Anti-tumor activity of wogonin, an extract from Scutellaria baicalensis, through regulating different signaling pathways

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[ABSTRACT] Wogonin is a plant flavonoid compound extracted from Scutellaria baicalensis (Huang-Qin or Chinese skullcap) and has been studied thoroughly by many researchers till date for its anti-viral, anti-oxidant, anti-cancerous and neuro-protective properties. Numerous experiments conducted in vitro and in vivo have demonstrated wogonin’s excellent tumor inhibitory properties. The anti-cancer mechanism of wogonin has been ascribed to modulation of various cell signaling pathways, including serine-threonine kinase Akt (also known as protein kinase B) and AMP-activated protein kinase (AMPK) pathways, p53-dependent/independent apoptosis, and inhibition of telomerase activity. Furthermore, wogonin also decreases DNA adduct formation with a carcinogenic compound 2-Aminofluorene and inhibits growth of drug resistant malignant cells and their migration and metastasis, without any side effects. Recently, newly synthesized wogonin derivatives have been developed with impressive anti-tumor activity. This review is the succinct appraisal of the pertinent articles on the mechanisms of anti-tumor properties of wogonin. We also summarize the potential of wogonin and its derivatives used alone or as an adjunct therapy for cancer treatment. Furthermore, pharmacokinetics and side effects of wogonin and its analogues have also been discussed.

[KEY WORDS] Anti-cancer; Apoptosis; Wogonin; Signaling pathways; Pharmacokinetics

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Introduction

Cancer has created havoc around the world. With increasing evidence of side effects and emergence of drug resistance with standard chemotherapy, the discovery of alternate therapies to overcome the disadvantages of conventional chemotherapy is urgently required [1-2]. Confronting with such issues, many researchers have incessantly studied extracts from plants, which have been traditionally prescribed for thousands of years, in order to achieve effective cure. A salient finding includes Scutellaria baicalensis (Fig. 1), which is commonly used in the East of Asia, including countries such as China, Japan, and Korea [3]. The first description of S. baicalensis was recorded in Shennong's Classic of Materia Medica (Shen Nong Ben Cao Jing) in ca. 100 BC and it is also listed in the Pharmacopoeia of China. The beneficial effects of S. baicalensis have been demonstrated in various disease conditions, including anti-tumor, hepato-protective, anti-oxidant, anti-inflammatory, anti-convulsant, anti-bacterial, and anti-viral effects [4-5]. The herb extracts in the forms of tablets or capsules/drops are commercially available and popular among peoples. Studies on the root of S. baicalensis have identified wogonin, baicalein, baicalin and wogonoside as the main active components that have exhibited anti-cancerous properties [6]. Several studies on wogonin (Fig. 1) have affirmed its anti-oxidant, anti-viral, anti-inflammatory and neuro-protective activities [7].
Fig. 1 Chemical structures of wogonin, an extract from traditional herbal remedy \textit{Scutellaria baicalensis} and its derivatives

\textit{In vitro} and \textit{in vivo} studies on wogonin against cancer cells have brought a remarkable new approach to cancer prevention\cite{6, 8-9}. These satisfactory outcomes came from the modulation of several pathways and were also dependent on the dose of treatment either alone or as an adjunct therapy. Some of previous reviews have mentioned about anti-cancer properties of wogonin in different pathways, such as the up-regulation of intracellular ROS and p53 level, targeting PI3k/Akt and MAPK pathways, inhibition of NF-κB, cell-cycle arrest, and overcoming drug resistance\cite{6-9}. In this review, we summarize the role of wogonin in medical applications and analyze the pathways modulated during the treatment of cancer by wogonin. Furthermore, some of newly synthesized wogonin derivatives and their impacts on tumor development are also discussed. Besides, the roles of wogonin in anti-angiogenesis, anti-invasion, anti-metastasis, as well as regulations of drug resistance both in normoxia and hypoxia conditions are also reviewed. The effects of wogonin in combination with other anti-cancer agents, its opposite role in inflammation-cancer link, and its pharmacokinetics and side effects are also deliberated. It is hoped that well-documented knowledge about anti-tumor effects of wogonin will help open a new prospect for wogonin-based clinical intervention in the near future.

\textbf{Anti-cancer mechanisms for wogonin: Molecular insights}

The most important target in cancer treatment is to induce apoptosis (programmed cell death) and during the process, nucleus become condense and fragmented while apoptotic bodies are formed without damage on plasma membrane. Those cells are actively directed to be deceased and are naturally eliminated by phagocytes without any sign of inflammation\cite{10}. Another type of cell death is necrosis, which occurs and affects large field of cells and is mediated by the lack of energy supply or by the damage on the cell membrane. Therefore, necrosis results in the release of cytoplasmic contents/chemotactic signals into the tissue and facilitates to recruit inflammatory cells. Due to those differences, apoptosis is preferred and becomes one of required standards when a new anti-cancer drug is developed.

Numerous studies have been performed to increase the cytotoxicity of drugs on cancer cells during treatment. Physiologically, cytotoxicity may lead cancer cells to either go through necrosis, whereby their membranes are deteriorated, leading to cell lysis, or suspend cell proliferation or promote apoptosis. Many approaches to assessing the level of cytotoxicity in cancer cells have been studied, including assays for cell membrane integrity, cell metabolism and proliferation\cite{11-13}. For cancer treatment, numerous chemical agents have been found to induce cytotoxicity in cancer cells. However, the main impediments in their clinical application include side effects and drug resistance, leading to the obsession to patients and tumor relapse after treatment.
**Wogonin as a sheet-anchor for anti-tumor capability**

Recently, many phytochemicals have been shown to have anti-cancer properties. With acceptable safety profile, fewer side effects and high efficiency in cytotoxic induction, phytochemicals have recently attracted the world for enormous therapeutic uses [1-2]. Prominently, wogonin has been rising as a promising anti-cancer compound to induce the programmed cell death in various types of cancer. Several in vivo studies have demonstrated that wogonin inhibits malignant growth remarkably in different tumor models [14-22]. Wogonin drastically inhibits the development of malignancies such as leukemia, gastric cancer, hepatoma, breast cancer, melanoma, colon cancer, and lung cancer in the 10–200 mg·kg⁻¹ dose range (Table 1). Furthermore, when administered along with other anti-cancer agents such as 5-FU in in vivo models, wogonin enhances the anti-tumor efficacy [16-17]. Wogonin anti-inflammatory and anti-oxidant properties help reduce the impact of colitis on bowel, which is considered as main cause of colorectal carcinogenesis [19]. Therefore, these data advocate the therapeutic potential of wogonin in fighting cancer.

**Table 1 Anti-tumor effects of wogonin in mouse models**

<table>
<thead>
<tr>
<th>Tumor models</th>
<th>Animals</th>
<th>Doses and routes of treatment</th>
<th>Results</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human acute T-cell Leukemia CEM</td>
<td>Immunodeficient mice (H-2&quot;Rag&quot;/&quot;Rc&quot;/)</td>
<td>Wogonin dose: 200 mg·kg⁻¹, 6 doses, i.p 2 days/dose</td>
<td>No visible tumor after 2 weeks treatment</td>
<td>[14]</td>
</tr>
<tr>
<td>Human gastric cancer cellIMGC-803</td>
<td>Male BALB/c nude mice</td>
<td>Wogonin dose: 15, 30 or 60 mg·kg⁻¹·d⁻¹, i.v, 12 doses</td>
<td>Tumor size significantly decreased after 12 days</td>
<td>[15]</td>
</tr>
<tr>
<td>Human breast cancer cellMCF-7</td>
<td>BALB/c nude mice</td>
<td>Wogonin dose: 30 mg·kg⁻¹·d⁻¹, i.p; Combined 30 mg·kg⁻¹ Wogonin and 10 mg·kg⁻¹·d⁻¹ 5-FU</td>
<td>Tumor inhibition rate 30 mg·kg⁻¹ Wogonin, 49.28% 10 mg·kg⁻¹·d⁻¹ 5-FU, 29.37% 30 mg·kg⁻¹ Wogonin combined and 10 mg·kg⁻¹·d⁻¹ 5-FU, 91.69%</td>
<td>[17]</td>
</tr>
<tr>
<td>Human melanoma cell B16-F10</td>
<td>C57BL/6 mice</td>
<td>Wogonin dose: 60 mg·kg⁻¹·d⁻¹, i.v, 21 days administration</td>
<td>Tumor size is significantly reduced in 60 mg·kg⁻¹ dose of Wogonin</td>
<td>[18]</td>
</tr>
<tr>
<td>Human colon cancer cell HCT 116</td>
<td>Male BALB/c nude mice</td>
<td>Wogonin dose: 30 mg·kg⁻¹·d⁻¹, i.v, 2 days/dose, 3 weeks</td>
<td>Tumor inhibition rate 30 mg·kg⁻¹ Wogonin, 52.62% 60 mg·kg⁻¹ Wogonin, 43.69%</td>
<td>[21]</td>
</tr>
<tr>
<td>Human lung cancer cell A549</td>
<td>Male BALB/c nude mice</td>
<td>Wogonin dose: 30 or 60 mg·kg⁻¹·d⁻¹, i.p, 20 days</td>
<td>Tumor inhibition rate 30 mg·kg⁻¹ Wogonin, 64.23% 60 mg·kg⁻¹ Wogonin, 45.20%</td>
<td>[22]</td>
</tr>
</tbody>
</table>

i.p: intraperitoneal injection; i.v: intravenous injection; i.g: intragastric injection; AOM: azoxymethane; DSS: dextran sulfate sodium

**Wogonin’s effects on different signaling pathways involved in apoptosis**

Numerous evidences have indicated that wogonin induces apoptosis via different mechanisms including DNA fragmentation [23-26], PARP degradation [23, 27-29], activation of Caspase-3 (but not Caspase-1), induction of Caspase-9 or Caspase-8 cleavage, and reduction of Bcl2 family proteins (Table 2) [23-24, 26-30].

**Induction of apoptosis by attenuating anti-apoptotic proteins**

Programmed cell death (apoptosis) can be executed by intrinsic (also called mitochondria-mediated) or extrinsic (also called receptor-mediated) signaling pathway. Molecular studies have indicated that wogonin induces apoptosis via intrinsic pathway by repressing myeloid cell leukemia protein (Mcl-1), an anti-apoptotic protein in Bcl2 family, at the concentration range of 50–100 µmol·L⁻¹ treatment [31-32]. Consequently, the pro-apoptotic proteins such as Bim, Bak, and Bid, are released to induce apoptosis [33-34]. Recently, the mechanism for wogonin’s down-regulation of Mcl-1 has been elucidated [35]. This flavonoid inhibits CDK9 and buckles the phosphorylation at Ser² of carboxy-terminal domain of RNA polymerase II, thereby inhibiting Mcl-1 transcription (Fig. 2). Moreover, wogonin also down-regulates the expression of survivin in breast cancer cell line MCF-7, at 90 µmol·L⁻¹ [30]. Survivin is a member of the inhibitor of apoptosis family and has been identified as a p53 repressed gene [36]. Wogonin also represses MDM2 expression, resulting in stabilization of p53 down-regulation of survivin protein, and inducing apoptosis (Table 2).
<table>
<thead>
<tr>
<th>Cell lines</th>
<th>Concentration</th>
<th>Mechanism</th>
<th>Effects</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukemia HL-60</td>
<td>80 µmol·L⁻¹</td>
<td>Caspase 3 and 9↑, ROS↓, DNA fragmented, PARP activation</td>
<td>Intrinsic apoptosis, Inhibition of Telomerase activity</td>
<td>[25]</td>
</tr>
<tr>
<td></td>
<td>10–150 µmol·L⁻¹</td>
<td>C-Myc/ hTERT↑, Activation of Caspase</td>
<td>Intrinsic apoptosis</td>
<td>[43]</td>
</tr>
<tr>
<td></td>
<td>25–50 µmol·L⁻¹</td>
<td>Release of CHOP/ GRP94/GRP78, PI3K/Akt↓</td>
<td>ER stress, Intrinsic apoptosis</td>
<td>[94]</td>
</tr>
<tr>
<td>Drug resistance leukemia K562/A02</td>
<td>20 µmol·L⁻¹</td>
<td>P-gp or MDR↓</td>
<td>Combatted chemoresistance</td>
<td>[98]</td>
</tr>
<tr>
<td></td>
<td>10–40 µmol·L⁻¹</td>
<td>Down-regulation of Nrf2 and MRP1, PI3K/Akt↓</td>
<td>Combatted chemoresistance</td>
<td>[106]</td>
</tr>
<tr>
<td>Leukemia cell U-937</td>
<td>10–100 µmol·L⁻¹</td>
<td>PKCβ/ P21↓, CD1/CDK4/ pRb↓, Expressing CD11b/CD14↓</td>
<td>G1 phase arrest, differentiating malignant cells into granule cells</td>
<td>[48]</td>
</tr>
<tr>
<td>Malignant T cell CEM, HTLV-1, SP</td>
<td>100–300 µmol·L⁻¹</td>
<td>Release of Cytochrome C/ AIF, Caspase↑, PARP↑, PLC↑ activation, Ca²⁺ release</td>
<td>Intrinsic apoptosis, ER-stress</td>
<td>[14]</td>
</tr>
<tr>
<td></td>
<td>50–100 µmol·L⁻¹</td>
<td>CDK9 blocked, RNA synthesis↓, Mcd1↓</td>
<td>Intrinsic apoptosis, Inhibition of protein synthesis</td>
<td>[35]</td>
</tr>
<tr>
<td></td>
<td>5–10 µmol·L⁻¹</td>
<td>HIF-1α/HDII/ PDK1/ LDHA↓, PI3K/Akt↓</td>
<td>Intrinsic apoptosis, Inhibition of Hypoxia-related resistance and glycolysis metabolism</td>
<td>[21]</td>
</tr>
<tr>
<td></td>
<td>20–100 µmol·L⁻¹</td>
<td>Promote Nrf2 activation, inhibition of NF-κB, IL-6 &amp; IL-1β↓</td>
<td>Suppression of inflammation-associated colon carcinogenesis and cancer development</td>
<td>[19]</td>
</tr>
<tr>
<td>Prostate cancer (LNCaP, PC3)</td>
<td>50–100 µmol·L⁻¹</td>
<td>p53↑, PUMA↑, Bax↑, Cytochrome C↑, MDM2↓</td>
<td>p53-dependent intrinsic apoptosis</td>
<td>[24]</td>
</tr>
<tr>
<td>Breast cancer Estrogen receptor + and Estrogen receptor –</td>
<td>50–200 µmol·L⁻¹</td>
<td>Caspase-3↑, Bax↑, EndoG↑, PARP↑, G1 arrest, p27↑/ GSK-3β/ Cyclin D1↓</td>
<td>Intrinsic apoptosis, Inhibition of proliferation</td>
<td>[27]</td>
</tr>
<tr>
<td>MCF-7</td>
<td>80–320 µmol·L⁻¹</td>
<td>Bax↑, p53↑, G, arrest Bcl 2/ Survivin↓, PI3K/Akt↓</td>
<td>Inhibition of proliferation</td>
<td>[30]</td>
</tr>
<tr>
<td>MDA-MB 231</td>
<td>60 µmol·L⁻¹</td>
<td>PI3K/Akt↓, MMP-2/MMP-9↓, PKCβ translocation↓</td>
<td>Mobility and invasion inhibited</td>
<td>[76]</td>
</tr>
<tr>
<td>Lung carcinoma SGC-7901 H23</td>
<td>100 µmol·L⁻¹</td>
<td>Block VEGF pathway via serving as antagonist of VEGF receptor 2 in vessel-forming endothelial cells</td>
<td>Suppressed angiogenesis, Tumor volume reduced</td>
<td>[15]</td>
</tr>
<tr>
<td>Gallbladder Carcinoma GBC-SD</td>
<td>1–10 µmol·L⁻¹</td>
<td>MMP-2/ MMP-9↓, Maspin↑, pERK↑</td>
<td>Inhibition of invasion and migration</td>
<td>[75]</td>
</tr>
<tr>
<td>Hepatoma HepG2</td>
<td>100–300 µmol·L⁻¹</td>
<td>Releases of Cytochrome C/ AIF, Caspase 9↑, PARP↑, PLC↑ activation, Ca²⁺ release</td>
<td>Intrinsic apoptosis, Mitochondrial dysfunction</td>
<td>[26]</td>
</tr>
<tr>
<td>Osteosarcoma U-2OS</td>
<td>75–100 µmol·L⁻¹</td>
<td>Caspase 3/9↑, Bad/ Bax↑, Release of Cytochrome C/ AIF/ EndoG Bcl2/ Δανα↓, ROS/Ca²⁺↑, GADD53/ Calpain ↑↑</td>
<td>Intrinsic apoptosis, Endoplasmic reticulum stress, mitochondrial dysfunction</td>
<td>[28]</td>
</tr>
<tr>
<td>Glioma GBM-U87</td>
<td>10–100 µmol·L⁻¹</td>
<td>Caspase 9/8/3↑, DNA fragmentation, activation of AMPK pathway, suppression of mTOR pathway</td>
<td>Intrinsic apoptosis, Inhibition of cell growth and protein synthesis</td>
<td>[53]</td>
</tr>
<tr>
<td>U251, U87</td>
<td>25–100 µmol·L⁻¹</td>
<td>Caspase 9/3↑, PARP degradation, BAX/Bak↑, Bcl2/Bcl-xl↓, G_i arrest, GRP78/GRP94↑, Calpain1/elF↑↑</td>
<td>Intrinsic apoptosis, Endoplasmic reticulum stress</td>
<td>[29]</td>
</tr>
</tbody>
</table>

↑: An increase in target protein
↓: A decrease in target protein
Several studies have reported wogonin induces apoptosis in malignant cells at 50–100 µmol·L$^{-1}$, via Bax activation [24, 30, 37]. Wogonin-exposed human prostate cancer LNCaP cells show elevated levels of p21, p27, p53 and PUMA, which are involved in Bax signaling pathway. In cancer cells with null of either PUMA or p53, Bax oligomerization is decreased even in the presence of wogonin [24]. This explains that both PUMA and p53 are strongly related to apoptosis induced by wogonin, resulting in Bax oligomerization and intrinsic apoptosis pathway activation. Moreover, this data has also demonstrated that p53 binds to PUMA promoter region, resulting to enhance PUMA gene transcription. This verdict is in agreement with the previous results published by Yu et al. [38]. As a result, level of enhanced PUMA in turn intensively interacts with Bcl-2 or Bcl-xL at BH3 domain and consequently promotes liberation of Bax from the Bax/Bcl-xL complex. Further, Bax migrates to mitochondrial membrane, oligomerizes, and facilitates the release of cyt-C as well as multimerization of Apaf-1 to form apoptosome in intrinsic pathway (Fig. 2) [37, 39]. However, in the same concentration range of wogonin (5–50 µmol·L$^{-1}$), it has no effects on Akt pathway, indicating that the Akt pathway is probably not related to apoptosis in the treated cancer cells, at the current concentrations.

Further experiment by Chung et al. has demonstrated that wogonin at higher concentrations (50–200 µmol·L$^{-1}$) induces apoptosis in breast cancer cells, via an attenuation of Akt phosphorylation without estrogen receptor (EsR) involvement [27]. This study shows that the proportion of breast cancer cells in sub-G1 is increased effectively in both EsR$^+$ and EsR$^-$ cell lines [27, 40]. Their results also exhibits the signs typical for apoptosis in the intrinsic pathway, encompassing Caspase-3 activation, PARP cleavage, Bax increment, Bcl-2 down-regulation, and the release of Endo G. In addition, a series of genes related to tumorigenesis such as CCND1 encoding Cyclin D1 and ESR1 for estrogen receptor alpha in EsR$^+$ population and cErbb-2 (also known as hEsR-2) in EsR$^-$ population are down-regulated by wogonin at transcriptional level. Thus, this finding provides a promising evidence for wogonin to be used in the treatment of breast cancer, regardless of estrogen receptor status (Table 2).

**Increases in H$_2$O$_2$ generation and Ca$^{2+}$ accumulation**

Several studies have revealed the generation of H$_2$O$_2$ and Ca$^{2+}$ overloading as anti-tumor mechanism following the treatment with wogonin in malignant T cells [14, 26]. A study of Baumann et al.indicates that the malignant lymphocytes produce higher level of free radicals (O$_2^-$), compared to the normal cells while Wogonin has the ability to convert O$_2^-$ into H$_2$O$_2$, leading to the selective accumulation of H$_2$O$_2$ in malignant T cells, instead of normal T cells, thereby inducing apoptosis selectively in malignant cells [14]. A separate study by Yang et al. has suggested that the wogonin works as a catalase inhibitor, partly preventing H$_2$O$_2$ loss [41]. Consequently, PLC$_7$-1 is...
activated by above H$_2$O$_2$ sources and converts PIP$_2$ into IP$_3$, which in turn targets IP$_3$ receptor on ER membrane and triggers the release of Ca$^{2+}$ into cytosol [14, 28]. The Ca$^{2+}$ overloading leads to apoptosis via intrinsic pathway by the release of cyt-C, Endo G, and AIF and activation of Caspase-9 [26, 28-29]. The same phenomenon is also observed in malignant hepatoma and glioblastoma cells in the presence of a suitable concentration of wogonin (Fig. 2) [26, 29].

**Induction of apoptosis via endoplasmic reticulum stress (ER-stress) pathway**

Beside effects on mitochondria and Ca$^{2+}$ releasing cascade related in the intrinsic apoptosis pathways, wogonin induces apoptosis in tumor cells via ER-stress pathway [28, 42-43]. ER plays a crucial role in the regulation of intracellular activities besides participating in lipid storage, translational and post-translational modification of proteins [44]. Growing evidences have elucidated the involvement of ER in apoptosis process through its membrane proteins, such as GRP-78, GRP-94, GADD153, Calpain-1, Calpain-2, and ATF-6 (Table 2) [44-45].

ER-stress markers such as GADD153, GRP-78, ATF-6α, Calpain-1 and Calpain-2 are significantly augmented in osteosarcoma cells, along with many other intrinsic apoptosis related proteins within 48 h of wogonin treatment [28]. Tsai et al. have revealed that wogonin is additionally responsible for the ER-stress by the intensification of GRP-78, GRP-94 and Calpain-1 in glioma U251 cells [29]. These results strongly consolidate a hypothesis that wogonin is able to target ER-stress, collaterally inducing intrinsic apoptosis pathway. Besides, Hu et al. have found the activation of multiple branches of ER stress transducers (IRE1α, eIF2α and ATF6) in tumor cells after wogonin treatment [43]. In addition, wogonin induces apoptosis via the activation of JNK pathway (Fig. 2) [46].

Indeed, Ge et al. have observed that the treatment of human malignant neuroblastoma cells with wogonin induces apoptosis via IRE1-α dependent pathway [42]. Along with ER-stress and mitochondrial dysfunction related proteins elevation and increasing the expression of IRE1-α and TRAF2, wogonin also concurrently enhances the activation of ASK1 and JNK in neuroblastoma cells. In contrast, IRE1-α siRNA inhibits anti-tumorigenic effect of wogonin and leads to decreased activation of ASK1 and JNK in tumor cells (Fig. 3). Thus, it is obvious that wogonin targets many pathways, including intrinsic apoptosis induced by the dysfunction of mitochondria or ER-stress or via IRE1α/TRAF2/JNK pathway.

![Fig. 3 Wogonin induces endoplasmic reticulum stress and mitochondrial dysfunction-related apoptosis in tumor cells](image.png)

It is obvious that the efficient dose for treatment depends on the type and stage or the malignance of tumor. The useful dose of wogonin can be calibrated so as to orient desired apoptosis signaling pathway and to safely eliminate tumor cells as a natural disposition from the body (Fig. 2).

**Cell growth inhibition and cell cycle arrest**

Due to the fast growth, malignant cells are likely to alter the expression of cell-cycle proteins in efforts to short the cell-cycle events and CDKs and Cyclins proteins play an important role in such transition [47]. These proteins are...
genetically and epigenetically modulated, and hasten the growth rate in malignant cells by promoting the handover between checkpoints, i.e., G1/S or G2/M. Thus, disruption of those checkpoints or cell cycle arrest is also considered as a therapeutic strategy for cancer treatment.

Several studies have indicated that wogonin interferes with the cell cycle, arresting the cells in G1 phase and induces differentiation leading to decreased malignant cell growth [23, 47-49]. Experiments have demonstrated that wogonin (80 µmol·L⁻¹) increases the number of cells in G1 phase significantly following a 72-h treatment [49]. In addition, the study has also demonstrated that the phosphorylation of proteins serving as negative markers in the cell-division such as PKCδ and p21 are increased while decreasing phosphorylated Rb, a form of inactive Rb protein, which is highly related to cell cycle checkpoint [50-52]. Thus, it is obvious that wogonin modulates PKCδ and inhibits the phosphorylation of protein Rb, thereby inhibiting tumor growth (Table 2). Correspondingly, proteins considered as key regulators of G1-S phase, Cyclin D1/CDK4 for instance, are decreased in a time-dependent manner following treatment with wogonin, while CDK2 is unchanged [49]. Therefore, it is presumable that wogonin arrests the cell cycle in G1 phase through PKCδ phosphorylation, p21 up-regulation, and restricting the Cyclin D1-CDK4/6 checkpoint (Fig. 4).

**Fig. 4** Effects of wogonin and its derivatives on cell cycle in cancer cells

Beside up-regulation of p21 and induction of G1 phase arrest, Lee et al. have reported that the treatment of glioblastoma multiform cells with low concentrations of wogonin (1–50 µmol·L⁻¹) induces AMPK activation, resulting in inhibition of mTOR and 4EBP and inhibiting cell proliferation (Fig. 2) [53-55]. More interestingly, wogonin induces differentiation of human leukemia cells U-937 into granulocyte-like cells and increases CD11b/CD14 ratio; the results are significantly different when compared to all-trans-retinoic acid (ATRA), which is usually prescribed as differentiating inducer in leukemia cells [56]. Hence, the above evidences open a new scope in combination therapy with between chemotherapeutic agents and differentiation agents to diminish cancer cells’ renewal property, especially in cancer stem cells, by targeting cancer cell-cycle and cell proliferation.

**Inhibition of angiogenesis**

The development of tumor requires angiogenesis to generate new blood vessels in order to meet the need of fast proliferation and metastasis. Such a process is complicated and several pro- and anti-angiogenesis factors from blood vessels and endothelial progenitors are involved [57-59]. The well-known factors involved in this vascularization are VEGF proteins and their receptors. Produced in tumor cells, endothelial cells, and TAMs; VEGF helps to recruit endothelial cells, proliferating and migrating to form new blood vessels, and thereby leading to angiogenesis in tumor [49-50]. Thus, inhibition of VEGF/VEGFR is one of the molecular targets in anti-tumor strategies [58-60].

Lu et al. have demonstrated anti-angiogenesis property of wogonin in both in vitro and in vivo experiments [15]. Ex vivo immunohistochemistry analyses of the tumor tissues affirm that the expression of CD31 protein, a marker for malignant endothelial cells in angiogenesis, is dramatically reduced after the treatment with 60 mg·kg⁻¹ of wogonin [60]. Furthermore, the study has also indicated that wogonin can block the effect
of VEGF in HUVECs and the transduction signaling of VEGFR via Akt pathway. Indeed, less phosphorylation of VEGF receptor 2 has been found in the VEGF/Wogonin group as compared to the VEGF/Wogonin group. At the downstream of VEGF/VEGFR2 pathway, the activation of p38 or ERK1/2 is attenuated without any change in their total forms [15]. Such a chain of events suggests that wogonin might be involved in the intervention of VEGF-triggered signaling in the blood vessel. Thus, fewer endothelial cells are able to transform into the EMT phenotype and migrate into the tumor environment for the formation of new blood vessel. Another study has proposed that wogonin also interferes with angiogenesis induced by H$_2$O$_2$, decreasing the ability of new tube formation in endothelial cells [61]. Alike to VEGF-stimulated angiogenesis discussed above, wogonin notably inhibits the H$_2$O$_2$-induced angiogenesis via PTEN/PI3K/Akt axis and hinders the translocation of NF-$\kappa$B, thereby inhibiting the effect of VEGF (Fig. 5).

**Fig. 5** Anti-angiogenesis activity of wogonin in vessel forming endothelial cells

Furthermore, some of the chemokines produced during inflammation are able to promote tumor growth due to their angiogenesis activities in micro-environment [62]. Monocyte chemo-attractant protein 1 (MCP-1) or chemokine (C-C motif) ligand 2 (CCL2) is thought to be the most important chemokine for recruitment of malignant macrophages to the tumor micro-environment [63]. A putative inflammation created by MCP-1 helps to maintain the nutrients supply efficiently for the fast growing tumor, because of its pro-angiogenic activity. Chang et al. have reported that MCP-1 production in endothelial cells is inhibited by the treatment with 30–70 µmol·L$^{-1}$ of wogonin [63-65]. Wogonin targets ERK1/2 and c-Jun amino-terminal kinase pathway in a dose-dependent pattern and in turn, ERK1/2 and c-Jun attenuates the transcription of MCP-1 at activator protein -1 (AP-1) site of promoter, subsequently reducing MCP-1 expression level (Fig. 5).

In addition to suppression of the autocrine secretion of VEGF in endothelial cells, wogonin also inhibits VEGF secretion from tumor cells and push-back angiogenesis process [66]. Under the stimulation of LPS, MCF-7 cells produce more VEGF, resulting in the accelerated tumor development [67-69]. However, exposure to 40 µmol·L$^{-1}$ of wogonin shows a decrease in the level of VEGF secretion from tumor cells. Moreover, wogonin also diminishes the interaction between HUVECs with MCF-7 in both *in vitro* and *in vivo* experiments. The rate of HUVECs migration, new tube formation on matrigel and rat aortic ring micro-vessel sprouting are decreased, resulting in the inhibition of the replenishment supplies from endothelial cells during tumorigenesis [66]. In-depth analysis of the signaling pathway has indicated that the wogonin-induced inhibition of angiogenesis is via TLR4 activation, leading to the attenuation of PI3K/Akt/NF-$\kappa$B activation [66]. It partly reveals the mechanism of the level of decreased VEGF secretion from tumor cells and angiogenesis inhibition, a hallmark feature of tumorigenesis (Fig. 6).
Numerous evidences also indicate that hypoxia has an intimate link with tumor angiogenesis [70]. Once the tumor develops to the size over 1 mm, it requires newer blood supplies, and thereby hypoxia condition is created naturally inside of the tumor [71]. Activated in hypoxia condition, HIF-1α translocates from cytoplasm to the nucleus and interacts with HIF-1β to drive downstream genes such as VEGF gene at HRE site, which is propitiously associated to tumor angiogenesis, invasion, and metastasis [18, 72-73]. Song et al. have observed decreased instability/ translocation of HIF-1α and less VEGF release following the treatment with wogonin [18]. Wogonin significantly reduces the level of HIF-1α under hypoxic condition via provoking prolyl-hydroxylation on HIF-1α and halts the binding of HIF-1α with Hsp90 and its client proteins such as EGFR, CDK4 and Survivin. Thus, the angiogenesis as well as the growth of tumor in vivo are efficiently impeded (Table 2).

Therefore, the use of wogonin as an anti-angiogenesis adjuvant not only blocks the activity of VEGF signaling but also reduces VEGF secretion, which is derived from tumor and endothelial cells. Besides, by halting the MCP-1 expression in endothelial cells, wogonin also helps to ease the MCP-1 mediated-inflammation, and as a result, supports from migrating TAMs for tumor development are efficiently impeded. Wogonin is also involved in hypoxic anti-angiogenesis by inactivating the translocation of HIF-1α at HRE site (Fig. 6). Thus, all above evidences corroborate the properties of wogonin as an anti-angiogenesis agent.

**Effects on invasion and metastasis**

The process of change in morphology and transition to EMT phenotype is strongly correlated with reduced blood supply to rapidly growing malignant cells. This is a multistep process and, as a starting point for metastasis, allows cancer cells to re-organize cytoskeleton to facilitate migration [74]. These migratory cells start an invasion to reach suitable place and colonize to form new tumors. During the EMT process, modulations of various genes related to maintenance of mesenchymal phenotype, such as over expression of N-cadherin and vimentin or decrease of E-cadherin or MMPs, related to ECM breakdown markers, take place. In addition, activation of some transcription factors, including Snail, Slug, Twist, and ZEB1, regulates the EMT process of malignant cells. Therefore, curing cancer essentially involves the inhibition of EMT process at early stage.

Dong et al. have highlighted the inability of wogonin at very low concentrations (1−10 µmol·L⁻¹) to induce apoptosis, but have demonstrated its potential to inhibit the mobility and invasion of human gallbladder carcinoma (GBC-SD) cells via ERK1/2 pathway [75]. There are significant diminutions in both migrating and invading cells (up to 73% and 71%, respectively) within 24h. Further results also indicate that phosphorylation of ERK1/2 is distinctly reduced by the up-regulation of Maspin, a protein thought to be an inhibitor of serine proteinase, and, as a result, downstream targets of ERK1/2, such as MMP-2 or MMP-9, are dramatically suppressed. Furthermore, there is a disruption of PKCδ

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*Fig. 6 Wogonin’s anti-angiogenesis effects under both normoxia and hypoxia conditions*
translocation and subsequently diminished ERK1/2 phosphorylation [76]. Along with both trends, the levels of MMP-2 and MMP-9, which are considered as markers for early metastasis and invasion, are significantly decreased in a concentration-dependent fashion [77].

B16-F10 melanoma cells treated with 60 μmol·L⁻¹ of wogonin reveal the down-regulation of Ras protein and subsequent attenuation of PI3K/Akt and ERK1/2 pathways, followed by the decrease in MMP2 level [20]. Wogonin exposure inhibits the translocation of transcription factors, such as NF-κB and c-Jun/c-Fos complex on MMP2 promoter (Fig. 7). This partly explains the down-regulation of PDKδ protein via Ras signaling as observed by Chen et al. [20, 76, 78].

Fig. 7 Effects of wogonin on angiogenesis, invasion, and EMT signaling in tumor cells

Other observations suggest that wogonin possesses the ability of inhibiting EMT process and metastasis in inflammatory micro-environment [22]. It is well-known that inflammation is one of the elements linked to tumorigenesis [61, 79]. The role of various inflammatory cytokines, such as TNF-α, IL-6, and IL-1β, in promoting cancer growth has been elucidated by various research groups [22, 81-82]. Interaction of IL-6 with IL-6 receptors on tumor cell membrane induces signal transduction via intracellular IL-6R-enclosed JAKs/gp130 complex and activates STAT3 translocation, and in turn regulates down-stream EMT marker genes, including E-cadherin, Snail, and Twist, promoting the EMT [82-83]. Hence, suppression of IL-6-induced STAT3 activation is one of the targets to inhibit cancer metastasis.

Zhao et al. have observed that wogonin at a low concentration (20 µmol·L⁻¹) has the ability to prevent EMT activation via attenuation of STAT3 pathway in tumor cells, when stimulated by IL-6 or THP-1 conditioned medium [22]. Wogonin enhances E-cadherin expression, whereas N-cadherin, vimentin and other EMT-controlling transcription factors such as Snail and Twist are reduced. Moreover, in vivo study confirms in vitro findings by demonstrating down-regulation of STAT3/N-cadherin/vimentin and enhanced E-cadherin level and reduced tumor nodules in wogonin-treated group as compared to the control group [22]. Above evidences uncover the fact that wogonin plays a role in the prevention of tumor metastasis, concretized by less migration of tumor cells and modulating IL-6/STAT3 signaling pathway in inflammatory condition (Fig. 7).

In summary, low level of wogonin is unable to induce tumor apoptosis, but effectively halts tumor invasion via up-regulating Maspin, repressing PKCδ translocation and the expression of MMP2 and MMP9, as well as down-regulating MCP-1 via ERK1/2 and c-Jun pathway. In addition, wogonin could be a metastasis inhibitor via STAT pathway in tumors. Such pathways need further studies in various malignant cells used alone or in combination with other anti-cancer drugs. 

Effects on telomerase activity

It is well-known that human telomerase activity is constituted by hTP1, hTR, and hTERT, and participates in elongation of telomeres in cancer cells, a step to extend lifespan of cancer cells [86]. A study of Snow et al. has indicated that the inhibition of hTP1-hTERT complex could regulate telomerase activity and inhibit cell growth [85-86].
Wogonin is not only responsible for apoptosis, but also inhibits telomerase activity. Indeed, Huang et al. have illustrated that the viability of leukemia HL-60 cells is reduced by nearly 80%, along with increase in Caspase-3 activity and DNA fragmentation, within 24h after the treatment with 100 µmol·L⁻¹ of wogonin [25]. Moreover, the results from telomere repeat amplification protocol (TRAP) assay has also demonstrated that wogonin decreases the telomerase activity at 100 µmol·L⁻¹ [25] (Table 2). The expressions of hTERT, hTP1, and Bcl2 are attenuated at transcriptional level [25]. Besides, C-myc, one of the enhancer at hTERT promoter and responsible for increasing the expression of hTERT, is also down-regulated [25,86]. Thus, it is reasonable to speculate that telomerase with elongation ability is inhibited by wogonin (Fig. 2).

Telomerase has been linked to cancer and ageing [87]. Wogonin participates in anti-oxidant and anti-anxiety via modulation of p53 expression, which in turns has dual impact on ageing [24, 30, 37, 88-93]. However, the role of wogonin in dampening telomerase activity while delaying ageing needs more research.

**Diminution in carcinogenesis via anti-DNA adducts formation**

Enhancement of N-acetyltransferase activity is associated with cancer [94]. N-acetyltransferase (NAT) participates in the metabolism of Arylamine compounds and indirectly intensifies DNA adduct formation in some specific organs due to its ability to convert and generate carcinogenic compounds. The treatment of human pro-myelocytic leukemia HL-60 cells with wogonin reduces the expression of NAT at both transcriptional and translational levels in a dose-dependent fashion, corresponding with a significant inhibition of AAF (N-acetyl-2-aminoflourene) production [94]. Concretely, microarray hybridization shows the down-regulation of NAT gene after treatment with 25 µmol·L⁻¹ of wogonin (Table 2). These results suggest that wogonin inhibits NAT expression, which may help control cancer (Fig. 2).

However, more studies are warranted to explicate the role of wogonin in detoxifying carcinogenic compounds in the body. Besides, the establishment of standard procedure to use wogonin at low dose for such a purpose is also considerable. These studies would open a new promising field to detoxify the carcinogenic compounds by natural flavonoids without side effects.

**Wogonin as a chemosensitizer**

One of the main impediments in cancer treatment is drug resistance [95]. Some cancer cells possess ABC transporter which removes anti-cancer drugs out of the cancer cells via ATP-dependent drug efflux pump, thereby neutralizing the efficacy of cancer treatment [96]. Enomoto et al. and Cheng et al. have demonstrated that wogonin can be used as an adjuvant at low doses (10–30 µmol·L⁻¹) in combination with anti-cancer agents, concomitantly accumulating drugs intracellularly and thereby leading the cells toward apoptosis [97-98]. This is a corollary originated from down-regulation of MDR I gene at both transcriptional and translational levels. Surprisingly, wogonin can protect normal cells from side effects when used in combination with anti-cancer drugs. Etoposide, for instance, in combination with 100 µmol·L⁻¹ of wogonin witnessed very less proportion of apoptosis in bone marrow cells or thymocytes as compared to the control cells without wogonin treatment (Table 3). Similarly, Wang et al. have shown that dihydrodiol dehydrogenase (DDH), which is involved in drug resistance, is suppressed by wogonin at low dose for such a purpose is also considerable.

Table 3  Effects of wogonin when used in combination with other anti-cancer agents for cancer treatment

<table>
<thead>
<tr>
<th>Co-treatment</th>
<th>Wogonin concentration</th>
<th>Cancer cell lines</th>
<th>Major mechanism</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Etoposide (10 µmol·L⁻¹)</td>
<td>100 µmol·L⁻¹</td>
<td>HL-60, Jurkat (Blood)</td>
<td>Inhibition of P-gp activity</td>
<td>[97]</td>
</tr>
<tr>
<td>TNF-α and TRAIL (100 ng·mL⁻¹)</td>
<td>50–100 µmol·L⁻¹</td>
<td>Jurkat, CEM (Blood)</td>
<td>Caspase-8 extrinsic pathway, inhibition of NF-xB</td>
<td>[132]</td>
</tr>
<tr>
<td>TRAIL (50 ng·mL⁻¹)</td>
<td>25–50 µmol·L⁻¹</td>
<td>LNCaP (Prostate), HTLV-1-ATL, MT-2 (Blood)</td>
<td>Both extrinsic and intrinsic apoptosis pathway</td>
<td>[32, 37]</td>
</tr>
<tr>
<td>5-FU (40 µmol·L⁻¹) (2.25 mmol·L⁻¹)</td>
<td>100 µmol·L⁻¹</td>
<td>MGC-803 (Stomach) SMCC-7721 (Liver)</td>
<td>Down-regulation of DPD and NF-xB inhibition of PI3K/Akt pathway, intrinsic apoptosis</td>
<td>[16] [17]</td>
</tr>
<tr>
<td>Cisplatin (10 µmol·L⁻¹) (10-50 µmol·L⁻¹)</td>
<td>5–20 µmol·L⁻¹</td>
<td>A549 (Lung), Hela (Cervix) HTC-116 (Bowel)</td>
<td>H₂O₂ generation, intrinsic apoptosis pathway reducing hypoxia-induced resistance</td>
<td>[144] [121]</td>
</tr>
<tr>
<td>Doxorubicin (10 µmol·L⁻¹)</td>
<td>10–40 µmol·L⁻¹</td>
<td>K562/A02 (Blood)</td>
<td>Nrf2, MR1↓ PI3K/Akt↓ p53↑</td>
<td>[106]</td>
</tr>
</tbody>
</table>

↑: An increase in target protein
↓: A decrease in target protein
In breast cancer treatment, the resistance to doxorubicin is an ominous issue, which decreases its effectiveness. Recently, Zhong et al. have demonstrated the Nrf2 involvement in doxorubicin-resistance as evidenced by the high expression of Nrf2 and downstream genes. The treatment with 60 µmol·L$^{-1}$ of wogonin reduces Nrf2 expression in drug resistant cancer group and consequently its target genes, including detoxification and anti-oxidant genes, are down-regulated (Table 2).

Another study of Xu et al. has indicated that the resistance of doxorubicin in leukemia is associated with MRP1 expression and closely linked with Nrf2 expression. MRP1, belonging to ABCC transporter subfamily, is implicated in the resistance to chemotherapeutic agents in a variety of cancers, such as doxorubicin, vincristine, etoposide and daunorubicin. Indeed, Xu et al. have observed that MRP1 expression is decreased significantly after the treatment with 40 µmol·L$^{-1}$ of wogonin, paralleling to the down-regulation of Nrf2 pathway and its target genes. Furthermore, suppression by PI3K inhibitor or overexpression of PI3K vectors has demonstrated a corresponding change in Nrf2 expression, suggesting that there is an association between Nrf2 and PI3k/Akt axis, resulting in the regulation of down-stream genes such as MRP1, HO-1 and NQO1. These experiments have demonstrated an enhanced chemo-sensitization effect on drug resistant cancer cells, following the treatment with wogonin as adjuvant at appropriate doses (Fig. 8).

Qian et al. have revealed the inhibitory effect of wogonin on hepatoma cell proliferation via suppressing Nrf2 signaling through ROS. Wogonin is one of the well-known intracellular ROS-inducer and studies have indicated that wogonin partly contributes to the elevation of ROS in cytoplasm and suppression of Nrf2. Introduction of tert-butylhydroquinone, an anti-oxidant and Nrf2 inducer reverses wogonin’s effects, confirming mode of action for wogonin. Moreover, Nrf2 expression is inversely correlated with p53 overexpression, and, therefore, it is presumable that the primitive issue of Nrf2 down-regulation is associated with p53 elevation induced by wogonin. Treatment of tumor cells with wogonin decreases the expression of down-stream genes, such as MRP1, MRP2, MRP3, MRP4, and ABCG2, which is correlated with enhanced p53 level, explaining the potentiation of cytotoxic effect on cancer when it is used in combination with hydroxycamptothecin, cisplatin (cis-diamminedichloroplatinum, DDP) or etoposide (Fig. 8).

Overcoming hypoxia-mediated drug resistance

Hypoxia is a micro-environment inside solid tumor, whereby cancer cells are frequently deprived of oxygen and able to maintain the “naïve” of cancer stem cells and activate numerous genes responsible for malignancy. HIF-1α, a nuclear transcription factor, is activated under hypoxia, which is translocated and then activates several genes involved in angiogenesis, invasion, tumor growth, and energy metabolism, especially glycolysis. In addition, several studies have suggested that hypoxia has a strong link with drug resistance in cancer. Recent studies have suggested that Akt

![Fig. 8  Effects of wogonin on both Nrf2/Keap 1 and NF-κB signaling pathways](image-url)
signaling is required for the activation of HIF-1α, leading to tumor growth and metastasis [116]. Thus, inhibition of Akt can be one of the strategies to inhibit HIF-1α to overcome hypoxia-mediated drug resistance.

Wang et al. have revealed that wogonin at concentration of 100 µmol·L⁻¹ reduces the expression of HIF-1α under hypoxia condition dramatically, along with inhibition of downstream genes such as hexokinase II (HKII), pyruvate dehydrogenase kinase 1 (PDHK1), and lactate dehydrogenase A (LDHA) [21]. As a result, the lactate generation and glucose uptake are decreased, leading to reduced glycolysis in cancer cells. Furthermore, Wang et al. have also demonstrated that, in the presence or absence of IGF-1, a ligand for tyrosine kinase receptor, wogonin is able to inhibit the activation of HIF-1α through PI3K/Akt pathway, efficiently reversing the cisplatin resistance in colon cancer (Fig. 6). Correspondingly, in vivo study has consistently indicated the wogonin-mediated tumor inhibition even under hypoxia condition, with less alteration in body weight [21]. Thus, the above evidences suggest the therapeutic effect of wogonin under hypoxic condition is via modulation of signaling process, substantiating its role as adjunct therapy to prevent drug resistance induced by hypoxia.

Obviously, minimizing the side effects during cancer treatment is a top priority while enhancing therapeutic activity and it is conceivable to consider that low-dose wogonin can be an ideal candidate to enhance the effects of anti-cancer drugs while reducing the dose needed.

**Inflammatory and immune regulations against cancer**

TGF-β1 is a well-known cytokine suppressing immune-defense system, leading to the tolerance in T cells with malignancy. TGF-β1 has also been considered as a hallmark of tumor. By cell to cell interaction or secretion of TGF-1β and IL-10, tumor cells can modulate regulatory T cells (T reg), dendritic cells [117]. Through this mechanism, tumor cells can escape from the natural immune defense system with tolerance development. In addition, many reports have claimed that the TGF-β1 could be a trigger of EMT and other metastasis. TGF-β1 reduces the expression of HIF-1α of LPS [19]. In an in vivo colitis-associated cancer model, wogonin effectively impedes the impact of chemical-induced inflammation and pro-inflammatory mediators, such as IL-6 and IL-1β in both tumor and surrounding tissues [19]. These evidences suggest that wogonin can protect the surrounding tissues from inflammation and cancer metastasis. Accordingly, NF-xB, a shuttle between inflammation and tumorigenesis, is down-regulated [79]. In vitro studies in HCT116 cells affirm the above findings, and wogonin is able to halt the proliferation even in the presence of 10 µg·mL⁻¹ of LPS [19].

In terms of signaling pathway, all evidences end up with a conclusion that wogonin (50 µmol·L⁻¹) targets NF-xB pathway by suppressing the activity of IKK, and as a result, IκB, a repressor of NF-xB, encloses the nuclear-translocation. Wogonin promotes IKK activity and thereby counteracts the effect of Akt (Fig. 8) [19]. Taken together, these pathways lead to a down-regulation of downstream genes related to inflammation and tumorigenesis following wogonin treatment.

Furthermore, growing evidences suggest that the maintenance of inflammatory environment is essential inside the tumor via the presence of TAMs [118-120]. This helps enhance angiogenesis and trigger metastasis. In an attempt to decrease the impact of inflammation on tumor, a driver for detoxifying and antioxidant genes, Nrf2 is found to be up-regulated after wogonin treatment [19]. The results show that wogonin promotes Nrf2 activity at ARE site in the nucleus of HTC-116 cells and acute monocytic leukemia THP-1 cells; those cells are considered as an emblem for TAMs in tumor environment for in vitro studies [121-122]. Subsequently, inflammatory agents such as reactive quinine and reactive peroxides dramatically decreased or turned into other metabolites which are safe, water-soluble, less harmful and readily excreted in the condition of inflammation [123-124]. Concurrently, the NF-xB activity in nucleus is decreased, due to reduced phosphorylation of IKK and IκB. This response significantly intercepts either the production of IL-6 and IL-1β in THP-1 cells or the interaction between THP-1 cells and colon cancer cells, resulting in an inhibition of inflammation and prevention of colitis-related colon cancer [19].

The molecular link between Nrf2 and NF-xB is not fully understood. Several studies have documented the contrary relation between NF-xB and Nrf2 [125-126]. Ordinarily, Nrf2 promotes tumor survival and development [105-106]. On the
other hand, Nrf2 acts as a transcriptional factor for detoxification and antioxidant genes and helps to reduce the pathological effects of inflammatory agents in tumorigenesis [124]. Studies in an inflammation-associated cancer model denote the enhancement of Nrf2 activity following wogonin treatment [109]. Possibly, ROS produced by wogonin treatment could induce Nrf2 activation and, subsequently, such event partly constrains the effect of NF-κB [123, 127]. Besides, wogonin is also a well-known inhibitor of NF-κB, leading to the subduction of inflammation-associated cancer via down-regulation of NF-κB and induction of Nrf2 signaling pathway [16, 19, 41, 66]. However, it needs further experiments to validate the findings and to get new insight in overall about the relation between inflammation and tumorigenesis in attempt to cure cancer more effectively.

**Wogonin as an adjunct therapy**

Despite revolutionary efforts around the world for developing new and effective therapies for cancer treatment, their anti-tumor efficiency is still limited. Various strategies to combine conventional therapies with other agents have been considered and obtained satisfactory results, compared to single agent therapies [128-131].

Several studies have been performed incorporating wogonin with other anticancer agents to treat cancer cells in vitro and tumors in vivo [16-17, 132]. For instance, Fas et al. have used wogonin to overcome the resistance to TNF-α and TRAIL in leukemia cells, without side effects on normal cells [132]. TNF-α acts as double edged sword: at one side it promotes inflammation via activation of NF-κB, one of the major factors regulating the expression of anti-apoptotic proteins, and contributing to the survival and anti-apoptosis of tumor [133-134]; on the other side, TNF-α serves as an apoptosis-inducer. Therefore, its contradictory roles in cancer therapy may explain unsatisfactory outcomes observed in experiments against malignant cells [81]. Wogonin used in conjunction with TNF-α decreases IκB phosphorylation and reduces NF-κB activation, prompting the effects of TNF-α towards cure cancer [132]. Similarly, wogonin also sensitizes leukemia cells to TRAIL and potentiates apoptosis through the extrinsic pathway (Table 3).

The combination between TRAIL and wogonin also provokes another apoptosis pathway related to up-regulation of p53 and PUMA, which is mediated by ROS, leading to activation of series of Caspases (Caspase-9, Caspase-8 and Caspase-3) in TRAIL-resistant human prostate cancer LNCaP cells [37]. Interestingly, Ding et al. have provided evidence supporting that wogonin enhances the effect of TRAIL on malignant T cells by down-regulation of c-FLIP, an anti-apoptotic factor overexpressed in some tumor cells counteracting the TRAIL effect [32, 135]. However, there is no association between c-FLIP down-regulation by NF-κB and Tax, a trans-activator originated from human T-cell leukemia virus type-I (HTLV-1) [32]. Thus, it is a probable that c-FLIP is repressed by wogonin, which would be correlated with NF-κB down-regulation. Another possible mechanism would be the conversion of free oxygen radicals (O₂ ·), responsible for NF-κB activation, to H₂O₂ by wogonin, leading to c-FLIP down-regulation (Fig. 2) [43, 136-137]. Wogonin used in combination with TRAIL significantly enhances apoptosis and the mechanism is ascribed to increased stabilization of p53 by TRAIL receptor 2 (TRAIL-R2) and death receptor 5 (DR5) (Table 3) [32, 138].

5-FU, first introduced in 1957 for solid tumor treatment, has been widely used to inhibit the proliferation of tumors due to its property of targeting the DNA synthesis [139]. However, along with various side effects, it is less effective as an anti-tumor agent, because of the presence of drug-resistant phenotypes in tumor cells [140]. Thus, finding new anticancer drugs and new therapies now is extremely urgent in current scenario.

Zhao et al. have attempted a combination between wogonin and low-dose of 5-FU in order to minimize the side effects and increase the efficiency of treatment in both in vitro and in vivo models [160]. The combination brings a higher percentage of human gastric cancer MGC-803 cells into apoptotic phase via more PARP cleavage, Bax inactivation, with undetectable level of intracellular pro-caspase-3 and Bcl2. In addition, the expression of Dihydropyrimidin dehydrogenase (DPD), an enzyme tightly involved in 5-FU-resistance of cancer, is also decreased (Table 3). Besides attenuating NF-κB expression in in vitro, the in vivo combination study (60 mg·kg⁻¹ of wogonin plus 10 mg·kg⁻¹ of 5-FU) also significantly inhibits tumor growth. However, this combination does not alter other enzymes such as orotate phospho-ribosyltransferase and thymidylate synthetase [160].

Recently, another group has demonstrated an enhanced efficacy of wogonin in combination with 5-FU in hepatocellular carcinoma cells with high COX-2 expression [17]. Numerous evidences have implicated the role of COX-2 in tumorigenesis, which has been identified as a new target for cancer therapy and inflammation management [141-142]. In hepatocellular carcinoma SMMC-7721 cells expressing higher COX-2, the combination of wogonin and 5-FU interferes the PI3K/Akt axis and decreases NF-κB translocation and COX-2 expression, thereby inhibiting malignant cell proliferation [17]. The combination also enhances the activity of PTEN, a tumor repressor involved in PI3K/Akt pathway, and as a result, the phosphorylated proteins, such as pAkt, pIkB and pIKK, are partly decreased, facilitating apoptosis in intrinsic pathway [143].

The synergism between wogonin and cisplatin is also observed in non-small cell lung cancer A549 cells and cervical cancer HeLa cells [144]. Although widely used as a chemotherapeutic agent for treatment in a variety of solid tumors, numerous incidences of chemo-resistance against...
cisplatin have been recorded [145]. However, He et al. have indicated that low concentration of wogonin (5–20 µmol·L⁻¹) potentiates cisplatin cytotoxicity and enhances intrinsic apoptosis in tumor cells [144]. By accumulating intracellular reactive oxygen species, wogonin helps to drive the activation of Caspase-3 and PARP, leading to a significant increase in cell death, as compared to cisplatin treatment alone. This evidence affirms distinctly the potential use of wogonin in cisplatin resistant cancer, whereby the treatment efficiency is notably heightened.

The above evidences suggest the synergistic effect of wogonin in combination with anti-cancer drugs, without any side effect. Further experiments are required to confirm above findings. Moreover, the combination index between low-dose wogonin and anticancer drugs needs to be determined preclinically and choosing the best candidate anti-cancer drugs is needed, which can be worked out in details in ex vivo studies before moving to clinical trial.

**Wogonin derivatives**

As discussed above, wogonin has been demonstrated to have anti-cancer effects in variety of malignancies, including breast cancer, hepatoma, gastric cancer, osteosarcoma, leukemia, and cervical carcinoma [6, 14, 25-30, 72, 109]. However, the major setback for its use as anticancer agent is its low water solubility and less effect in certain type of tumors [150]. Thus, it is necessary to develop new wogonin analogues with similar or enhanced anti-tumor activity and minimal side effects. For these reasons, a few wogonin derivatives have been developed [146-150]. The following section will discuss several lead compounds.

**LYG-202**

Chen et al. have synthesized flavonoid-LYG-202 based on wogonin structure, which induces significant apoptosis in hepatocellular carcinoma cells (Fig. 1) [147]. LYG-202 (20–40 µmol·L⁻¹) helps increase intracellular ROS tremendously and the proportion of apoptotic cells. Moreover, this compound also significantly induces the loss of mitochondrial membrane potential and intracellular ATP, directing mitochondria-related apoptosis, as evidenced by the increase of AIF and cyt-C in cytoplasm, followed by the activation of Caspase 3, Caspase 9, and PARP. In addition, there is up-regulation of phosphorylated proteins, such as JNK, ERK1/2 and p38, suggesting that the effect of LYG-202 on hepatocellular carcinoma cells is also associated with the MAPK/JNK signaling pathway (Table 4) [151].

**Table 4  Wogonin derivatives and synthesized flavonoids with anti-tumor activities**

<table>
<thead>
<tr>
<th>Derivatives &amp; Concentrations of treatment</th>
<th>Cancer cell lines</th>
<th>Major mechanisms</th>
<th>Effects</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>LYG-202 20–40 µmol·L⁻¹</td>
<td>Hepatocellular carcinoma HepG2</td>
<td>ROS↑, Δψm↓, Release of cyt-C/AIF, MAPK/JNK/p38↑</td>
<td>Intrinsic apoptosis, mitochondrial dysfunction</td>
<td>[147]</td>
</tr>
<tr>
<td>LYG-202 22 µmol·L⁻¹</td>
<td>Colorectal carcinoma HT116</td>
<td>CDK2/4, Cyclin D1 &amp; Cyclin E↓, Rb/P21/p53↑</td>
<td>G1/S phase arrest, Intrinsic apoptosis</td>
<td>[148]</td>
</tr>
<tr>
<td>LW-214 15–50 µmol·L⁻¹</td>
<td>Breast cancer MCF-7</td>
<td>Trx-1/ Bcl2↓, ROS/ASK1/JNK↑</td>
<td>Intrinsic apoptosis, mitochondrial dysfunction</td>
<td>[149]</td>
</tr>
<tr>
<td>LL-202 8–25 µmol·L⁻¹</td>
<td>Breast cancer MCF-7</td>
<td>CyclinA/CyclinB1/CDK1↓, P21, Bid↑, Cleavage Caspase 3/9↑, degradation of PARP</td>
<td>G2/M phase arrest, mitochondrial dysfunction, Cell proliferation inhibited, intrinsic apoptosis</td>
<td>[150]</td>
</tr>
<tr>
<td>LZ-207 20–100 µmol·L⁻¹</td>
<td>Breast cancer MCF-7</td>
<td>Cleavage Caspase 3/9↑, PARP, Bcl2↓, Release of cyt-C, NF-κB/pIKK/pIκB, pro-inflammatory cytokines production in TAMs↓</td>
<td>Intrinsic apoptosis Decrease of cancer related inflammation</td>
<td>[146]</td>
</tr>
</tbody>
</table>

↑: An increase in target protein  
↓: A decrease in target protein

Furthermore, another study has shown that LYG-202 helps arrest cells at G1/S phase and induces apoptosis in human colorectal carcinoma HCT-116 [148]. Low dose of LYG-202 significantly increases the number of cells in G1 phase as compared to control group, leading to the attenuation of cell proliferation. Treatment with LYG-202...
Table 5 Pharmacokinetic parameters of wogonin and its formulations in rats

<table>
<thead>
<tr>
<th>Dosage</th>
<th>T_{1/2}/min</th>
<th>V_d/(L·kg⁻¹)</th>
<th>C_{max}/(mg·L⁻¹)</th>
<th>CL/(L·min⁻¹·kg⁻¹)</th>
<th>AUC_{0-∞}/(mg·min·L⁻¹)</th>
<th>MRT/min</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wogonin 10 mg·kg⁻¹ (i.v)</td>
<td>14.13 ± 4.22</td>
<td>2.9 ± 1.61</td>
<td>7.12 ± 1.22</td>
<td>0.09 ± 0.02</td>
<td>112.13 ± 18.92</td>
<td>8.15 ± 5.72</td>
<td>[159]</td>
</tr>
<tr>
<td>Wogonin 20 mg·kg⁻¹ (i.v)</td>
<td>14.58 ± 5.27</td>
<td>1.52 ± 0.96</td>
<td>19.13 ± 3.06</td>
<td>0.07 ± 0.01</td>
<td>286.75 ± 55.03</td>
<td>6.43 ± 2.94</td>
<td>[159]</td>
</tr>
<tr>
<td>Wogonin 40 mg·kg⁻¹ (i.v)</td>
<td>13.44 ± 3.55</td>
<td>1.03 ± 0.27</td>
<td>43.08 ± 6.14</td>
<td>0.05 ± 0.01</td>
<td>758.19 ± 26.91</td>
<td>6.55 ± 0.71</td>
<td>[159]</td>
</tr>
<tr>
<td>Wogonin 100 mg·kg⁻¹ (i.v)</td>
<td>27.97 ± 4.73*</td>
<td>24.19 ± 60.2*</td>
<td>0.3 ± 0.08*</td>
<td>5.97 ± 0.87*</td>
<td>17.02 ± 2.12</td>
<td>n/a</td>
<td>[159]</td>
</tr>
<tr>
<td>Wogonin-albumin sphere 4 mg·kg⁻¹ (i.v)</td>
<td>33 ± 7.68*</td>
<td>n/a</td>
<td>n/a</td>
<td>&lt; 0.000 001b</td>
<td>7575.8 ± 570b</td>
<td>n/a</td>
<td>[166]</td>
</tr>
<tr>
<td>Wogonin-SLN 4 mg·kg⁻¹ (i.v)</td>
<td>33.06 ± 7.48*</td>
<td>n/a</td>
<td>n/a</td>
<td>&lt; 0.000 001c</td>
<td>7545.6 ± 574c</td>
<td>n/a</td>
<td>[168]</td>
</tr>
</tbody>
</table>

\( T_{1/2}/\text{min} \): eliminated half life time; \( V_d/(L·kg^{-1}) \): Volume of distribution; \( C_{max}/(mg·L^{-1}) \): peak concentration of drug in plasma; \( CL/(L·min^{-1}·kg^{-1}) \): apparent clearance; \( AUC_{0-\infty}/(mg·min·L^{-1}) \): Area under the curve; \( MRT/min \): mean residence time; SLN: solid lipid nanoparticle; i.v: intravenous injection; i.g: intragastric injection; a: data are relative to oral bioavailability (\( F = 1.1\% ± 0.18\% \)); b, c: data are re-calculated to be consistent with units in table; n/a: not available

** LW-214 **

Human breast cancer MCF-7 cells treated with LW-214, another analog of wogonin (Fig. 1), show several signs of mitochondria-related intrinsic apoptosis, such as loss of mitochondrial membrane potential (ΔΨm), elevation of Bax, and cyt-C release as well as AIF transposition [149]. Low dose of LW-214 also decreases the stability of Trx/ASK1 complex, which helps activate ASK1 in free form, resulting in JNK-induced apoptosis [149, 151].

Known as a major antioxidant protein to maintain the intracellular redox state, Trx-1 annihilates ROS, which is necessary for the apoptosis induced by wogonin and its analogues [29, 37, 147, 149]. Once oxidized by LW-214, Trx-1 becomes inactive and unable to decrease intracellular ROS content. Besides, ROS is believed as an inducer for JNK-related apoptosis [151], and therefore, ROS induced by the suppression of Trx-1 partly contributes to increase in cytotoxic effect in tumor [149]. More interestingly, LW-214 (10 mg·kg⁻¹) generated the ideal result during in vivo nude mouse study, compared with Paclitaxel (10 mg·kg⁻¹), without changing body weight. These evidences demonstrate the potential for LW-214 in cancer treatment (Table 3).

** LL-202 **

LL-202, a newly synthesized analog of wogonin (Fig. 1), shows anti-cancer activities in human breast cancer MCF-7 cells in time- and concentration-dependent manner [159]. Low dose of LL-202 (15–25 μmol·L⁻¹) promotes mitochondria dysfunction, mediates apoptosis, and inhibits the expression of cell cycle proteins like cyclin B1, cyclin A, and CDK1, while up-regulating p21 translation, resulting in G2/M phase arrest (Fig. 4). Moreover, LL-202 also effectively induces intracellular ROS and decreases mitochondrial membrane potential, as compared to wogonin. In addition, LYG-202 highly up-regulates the expression of p53, a master protein tightly controlling the expression of p21 and regulating G1/S phase in cell cycle, partly explaining how LYG-202 works to arrest cell at G1/S phase and induce apoptosis (Fig. 4) [155, 152].
study, LZ-207 promotes the decreases in both tumor volume and weight, while the morphology of normal organs and body weights remain unchanged. These evidences fortifies that LZ-207 might be a promising synthesized flavonoid with remarkable anti-tumor activity (Table 4).

Obviously, the convincible achievements evidenced by studies with LYG-202, LW-214, LL-202 and LZ-207 have urged to exploit the new strategies of using wogonin analogues. However, it is required to have more supporting studies to discover the optimal wogonin derivative with high and broad anti-tumor activities, no side effects, as well as high water-solubility. Despite few “dark sides” in wogonin-derivatives-based therapies, wogonin at present is one of the safe options for further studies to identify new anticancer drugs for clinical use.

Wogonin Pharmacokinetics

Studies about pharmacokinetics of drugs are essential for understanding the in vivo toxicological and pharmacological impacts in the body. Hence, knowledge about risky potentials or bioavailability of drugs would facilitate to examine the treatment efficiency. Pharmacokinetics is constituted by the liberation, absorption, distribution as well as metabolism and excretion of drug in the body. Well-understanding of drug pharmacokinetics helps establish the appropriate dose and frequency of treatment and avoid the unwanted effects during clinical application.

Wogonin can be administrated by oral (p.o.) or intravenous (i.v.) routes and its concentration in plasma can be measured by liquid chromatography [155-156]. Indeed, Tian et al. have indicated that wogonin binds highly and reversibly to subdomain IIA of human serum albumin (HSA) in plasma by hydrophobic force and hydrogen bond between the hydroxyl residue of wogonin and the Arg 257 of HSA, whereas this protein serves as a drug-transport carrier in circulation system, and as a result, wogonin displays a rapid absorption and distribution in the body [157-159]. Hou et al. have revealed that the wogonin is mainly accumulated in the liver, kidneys and lungs after oral gavage administration of S. baikalensis radix [158]. The dosing schedule (2 g·kg⁻¹ thrice daily for seven doses) in rats would allow enough time for wogonin transportation through HSA to different organs, leading to undetectable concentration in rat plasma. However, wogonin glucuronide and sulfate are detected in plasma, kidneys, and liver. These evidences point towards that unbound fraction of wogonin, which is considered to exhibit pharmacological effects, is partly metabolized in the liver, followed by circulation in plasma and excretion from kidneys [160].

Reinforcing such evidences, Talbi et al. have administrated 20 mg·kg⁻¹ of wogonin intravenously into rats and found that there is early dispersion of wogonin within 10 min post-intravenous injection into almost all organs, except for brain and fat tissues, and the main sites are liver, lungs and kidneys [159]. Furthermore, the similar amount of wogonin is found in testis compared to heart, spleen, and stomach. This observation suggests that wogonin could effectively displace through blood-testis barrier. This finding may be valuable for studies related to testis cancer. However, at 60 and 120 min after doing, the wogonin levels in the organs are decreased. The study suggests that the elimination-half life time for i.v. administration at 10, 20, 40 mg·kg⁻¹ concentration is short, approximately 14 min, followed by a disproportional increase in area under curve (AUC₀-∞). The results reveal high peak drug concentration (Cₘₐₓ) in plasma and high apparent clearance, following i.v. administration of wogonin. Thus, above findings elaborate that administrated wogonin is readily absorbed following injection and also rapidly distributed in almost all organs, followed by quick elimination. Further studies also reveal that unconverted form (approximately 21%) is found in feces, urine, and bile, partly contributing to the wogonin elimination following administration.

Wogonin has low oral bioavailability (1.1% after intragastric administration at 100 mg·kg⁻¹), because of various hindrances, including quick elimination and excretion of unconverted form via feces, urine, and bile, abundant loss during liver metabolism, and inactivation during interaction with HSA [159-160]. However, the lack of correlation between excretion ratio (21%) and wogonin binding with HSA (over 90%) suggests that the bound fraction in HSA-wogonin complex may act as a reservoir or depot and thereby maintain the equilibrium. It is optimistic to develop new approach to target the binding between HSA and wogonin, in order to intensify the unbound fraction at an appropriate time and to increase its pharmacological effects.

Another approach to modulate the HSA-drug interaction is simultaneous administration of two or more compounds that are HSA-binding drugs. The competition between them for HSA interaction may increase the proportion of unbound fraction and bioavailability [161]. Zou et al. have reported that the constituents of Huangqin-Tang compound prescription help delay absorption and elimination and increase mean residence time, Cₘₐₓ as well as AUCₐ₀-∞ as compared to single herb decoction [162]. These may increase the bioavailability of each constituent in Huangqin-Tang. However, other compounds, such as baicalin, baicalein, and chrypsin, also potentiate anti-cancer effects and the combination ratio between them and wogonin to enhance bioavailability of each other is still a void space which needs to be filled by further studies.

Besides, new approaches include the use of natural drug-carrier in plasma to become more efficient drug carrier [163-164]. These methods could be the coupling of low-molecular weight drugs to exogenous or endogenous albumin, the conjugation of drug and bioactive proteins, or the encapsulation of drugs with albumin or lipid nanoparticles. Chen et al. have successfully developed wogonin-albumin microspheres and reported significantly improved wogonin pharmacokinetics in rats via increases in elimination-half life and AUCₐ₀-∞ as well as decrease in apparent clearance [165-166]. Another group has prepared wogonin loaded solid lipid
nanoparticles and in vitro studies have demonstrated 80% drug release within 48h and increased wogonin bioavailability in a rat model [167-168]. Furthermore, the study also indicates the presence of wogonin in different rat organs, such as liver, spleen, heart, lungs and kidneys. These results suggest a new style in wogonin delivery to tumor sites, without alteration of its bioavailability.

**Side Effects Associated with Wogonin Therapy**

Many reports have confirmed that wogonin in the safe range (10–100 µmol·L⁻¹) can induce apoptosis in various cancer cells without any cytotoxicity to normal cells [14, 24, 26, 35, 43, 169-170]. Moreover, numerous in vivo studies have shown that wogonin effectively and safely eliminates tumor without altering body weight [6, 14-18, 20-22, 27]. However, there are very few studies exploring therapeutic index with respect to wogonin use and it is important to document the effect of an overdose on the living animals before entering into clinical trial.

Non-experimented clinical evidences have recorded some side effects that *S. baicalensis* may cause, such as drowsiness, confusion or giddiness in patients. Chang and But have reported that *S. baicalensis* induces raregastric discomfort and diarrhea if taken in high doses [171]. Recently, Tian *et al.* have examined the effect of *S. baicalensis* on embryonic development in mice and the study showed that the aqueous extract at the dose of or below 32 g·kg⁻¹·d⁻¹ to ICR mice during organogenesis, did not significantly change fetal external or skeletal malformations [172]. However, 32 g·kg⁻¹·d⁻¹ presented potential maternal toxicity (Fig. 9). This study raises the issue of more detailed safety studies needed to explore convincible safety profile with acceptable pharmacodynamics to endorse its use for future cancer therapy.

Existed as one of the main and active ingredients from *S. baicalensis* possessing therapeutic properties, wogonin also has been examined for toxicity, a critical step before clinical application. Qi *et al.* have documented that the general indicator of a substance’s acute toxicity -lethal dose (LD₅₀) of wogonin via intravenous injection (i.v.) in mice is 286.15 mg·kg⁻¹ and the confidence limit is 278.27–295.26 mg·kg⁻¹ [172]. The results bolster its relative safety in clinic. However, rats dosed with wogonin (120 mg·kg⁻¹) induces heart injury and other signs like myocarditis, inflammatory cell infiltration or side effects such as tachypnoea, astasia, blepharon retraction, while there is no significant change in body weight. Furthermore, although red blood cells and platelets counts are decreased significantly after wogonin treatment, all plasma biochemical parameters fall within normal range in long-term exposure for 90 days, except for creatinine phosphatase, in the dose range of 60–120 mg·kg⁻¹. In term of organs, no pathological findings or changes in organ/bodyweight ratios are observed. This study is an initial step to define the non-toxic dosage of wogonin administered via intravenous injection, which is a foundation for future clinicaltrial of wogonin-based therapy (Fig. 9).

Peng *et al.* have attested the innocuousness of wogonin on dogs administered intravenously in dose range of 15–60 mg·kg⁻¹ [174]. Alike to experiments in mice and rats, dogs treated with wogonin show no changes in organ/bodyweight ratio ormorphology or the levels of plasma biochemical parameters after long-term treatment (90 days), compared to the control group. This non-organ dosage is much higher than that used for human trials (50 mg/60 kg), anticipating a safe zone for further human clinical research.

![Dose-dependent toxicities of Wogonin following intravenous administration in rats and safe dose range for human clinical](image-url)

**Fig. 9** Dose-dependent toxicities of Wogonin following intravenous administration in rats and safe dose range for human clinical
Proceeding to potential toxicity of wogonin and based on its safe properties in mature animals, Zhao et al. have further examined the in vivo impact of wogonin on the development of fetus in pregnant mice [175]. The study shows that i.v. dose of 40 mg·kg⁻¹ causes the maternal weight gains and affects significantly fetus development, including body weight, resorptions, live birth index, fetal skeletal alterations as well as fetal genotoxicity. However, there is no mutagenicity or positive findings in vitro and in vivo micronucleus assays following wogonin treatment. Although wogonin induces in vitro weak potential of chromosome aberration, low dose of wogonin is a relative safe drug described for sensitive cases during pregnancy [175]. Thus, upon the targets of treatment, wogonin concentration should be fine-tuned to obtain the desired efficiency (Fig. 9).

Recently, the convincible results indicate that newly synthesized flavonoids derived from wogonin have shown their safety and application potential as anticancer drugs in vitro and in vivo [146-150]. Furthermore, those derivatives are more water-soluble which is considered to increase its absorption in the body. However, it is necessary to elucidate safety profile and possible side effects of these new drugs before applying for potential cancer therapy. Moreover, pharmacokinetics of these compounds should be investigated before clinical treatment.

Conclusion Remarks and Future Perspectives

Association of conventional anti-cancer therapies with numerous side effects to healthy cells as well as other distant organs has drawn great attention from biomedical scientists. Quest for safer medicine is the top priority and to pursue the same, many endeavors have been exploited. The great vogue in current cancer treatment is extracts from herbs which contain single or mixture of therapeutic compounds. The radix of S. baicalensis, a remedy traditional Chinese medicine, has been prescribed since ancient era and possesses anti-tumor, hepato-protective, anti-oxidant, anti-inflammatory, anticonvulsant, anti-bacterial, and anti-viral effects, with low toxicity to humans [4-5]. Present as one of the main active components in the root of S. baicalensis, wogonin, along with baicalein, baicalin, and minor flavonoids such as chrysulin, luteolin, and apigenin, has potential anti-tumor effects by engaging various pathways, such as those involved in mitochondrial induced apoptosis, anti-angiogenesis and invasion, cell-cycle arrest, and anti-drug resistance [12, 176-180].

Many researchers have tried to adequately evaluate wogonin as a potential agent for cancer therapy and as an adjunct therapy with an expectation to bring a new hope to treat cancer. With initial achievements in in vitro and in vivo studies, it can be stated that wogonin has a broad spectrum of anti-tumor properties targeting different types of cancers utilizing numerous pathways. Besides, wogonin is involved in inhibition of angiogenesis, invasion, and metastasis, halting tumor growth, and inducing cell-cycle arrest as well as modulating telomerase activity. Multidrug resistance is significantly repressed by down-regulation of drug resistant genes, like Nrf2, MRP s, ABCG2 or hypoxic genes [102, 106, 109]. Furthermore, wogonin enhances treatment efficacy of conventional anti-cancer drugs. However, the general picture of changes in signaling pathways following wogonin treatment is not fully elucidated and needs further studies. For instance, wogonin induces Nrf2 activation in inflammation-related cancer models and subdues its cancer-promoting activities efficiently in drug resistance models [19, 102, 106, 109]. A better understanding can open a new scope to exploit wogonin properties in anti-cancer therapy. Furthermore, the triangle relationship among wogonin, Nrf2, and p53 is also heeded. Many evidences support that wogonin helps elevate intracellular p53 in tumor cells while p53 has dual impact on Nrf2-mediated survival responses [14, 29, 38, 111]. In inducing phase, relatively low p53 supports the expression of Nrf2 whereas higher p53 expression inhibits it. These events partly explain the different impacts of wogonin in drug resistance model and inflammation-related cancer model. However, all these interpretations are presumable and more studies are warranted to verify such supposition.

In addition, Wang et al. have observed that wogonin is able to down-regulate glycolysis in cancer cells [21]. Playing an important role in metabolism during malignant transformation, extraordinary glycolysis in most cancer cells attempts to meet large demand of ATP generation and, as a result, this metabolic event could be a preferred target in cancer therapy [181]. By inactivating PI3K/Akt/HIF-1α, wogonin impacts on glucose uptake, lactate generation, and glycolysis-related proteins, leading to suppression of tumor growth. However, there are some potential problems which may be a concern for glycolysis based therapies. It is obvious that some normal tissues also metabolize glycolysis-derived products as the main energy source. Future studies should address whether normal tissues will also be influenced by wogonin-induced glycolysis inhibition and by how wogonin targets selectively on malignant cells instead of normal cells.

Moreover, there are a few potential issues necessary to be cleared before entering into clinical trials. Wogonin analogs have been developed because of wogonin low solubility and weak cytotoxic effect in some types of tumors. Although derivatives are more dispersible and display higher efficacy both in vitro and in vivo, pharmacokinetic studies are warranted to answer the questions pertaining to their bio-distribution, bioavailability, and impact on other organs in the body. In addition, process of resonant effect between wogonin or its derivatives and other specific therapies in different stages or types of cancers needs to be elucidated in detail. The ideal flavonoid should meet basic expectations: no side effects, overcoming drug resistance, high solubility, and possessing anti-tumor efficacy. Thus, more studies should be warranted to discover the genuine evidences for wogonin-based antitumor therapies. We firmly believe that, once
harmonizing those requirements in a synthesized flavonoid, it would be a great achievement against broad spectrum of tumors, heading to “various cancers cured by one remedy”.

While waiting-and-seeing the emergence of ideal flavonoids, wogonin is a promising approach and is relatively safe. A good understanding of the pathways modulated by wogonin will help achieve desired outcomes, depending on tumor stages and the situation of patients. For instance, after diagnosis of tumor type as well as tumor size, one of the urgent aims is to prevent the metastasis and angiogenesis. By future convincible clinical trials, appropriate dose of wogonin would stop tumor spread and extend the lifespan of patients. Furthermore, serving as a good adjuvant anti-cancer drugs, the combination of wogonin and other agents might bring a new hope for cancer treatment, with minimal side effects.

Development of new drugs requires detailed studies on pharmacokinetics and side effects, determining its further clinical application. High dosage and long-term use of wogonin may cause side effects including organ injuries, while its low dose may induce low pharmacological effects, due to its low bioavailability. Therefore, development of new delivery system to introduce wogonin toward tumor sites without loss is the need of hour. Albumin and lipoprotein are natural drug carriers in circulation system and are considered as best candidate to improve pharmacological effects of drugs with low bioavailability such as wogonin. With initial achievements, bioavailability of wogonin has been notably improved by the increase in elimination time or $AUC_{0-\infty}$ and the decrease in drug apparent clearance. Yet, there are wide gaps in our knowledge about drug delivery and design of nanoparticles, which are very important for further clinical trials.

Despite less understanding of wogonin doses used alone or in combination with other agents for tumors at different stages, there is no doubt that the usage of wogonin would be dynamic. Moreover, starting from a precept “prevention is much better than treatment”, wogonin can probably be developed as a supplement in beverages, whereby users can select varieties of drinks to boost the health, or can be used as an oral remedy for cancer treatment.

**Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>4EBP</td>
<td>Phosphorylation of 4E-binding protein 1</td>
</tr>
<tr>
<td>5FU</td>
<td>5-Fluorouracil</td>
</tr>
<tr>
<td>ABC</td>
<td>ATP-binding cassette</td>
</tr>
<tr>
<td>AIF</td>
<td>Apoptosis-induced factor</td>
</tr>
<tr>
<td>AMPK</td>
<td>AMP-activated protein kinase</td>
</tr>
<tr>
<td>AP-1</td>
<td>Activator protein-1</td>
</tr>
<tr>
<td>ARE</td>
<td>Antioxidant response element</td>
</tr>
<tr>
<td>ASK1</td>
<td>Apoptosis signal-regulating kinase 1</td>
</tr>
<tr>
<td>ATF6</td>
<td>Activating transcription factor 6</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>Bak</td>
<td>BCL2-antagonist/killer</td>
</tr>
<tr>
<td>Bax</td>
<td>BCL2-associated X protein</td>
</tr>
<tr>
<td>Bcl2</td>
<td>B Cell lymphoma/leukemia</td>
</tr>
<tr>
<td>Bcl-xL</td>
<td>B-cell lymphoma-extra large</td>
</tr>
<tr>
<td>Bid</td>
<td>BH3 interacting-domain death agonist</td>
</tr>
<tr>
<td>Bim</td>
<td>Bcl-2 interacting mediator of cell death</td>
</tr>
<tr>
<td>CDKs</td>
<td>Cyclin-dependent kinases</td>
</tr>
<tr>
<td>c-FLIP</td>
<td>FLICE-cellular-like inhibitory protein</td>
</tr>
<tr>
<td>COX-2</td>
<td>Cyclooxygenase-2</td>
</tr>
<tr>
<td>cyt-C</td>
<td>Cytochrome C</td>
</tr>
<tr>
<td>ECM</td>
<td>Extra-cellular matrix</td>
</tr>
<tr>
<td>EGFR</td>
<td>Epithelial growth factor receptor</td>
</tr>
<tr>
<td>eIF2</td>
<td>Eukaryotic initiation factor 2</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>EMT</td>
<td>Epithelial-Mesenchymal Transition</td>
</tr>
<tr>
<td>Endo G</td>
<td>Endonuclease G</td>
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<tr>
<td>ERK</td>
<td>Extracellular regulated protein kinase</td>
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<td>EsR</td>
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<td>GSK3β</td>
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<td>Human epidermal growth factor receptor</td>
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<td>Hemeoxygenase-1</td>
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<tr>
<td>HRE</td>
<td>Hypoxia response element</td>
</tr>
<tr>
<td>Hsp90</td>
<td>Heat shock protein 90</td>
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<tr>
<td>hTERT</td>
<td>Human telomerase reverse transcriptase</td>
</tr>
<tr>
<td>hTP1</td>
<td>Human telomerase-associated protein 1</td>
</tr>
<tr>
<td>hTR</td>
<td>Human telomerase RNA</td>
</tr>
<tr>
<td>HUVECs</td>
<td>Human umbilical vein endothelial cells</td>
</tr>
<tr>
<td>IGF-1</td>
<td>Insulin-like growth factor 1</td>
</tr>
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<td>IKK</td>
<td>I kappa B kinase</td>
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<td>Interleukin-10</td>
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<td>IL-1β</td>
<td>Interleukin-1 beta</td>
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<td>IL-2</td>
<td>Interleukin-2</td>
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<tr>
<td>IL-6</td>
<td>Interleukin-6</td>
</tr>
<tr>
<td>IP3</td>
<td>Inositol 1, 4, 5-triphosphate</td>
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<tr>
<td>IREα</td>
<td>Inositol-requiring enzyme-1 alpha</td>
</tr>
<tr>
<td>IκB</td>
<td>Inhibitor of NF-κB</td>
</tr>
<tr>
<td>JAK2</td>
<td>Janus Kinase 2</td>
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<tr>
<td>JNK</td>
<td>c-Jun N-terminal kinases</td>
</tr>
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<td>LPS</td>
<td>Lipopolysaccharide</td>
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