NF-kB2 (p52/p100). In cancer, the functions of NF-kB are mostly dysregulated. Active NF-kB has been reported in several cancers, including colon, liver, breast, pancreas, prostate, ovarian, leukemia, and lymphoma cancers. NF-kB becomes activated when the DNA becomes damaged, which consequently activates a number of NF-kB-targeted genes, including inducible nitric oxide synthase (iNOS) and COX-2. Furthermore, binding of TNFα to TNFR leads to homotrimerization of receptors and adaptor proteins, resulting in cell proliferation and survival by increasing the expression of NF-kB and activator protein 1 target genes, including vascular cell adhesion molecule-1 (VCAM-1). NF-kB activation triggers the activation of chemokines and its related receptors, including C-X-C chemokine receptor 4 (CXCR4) and CCR7, which play important roles in the migration of cancer cells to target organs. These genes play pivotal roles in pro-survival anti-apoptosis. Therefore, NF-kB is a candidate for therapeutic resistance in different cancers. Different NPs have potential therapeutic efficacy against cancer by inhibiting NF-kB pathway activation in cancer cells.

In lipopolysaccharide-induced MDA-MB-231, MCF-7/adriamycin (MCF-7/adr), MCF-7 cells, Z138 cells, T24, THP1 cells, Pue inhibits proliferation and negates adhesion, migration, and invasion by regulating the NF-kB pathway. In the NF-kB pathway, Pue inhibits the expression of inflammatory factors TNF-α and IL-1β and abolishes NF-kB activation through inhibition of Phospho-IκBα/κBα, IkappaB, and p65 and upregulation of miR16. Pue also inhibits the NF-kB nuclear translocation, which leads to the inhibition of COX-2, MMP-2,9 CXCR4, CCR7, VCAM, and ICAM at the mRNA and protein levels, as shown in Table 1 and Figure 3A.

Inflammation Pathway
Inflammation is often associated with the progression and development of cancer. Inflammation leads to tumorigenesis via two basic ways, including extrinsic and intrinsic. There are many factors which cause tumor-extrinsic inflammation, including viral and bacterial infections, obesity, autoimmune diseases, asbestos exposure, excessive alcohol consumption and tobacco smoking. On the other hand, cancer intrinsic inflammation can be triggered through cancer-initiating mutations and increase tumor growth through the activation of inflammatory cells. These information show that both the extrinsic and intrinsic inflammations are providing a good background for cancer progression. Those cells which are responsible for cancer-causing inflammation are stable genetically and did not cause rapid emergence of drug resistance, therefore targeting of inflammation represents a good strategy for cancer prevention and therapy. Here, we focus on cancer-elicted intrinsic inflammation.
Macrophages play important roles in different types of inflammatory diseases and cancer progression.\textsuperscript{128} M1 and M2 are markers of inflammation, in which M1 macrophages intimate tumorigenesis through reactive oxygen and intermediates of nitrogen, while M2 macrophages increase tumor progression.\textsuperscript{129,130} Next, NF-kB is considered a central mediator in inflammation process while identification of its kinship with v-Rel oncogene, shows that the NF-kB is involved in cancer development.\textsuperscript{131} The dual role of Macrophages and NF-kB has made its therapeutic targeting a challenge. Therefore, the understanding of such interaction between immune signaling pathways and cellular metabolism can provide us the clues to develop potential therapeutic strategies for the treatment of inflammatory diseases, including cancer. Recent studies have reported that NPs target both cancer and inflammation.\textsuperscript{132}

Pue inhibits the tumor volume and its growth in the NSCLC xenograft model by upregulating M1 markers (such as iNOS+, cluster of differentiation 197, and CD40+) and decreasing M2 markers (including CD163+, Arginase 1, and CD206+).\textsuperscript{86} Furthermore, Pue increases TNF-α, pro-inflammatory cytokine interferon-γ, and interleukin-12 while reducing pro-tumor cytokines IL-4, IL-10 and

![Diagram](image-url)
transforming growth factor-β. Pue inhibits the change of pure macrophages from polarized to M2 phenotype without other auxiliary cell involvement. In THP1 macrophages, Pue dose-dependently inhibits the expression of oxLDL-activated pro-inflammatory genes, including toll-like receptor 4, and the ratio of Phospho-IκBα/IκBα. Furthermore, Pue inhibits the formation of foam cells and lipid deposition induced by oxLDL, which are associated with scavenger receptor CD36 downregulation. Therefore, these results reveal that Pue acts as an antiatherogenic and anti-inflammatory agent by downregulating CD36 expression and inhibiting the TLR4/NF-κB pathway. All these results show that the Pue inhibits the cancer progression through inhibition of inflammation. The inflammation pathway is summarized further in Table 1 and Figure 3B.

**Autophagy Pathway**

In normal cells, autophagy suppresses tumor growth by maintaining genomic stability. Once the tumor forms, the unbalance in autophagy contributes to tumor growth. In the complexity of autophagy, the ERK/MAPK, PI3K/MAPK, and other signaling pathways play important roles. The NP modulates autophagy in different diseases, including cancer. In NCI-H441 cells, Pue induces autophagy through the PI3K/Akt and ERK pathway via extreme inhibition of Akt and ERK phosphorylation, which was further inhibited through BEZ235, an inhibitor of PI3K/Akt. Activation of the PI3K/Akt pathway further increases Atg5 expression, but no obvious effect was observed in LC3I conversion to LC3II. However, rapamycin, an inhibitor of mTOR, increases the expression of Atg5 and LC3II, as depicted in Table 1 and Figure 3C.

**Conclusion**

Pue has potential anticancer activity. Available studies show that Pue might be a very good therapeutic drug for the treatment of different cancers because it is a good inducer of apoptosis. Additional preclinical and clinical studies must be designed and conducted to determine the definite dose of Pue for each type of cancer and establish a specific pathway or gene. The available anticancer information about Pue is summarized in Table 1 and Figures 2 and 3.

**Abbreviations**

NPs, Natural products; Pue, Puerarin; ROS, reactive oxygen species; MMP, mitochondrial membrane potential; Bel-2, B-cell lymphoma 2; AIF, apoptosis-inducing factor; IAP, inhibitors of apoptosis protein; XIAP, X-linked inhibitor of apoptosis protein; TNF, tumor necrosis factor; TNFR1, Fas or TNF receptor-1; CDKs, cyclin-dependent kinases; iNOS, inducible nitric oxide synthase; TNF-α, tumor necrosis factor; oxLDL, oxidized low-density lipoprotein; NF-kB, Nuclear factor kB; Rel, Reticulonetheliosis oncogene cellular homolog; VCAM-1, vascular cell adhesion molecule-1; CXCR4, C-X-C chemokine receptor 4; PI3K/akt/ mTOR, Phosphatidylinositol-3-kinase/protein kinase B/ mammalian target of rapamycin; MAPK/ERK, Mitogen-activated protein kinase/extracellular signal-regulated kinases; Ras, Rat Sarcoma; Raf, Rapidly Accelerated Fibrosarcoma; AMPK, AMP-activated protein kinase; ACC, acetyl-CoA carboxylase; GSK-3β, glycogen synthase kinase-3beta; CREB, cAMP-responsive element-binding protein; MDR1, multi-drug resistance 1; MCF-7/adr, MCF-7/adriamycin; CRE, cAMP-responsive element; JNK, c-Jun NH2 terminal kinase; P450 (arom), Aromatase P450; LC3, light chain 3; Atg5, autophagy protein 5.

**Author Contributions**

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

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**Disclosure**

The authors declare no conflict of interest related to this work.

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