Oridonin and its derivatives for cancer treatment and overcoming therapeutic resistance

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Abstract Cancer is one of the diseases with high morbidity and mortality on a global scale. Chemotherapy remains the primary treatment option for most cancer patients, including patients with progressive, metastatic, and recurrent diseases. To date, hundreds of chemotherapy drugs are used to treat various cancers, however, the anti-cancer efficacy and outcomes are largely hampered by chemotherapy-associated toxicity and acquired therapeutic resistance. The natural product (NP) oridonin has been extensively studied for its anti-cancer efficacy. More recently, oridonin has been shown to overcome drug resistance through multiple mechanisms, with yet-to-be-defined bona fide targets. Hundreds of oridonin derivative analogs (oridonalogs) have been synthesized and screened for improved potency, bioavailability, and other drug properties. Particularly, many of these oridonalogs have been tested against oridonin for tumor growth inhibition, potential for overcoming therapeutic resistance, and immuno modulation. This concise review seeks to summarize the advances in this field in light of identifying clinical-trial level drug candidates with the promise for treating progressive cancers and reversing chemoresistance.

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Introduction

Cancer has been a major health threat to human beings worldwide. According to the International Agency for Research on Cancer (IARC), there were 17 million new cancer cases diagnosed and 9.5 million cancer-associated deaths reported in 2018. Unfortunately, with a growing, aging population in most countries, the cancer incidence and mortality have been sustainably increasing over the past decades and are predicted to remain high in future years. To date, surgery, chemotherapy, and radiotherapy are among the major therapeutic options for cancer patients, with addition of the more recent targeted therapy and immunotherapy, which may benefit selective patient subpopulations. Chemotherapy has always been the mainstream treatment for the majority of cancer patients with the exception of some early-staged patients. With the prevalent use of chemotherapies, the drug-associated toxicities and adverse side effects became serious clinical concerns, particularly with acquired drug resistance, or chemoresistance, developed during and after chemotherapy administration, as the most significant contributor to such challenges. Chemoresistance seriously hampers the application of cancer drugs, in general, to achieve optimal and satisfactory anti-cancer efficacy in patients. Efforts to reduce toxicity, overcome resistance of chemotherapies, and enhance the efficiency of anti-cancer drugs are the vigorous focus of research via elucidation of the underlying mechanisms and development of agents capable of overcoming chemoresistance.

Natural products (NPs) have provided a rich source in developing a plethora of cancer drugs in use, and the NPs possessing low toxicity can be ideal candidates in the identification of initial chemical entities in the development of cancer drugs capable of overcoming chemoresistance. Design based on the structure of those NP entities and their structural modulation can target individual or multiple pathways critical for developing chemoresistance. In this review, we focus on one of these NPs, oridonin, and its derivative compounds developed in recent years by our team and other laboratories. While oridonin possesses moderate to potent anti-cancer efficacy, its potential clinical use has been limited due to its low aqueous solubility and bioavailability. To improve its overall drug-like properties, many oridonin analogs (oridonalogs) have been developed by modifying the A, B, and D rings of oridonin to achieve higher anti-cancer efficacy, lower toxicity, and the ability to overcome de novo and acquired chemoresistance. In fact, many of such oridonalogs have shown favorable capacity in suppression of tumor growth and progression, overcome of therapeutic resistance, and blockade of metastasis and recurrence.

Oridonin and its use for treating human diseases

Oridonin (C_{20}H_{28}O_{6}), a kaurene-type diterpenoid isolated from *Rabdosia rubescens*, is called “Dongjingcao” in Chinese or “Hara” in Japanese. Its chemical structure and antitumor effect were characterized by Fujita E. *et al* in 1976 (*Table 1*). Since its first report as a potential antitumor agent, accumulating evidence demonstrates that oridonin can modulate multiple biological functions by acting as anti-inflammatory, antibacterial, anti-fibrotic, auto-immune-regulatory, and neuro-regulatory agent in diverse diseases.

As a traditional Chinese herb, *Rabdosia rubescens* was firstly used as anti-inflammation herb for sore throat and tonsillitis and has been included in the Chinese Pharmacopoeia since 1977. The leaves of *Rabdosia rubescens* are still used for tea in China for their antibacterial function. It was reported that the anti-inflammatory effect of oridonin is primarily associated with suppressing the nuclear factor-kappa B (NF-κB) signaling pathway, reducing secretion of serum cytokines including interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), and inhibiting expression and function of toll-like receptors 4 (TLR4) as well as the p38-mitogen activated protein kinase (p38-MAPK) in endometritis, diabetic nephropathy, vascular inflammation, acute lung injury, liver injury, inflammatory bowel disease, and sepsis. Recently, oridonin was reported to inhibit NLRP3 which is a key component in the NLRP3 inflammasome by targeting the Cys279 residue of NLRP3 in the NACHT domain.

Oridonin was also found to suppress Th1/Th17 cells and the proliferation of CD4^+ T cells by inhibiting the NF-κB signaling pathway in a mouse Crohn’s disease model. There is increasing evidence that oridonin modulates the immune system by yet undefined mechanisms. Oridonin was reported to increase the weights of spleen and bursa, as well as the number of proliferating peripheral blood T and B lymphocytes and serum cytokines, such as IL-2, IL-4, and TNF-α in broiler chickens. Furthermore, in broiler chickens, oridonin improves immune functions against infection of *Salmonella pullorum*, by decreasing splenic inflammatory cytokines, through down-regulating the B lymphocyte stimulator (BlyS) which is a TNF family member. BlyS can irritate B cell proliferation and survival *in vitro* and adjust B cell homeostasis *in vivo* to regulate the Th1/Th2 balance. Oridonin also promotes CD4^+ /CD5^+ regulatory T cell differentiation, and modulates the Th1/Th2 balance, by inducing HO-1, an anti-inflammatory target. In an asthma mouse model, oridonin was used to treat asthma by regulating the Th1/Th2 balance. Oridonin inhibits the proliferation of fibroblast-like synoviocytes and induces mitochondria-dependent apoptosis in rheumatoid arthritis, a chronic inflammatory autoimmune disease. *In vitro* and *in vivo*, oridonin modulates immunity via down-regulation of B-cell activating factor and B-cell maturation and differentiation in systemic lupus erythematosus. The aforementioned research provides evidence that oridonin modulates immunity and may therefore impact its own ability to suppress cancer growth and progression, and may also facilitate other immunity-targeting drugs in additive or synergistic manner.

Furthermore, oridonin was reported to possess anti-osteoporotic ability, by inhibiting the NF-κB pathway and p65 nuclear translocation in degenerative diseases. Through inhibiting NF-κB signaling, oridonin was used for treating Alzheimer’s disease to prevent synaptic loss, by modulating β-amyloid *in vitro* and *in vivo*. Oridonin was reported to be potentially useful in cardiovascular disease and liver fibrosis. Also, oridonin induces...
Table 1  Approaches in optimizing oridonin.

| Modified Structure/Name of Compounds | Chemical Structures | Targeting Pathways | Models Tested | Ref |
|-------------------------------------|---------------------|---------------------|---------------|-----|
| Oridonin                            | ![Oridonin structure](image) | —                  | BGC-7901, SW-480, HL-60, BEL-7402, A549, B16 | 1  |
| 1-O- and 14-O-derivatives            | ![1-O- and 14-O-derivatives structure](image) | —                  | A549, Bel-7402, K562, MGC-803, CaEs-803 | 50 |
| Ent-6,7-seco-derivatives             | ![Ent-6,7-seco-derivatives structure](image) | —                  | EC9706, EC109, HEECs, HL-7702 | 52 |
| 13p                                 | ![13p structure](image) | Mitochondrial pathway | MCF-7 | 53 |
| DS2                                 | ![DS2 structure](image) | MMP, ROS           | EC9706, EC109, HEECs, HL-7702 | 54 |
| Geridonin                           | ![Geridonin structure](image) | ROS-mediated PTEN, PI3K/Akt pathway | MGC 803 | 55,56 |
| Furoxan-based nitric oxide-releasing derivative | ![Furoxan-based nitric oxide-releasing derivative structure](image) | —                  | Bel-7402, K562, MGC-803, CaEs-17 | 57 |
| Spirolactone-type diterpenoid derivatives | ![Spirolactone-type diterpenoid derivatives structure](image) | —                  | Bel-7402, K562, MGC-803, CaEs-17 | 58 |
| Enmein-type diterpenoid analogs     | ![Enmein-type diterpenoid analogs structure](image) | Mitochondria-related caspase-dependent pathway | Bel-7402, K562, MGC-803, CaEs-17 | 59 |
| Modified Structure/Name of Compounds | Chemical Structures | Targeting Pathways | Models Tested | Ref |
|---------------------------------------|---------------------|-------------------|--------------|-----|
| H$_2$S releasing ent-kaurane diterpenoid oridonin derivatives | ![Chemical Structure](image) | Extrinsic and intrinsic apoptosis pathways | HepG2, MCF-7, HCT-116, B16, K562, L-02, PBMC | 60 |
| Nitric oxide (NO)-releasing oridonin derivatives | ![Chemical Structure](image) | Apoptosis and cell cycle arrest at S phase | Bel-7402 | 63 |
| Seven-membered C-ring-expanded 6,7-seco-ent-kaurenes | ![Chemical Structure](image) | Apoptosis and cell cycle arrest | MCF-7 | 64 |
| Enmein-type diterpenoid amino acid ester derivatives | ![Chemical Structure](image) | Intrinsic apoptosis pathway | Bel-7402, SGC-7901, HL-60, PC-3, A549, K562, L-02 | 66 |
| Ent-kaurane and spirolactone-type 6,7-seco-ent-kaurane derivatives | ![Chemical Structure](image) | Intrinsic apoptosis pathway | K562, Bel-7402, SGC-7901, A549, L-02, PBMC | 67 |
| A-ring nitrogen-enriched modifications | ![Chemical Structure](image) | Mitochondria-dependent pathway; antifibrosis through NF-κB pathway; STAT3 | MDA-MB-232, MCF-7, MCF-7/ADR, HMEC, LX-2, HSC-T6, A2780, OVCAR3, OVCAR8, SKOV3 | 68-70, 78, 79 |
| CYD-6-17 (CYD0617) | ![Chemical Structure](image) | Wnt/β-catenin pathway; AKT/PDPK1 | T24-P, UMUC3 | 71 |

(continued on next page)
mitochondria-dependent apoptosis of pulmonary artery smooth muscle cells (PASMC) to reduce pulmonary artery pressure, and inhibits pulmonary artery structural remodeling in pulmonary arterial hypertension (PAH).\textsuperscript{32} It was reported that oridonin induces apoptosis of hepatic stellate cells (HSCs), which promotes liver fibrosis, through decreasing intracellular glutathione (GSH) and the production of the reactive oxygen species (ROS) in rats.\textsuperscript{8} Other than inducing apoptosis, oridonin inhibits the proliferation of HSCs time- and dose-dependently, arrests S-phase cell cycle progression, and reduces expression of extracellular matrix (ECM) proteins.\textsuperscript{9} Collectively, these research findings provide rich evidence that oridonin can treat inflammatory and immune diseases by targeting a number of pathways and genes, such as BLYS, HO-1, and NF-κB, that are important in inflammation and immune system function. This may be achieved through maintaining Th cell balance and cytokine secretion.

**Oridonin as cancer therapy**

Despite the evidence supporting oridonin’s therapeutic efficacy with many human diseases, a major focus for its potential clinical use is for cancer therapy. Using the term “oridonin”, we searched the PubMed.gov database and found 468 research articles published as of January, 2020, while searching “oridonin AND cancer” generated 341 articles. There were limited studies of oridonin for cancer therapy in the 1990s, only the cytostatic effects of oridonin

| Modified Structure/Name of Compounds | Chemical Structures | Targeting Pathways | Models Tested | Ref |
|--------------------------------------|---------------------|--------------------|---------------|-----|
| **D-ring modifications**              |                     | NRF-2/RHOA/ROCK signaling pathway | MDA-MB-231, GI101, GILM2, GILM3 | 74  |
| Oridonin A-ring-based diverse constructions of enone functionality | | p53-dependent apoptosis | LX-2 cells | 73, 76 |
| α-formylenone in the A-ring and introduction of an acetone moiety to 7,14-dihydroxyl HAO472, produg with an amino acid residue | | S-phase cell cycle arrest, apoptosis | LX-2 cells | 77  |
| Benzene analogues at C17 position | | Suppressing NF-κB pathway | mouse colitis model | 5   |
| 14-substituted oridonin analogs | | p53-MDM2 pathway | HCT116, BEL7402, MCF-7 | 82  |
in leukemia L1210 cells and gastric cancer cells were reported. Since 2004, there has been an increasing number of publications demonstrating oridonin’s anti-proliferative effects via induction of apoptosis in lung cancer cells, HeLa cells, and melanoma cells, via modulation of apoptotic signaling including the caspase family, Bcl-2/Bax family, and the mitogen-activated protein kinase (MAPK) pathway. In addition, oridonin stimulates phagocytosis of apoptotic tumor cells through regulation of macrophage function that involves TNF-α and IL-1β. Furthermore, oridonin induces apoptosis of breast cancer cells by enhancing autophagy. Oridonin was also shown to arrest G2/M cell cycle progression in cancer cells leading to apoptosis and inhibition of cancer progression. ROS is the initial and pivotal step in inducing apoptosis or autophagy with oridonin treatment, involving p53 and MAPK pathways, SIRT1, and NF-κB. A recent study showed that oridonin suppresses epithelial-mesenchymal transition (EMT) through TGF-β1/Smad2/3 signaling pathway in osteosarcoma 143B cells, while in pancreatic cancer SW1990 cells this effect is achieved through Wnt/β-catenin pathway. Additionally, oridonin induces autophagy in colorectal cancer cells via inhibition of glucose metabolism. These studies demonstrate that oridonin processes impressive anti-cancer activity in many cancer types through modulation of cell cycle progression and induction of phagocytosis and autophagy, eventually leading to apoptosis.

It is believed that its intrinsic chemical structure endows oridonin’s multiple efficacy against inflammation and cancers, by inhibition of cellular proliferation and induction of apoptosis. Although Rabdosia rubescens has been used as a natural anti-bacterial/anti-inflammatory therapy for a long time in China and Asia, its major active component, oridonin, has a low oral bioavailability, merely less than 5%, due to its low water-solubility. This property adversely impacted its potential clinical implications. To date, oridonin is only used as an over-the-counter herbal medicine for anti-inflammation in China. There are no drugs based on oridonin approved by U.S. Food and Drug Administration (FDA) for clinical use for cancer medication or other diseases.

**Oridonin derivative compounds as potential cancer therapies**

**Optimization of oridonin and new oridonin-derived oridonalogs**

With its discovered and verified anti-tumor effects, a number of laboratories attempted to modify the structure of oridonin in order to achieve improved efficacy and drug properties, such as better bioavailability, higher potency, and less toxicity, as summarized in Table 1. Xu J. et al in 2008, synthesized 1-O- and 14-O-derivative compounds from oridonin and found that these compounds were more effective than oridonin itself in inhibiting BGC-7901, SW-480, HL-60, BEL-7402, A549, and B16 cancer cell lines. In 2011, Liu H. et al reported three oridonin derivative compounds complexing with β-cyclodextrin (pCD), a commonly used drug delivery compound, to increase drug solubility; however no evaluation of their biological activity was reported. Later, Ent-6,7-seco- oridonin derivatives were synthesized in 2012 to enhance the activity of oridonin. It was reported that 13-p, an A-ring-modified analogue, showed 200-fold higher efficacy than oridonin, in breast cancer cell line MCF-7, by targeting the mitochondrial pathway. Another oridonin analog, DS2, was reported to induce apoptosis in human esophageal cancer cells, through modulating the mitochondrial membrane potential (MMP) and ROS. More recently, geridornin, an oridonin-based analog was reported to inhibit gastric cancer cells, alone or in combination with paclitaxel, via up-regulating PTEN and down-regulating the PI3K/Akt signaling pathway. Li D. et al used oridonin as a nitric oxide donor and designed furoxan-based nitric oxide-releasing (NO-releasing) derivatives, and also designed spirabolactone-type and enmein-type diterpenoid derivative compounds. These derivatives demonstrated more powerful anti-cancer effects than oridonin. Hydrogensulfide (H₂S) was also used to improve the bioavailability of oridonin, instead of the NO-releasing gasotransmitter. Recently, H₂S-releasing ent-kaurene diterpenoid oridonin derivatives were designed. Most of these derivatives were reported to induce apoptosis through a mitochondrial-dependent pathway in cancer cells, a same mechanism of action in antibacterial effect. Other oridonin-based derivatives were reported in which adiazin-1-ium-1,2-diolate nitric oxide donor moiety was added and were demonstrated to arrest S phase cell cycle progression in hepatoma Bel-7402 cells. In 2017, spiro-lactone-type ent-kaurene derivatives were reported to be able to induce apoptosis and arrest cell cycle progression in MCF-7 human breast cancer cells. Xu S. et al also reported a modification with the trans-cinnamic acid moiety on the oridonin structure, which rendered the derivatives stronger anti-mycobacterial activity. Recently, a series of enmein-type diterpenoid amino acid ester derivatives were developed and tested in multiple human cancer cell lines. Among them, the compound 19 was found to act against proliferation by inducing apoptosis and cell cycle arrest through a mitochondria-dependent pathway. Similarly, another two ent-kaurene derivatives, linking different H₂S donors, also induced mitochondria-dependent apoptosis. These studies demonstrate that there are aroused interest in the synthesis and identification of potent oridonalogs as potential cancer therapies (Table 1).

In 2013, our team systematically modified the A-ring of oridonin and developed a series of new oridonalogs as potential therapies for cancer and other human diseases. We incorporated a nitrogen moiety into the core scaffold of oridonin to improve solubility. Most of the nitrogen-enriched oridonalogs not only showed improved aqueous solubility, but also enhanced ability of inducing apoptosis in the triple-negative breast cancer cell line, MDA-MB-231, and other cancer cell lines, as well as tumors in mice. An important discovery was that our compounds, such as CYD0618, were able to overcome resistance in drug-resistant MCF-7 cells, consistent with the reported antichemosensitivity effect of oridonin. Another of our compounds, CYD0617, also showed inhibition on transition cell carcinoma (TCC) cells by targeting the Wnt/β-catenin pathway in vitro and in vivo. CYD0617 also regulates AKT by targeting 3-phosphoinositide-dependent protein kinase 1 (PDK1) in drug-resistant renal cell carcinoma in vitro and in vivo. Using an additional α, β-unsaturated ketone system
to modify the A-ring structure, we developed a new generation of oridonin-based compounds, which could more efficiently induce apoptosis in highly aggressive breast cancer cells. Another D-ring modification oridonalog, YDOS1, was discovered through aziridination and mediated covalent warheads, was shown to inhibit breast cancer metastasis to the lungs by targeting the nuclear factor erythroid 2-related factor 2 (Nrf2)/ROH/Rock signaling pathway. Oridonalog, CYD0682, was developed through oridonin ring A-based diverse constructions of enone functionality, which acquired a more anti-fibrogenic effect than oridonin in the LX-2 human hepatic stellate cell line. The anti-fibrogenic effects have also been observed in oridonalog CYD0692, which has an additional α-formylidene in the A-ring and an acetonide moiety attached to 7,14-dihydroxyl. Recently, CYD0618 was reported to inhibit fibrosis in hepatic stellate cells through suppression of the NF-κB pathway. CYD0618 was also reported to target STAT3 signaling in ovarian cancer. A water-soluble oridonin prodrug, HAO472, was recently reported in a Phase I clinical trial for treating acute myelogenous leukemia, as reviewed. HAO472 has been demonstrated to modulate inflammation by suppressing NF-κB signaling in a mouse colitis model at 5.0–7.5 mg/kg doses. An oridonin phosphate was recently reported to be able to induce apoptosis by up-regulating autophagy in breast cancer cells. Shen Q. et al. synthesized benzene analogs with modifications at the C17 position of the oridonin structure and the derivatives showed inhibition of proliferating cancer cells. Substitution on the 14-OH position of oridonin structure also rendered a new derivative compound to induce apoptosis and arrest cell cycle progression by suppressing p53-MDM2 signaling pathway. Together, vigorous efforts have been made in this field in the modification of different ring structures of oridonin to generate new compounds for better efficacy and drug properties (Table 1). Some of those have already shown promising profiles for further development into potential drug candidates for treatment of cancer and other diseases.

**Oridonin and its derivative compounds for treating cancers**

To date, hundreds of oridonin derivatives have been developed to improve solubility and enhance bioavailability of oridonin. The majority has shown improved efficacy against proliferation and fibrosis compared to oridonin. Targeting the intrinsic apoptosis pathway primarily contributes to the anti-cancer effect of oridonin and its derivative compounds. Here, we summarized the major modification strategies used in optimizing oridonin, chemical structures of oridonin and its derivative compounds, targeting pathways, and the model systems for testing the compounds (Table 1).

**Oridonin and its derivative compounds tested in combination with other chemotherapy drugs**

Chemotherapy has been the most important therapeutic option for treating cancers, particularly, for hematologic malignancies and certain cancer types reaching unresectable or advanced stage of disease. No matter how the chemotherapeutic drugs are used as adjuvant, cytotoxic, or palliative therapies, adverse side effects are unavoidable in chemotherapy, and drug resistance develops during and/or after regimens, becoming a serious barrier to achieve expected therapeutic outcomes and long-term survival. Thus, combination therapy with natural or non-/low-toxic herbs to enhance cytotoxicity or reverse drug resistance arouses continuous interest in this regard. There is a plethora of reports showing oridonin’s anti-tumor activities via promotion of ROS, induction of apoptosis and autophagy, inhibition of EMT, and modulation of cell cycle progression in various cancer cells. Oridonin has been investigated in the experimental settings for combination with other chemotherapeutic agents for synergistic anticancer activity and overcoming drug resistance or chemoresistance. As summarized and reviewed above, oridonin plays an important role in inducing apoptosis, which would be a significant contribution in combination therapy. Either the classical apoptosis pathway, such as the caspase-dependent pathway or the mitogen-activated protein kinase (MAPK) pathway, which includes ERK, c-Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK) and p38 kinases, is involved in inducing cell death by oridonin. Oridonin was shown to enhance the effects of gemcitabine in pancreatic cancer via mitochondrial caspase-dependent signaling pathway. Oridonin synergizes with JQ1, a bromodomain and extra-terminal domain (BET) inhibitor, to enhance the sensitivity of JQ1 in hepatocellular carcinoma (HCC) cells by inducing apoptosis involving NF-κB signaling oridonin and other chemotherapeutic agents has been widely tested in experimental hematological malignancies. Intriguingly, in a recent study with the combination of oridonin and valproic acid (VPA) in treating HL-60 leukemia cells, caspase-dependent apoptosis pathways contributed to cell death. Homoharringtonine (HHT) induces apoptosis through targeting c-KIT protein which is a member of type III tyrosine kinase subclass in t (8; 21) acute myeloid leukemia (AML), one of the most common AML subtypes. The combination of oridonin with HHT, showed synergistic inhibition in the same cell line through inducing MMP loss and enhancing HHT-induced c-KIT downregulation. Interestingly, HHT also increased intracellular oridonin level and enhanced its influx by inhibiting ATP-binding cassette (ABC)-mediated efflux of oridonin. In vivo, the combination therapy prolonged survival of mice bearing t (8; 21) AML. The combination of oridonin with HHT, showed synergistic inhibition in the same cell line through inducing MMP loss and enhancing HHT-induced c-KIT downregulation. Additionally, LYN/mTOR, Raf/MEK/ERK and SAK signaling pathways were reported to be involved in combining oridonin and imatinib for treating Ph+ acute lymphoblastic leukemia cells. These studies suggest that
oridonin and oridonals, whether in single or combination use, have huge potential for treating hematological malignancies.

Other than hematological malignancies, oridonin has been shown to trigger ROS generation in solid tumor cells. ROS generated by aerobic metabolism induces oxidative stress. Unfortunately, cancer cells especially those in advanced disease stages have higher tolerance to exogenous stress. Cancer cells with redox adaptation survive better than other cancer cells and are thought to be associated with chemoresistance. Arsenic trioxide (As$_2$O$_3$) induces ROS-dependent apoptosis in liver cancer cells and was adopted as the first-line treatment for acute promyelocytic leukemia (APL). HCC cell lines including Bel7402, HepaG2, and SMMC7721 became more sensitive to As$_2$O$_3$ after administration of oridonin. Synergistic elevation of cellular ROS level is related to low GSH level. Interestingly, low dose oridonin promotes ROS generation, but not induce cell death in Bel7402 cells, with undefined mechanisms. Nevertheless, oridonin can activate Nrf2 and attenuate arsenic-induced toxicity, through the Nrf2 signaling pathway at a low dose in vitro, whereas, at a high dose it induces apoptosis and inhibits the Nrf2-dependent survival pathway. A recent study reported that the combination of oridonin with cetuximab, a mouse-human chimeric anti-EGFR monoclonal antibody of epidermal growth factor receptor (EGFR), synergistically activates the ROS-mediated JNK pathway and downregulates p-EGFR in laryngeal squamous cell carcinoma cell lines, HEp-2 and Tu212. Thus, oridonin enhances chemotherapeutic efficacy and may simultaneously alleviate the adverse side effects of chemotherapy.

Radiotherapy has been an increasingly important modality among cancer therapies, especially for radiation-sensitive cancers. However, there are concerns and side effects, before and after radiotherapy, and radiation resistance compromises the clinical efficacy. Recently, oridonin was reported to enhance irradiation-induced cell death in non-small cell lung cancer (NSCLC) cells by promoting DNA damage. NSCLC H460 cells were treated with oridonin (1 and 2.5 μM) before exposure to different doses of gamma rays. A 2.5 μM oridonin dose with 4 Gy gamma rays showed strong inhibition of H460 cell viability. This was achieved by inducing ROS, which leads to DNA damage and apoptosis, together with consistent in vivo inhibition of tumor growth and enhanced sensitivity towards radiotherapy. This study suggests that oridonin and its derivatives may be used as radiosensitizer to enhance radiotherapy.

There is no doubt that a combination of multiple chemotherapeutic agents, not only potentially reduces the dose of oridonin needed, but also reduces the doses of combining chemotherapeutic agents, and hence diminishes adverse side effects as well. Oridonin was shown to sensitize cells to lentinan, a medicinal polysaccharide isolated from the Shitake mushroom. Combination of both NPs showed more effective anticancer activity, compared to treatment with oridonin, lentinan, or cisplatin alone in a human hepatoblastoma cell line, HepG2. The combination increased caspase-3, caspase-9, p53, and p21 levels, and inhibited NF-kB signaling which contributes to enhanced apoptosis. Other groups reported γ-tocotrienol as a nontoxic natural phytochemical in the vitamin E family, and combination of oridonin and γ-tocotrienol inhibits malignant and SA mammary epithelial cells, but displayed nearly no toxicity with normal CL-S1 mammary epithelial cell. These studies, as summarized in Fig. 1, Table 2, and Table S1, suggest that oridonin in combination with other anti-cancer agents may significantly increase the efficacy of those agents and also reduce side-effects in many occasions, thus providing
Current literature supports the notion that oridonin also in- 
duced apoptosis of many cancer cell types with potent effi- 
cacy. For example, platinum-based chemotherapeutic agents 
such as cisplatin (DDP), carboplatin, and nedaplatin are 
widely used in ovarian cancer chemotherapy. However, 
these drugs did not achieve satisfactory clinical outcomes 
to prolong survival, either due to chemoresistance or se- 
vere side effects. Oridonin in combination with DDP de- 
creases the IC50 of DDP in A278/DDP and SKOV3/DDP cells 
via induction of apoptosis and arrest of cells in G0/G1 phase. 
This was also evident in cisplatin-resistant AML cells 
via apoptosis induction. Zhao Y. et al reported that oridonin 
enhances the sensitivity of A2780CP and SKOV3/ 
DDP cells to cisplatin via cisplatin-mediated autophagy. 
Intriguingly, the paclitaxel-resistant cell line PTX10 
showed higher sensitivity to oridonin compared to the chemotherapy. As intrinsic or acquired chemoresistance is 
related to poor prognosis in cancer patients. Intrinsic drug 
resistance occurs in initial chemotherapy while acquired 
chemoresistance relates to patients that may be initially 
sensitive to chemotherapy, but later become resistant to 
the same or similar drugs. 
Other drug properties and cancer stem cells also contribute to che- 
9. Current literature supports the notion that oridonin, used alone or in combination with other chemotherapeutic agents, 
primarily exhibits anti-chemoresistance activity via induction of apoptosis, 
potentially by regulating drug resistance-associated mole- 
cules such as P-glycoprotein (P-gp) and MDR-associated protein 1 (MRP1).

Nearly all chemotherapeutic drugs are able to induce 
apoptosis of cancer cells. However, after cancer cells 
develop chemoresistance, they become resistant to 
apoptosis, which eventually lead to the escape from 
immune surveillance. Oridonin has been shown to induce 
apoptosis of many cancer cell types with potent efficacy.

### Oridonin and oridonanalogs as potential therapies to sensitize cancer cells and overcome therapeutic resistance

Chemoresistance is one of the major challenges in cancer 
therapy, as intrinsic or acquired chemoresistance is 
related to poor prognosis in cancer patients. Intrinsic drug 
resistance occurs in initial chemotherapy while acquired 
chemoresistance relates to patients that may be initially 
sensitive to chemotherapy, but later become resistant to 
the same or similar drugs.

| Table 2 | Targeting therapeutic resistance with oridonin and its derivatives in vitro. |
|---------|--------------------------------------------------------------------------------|
| aCell Lines Tested | Potential Mechanisms | Ref |
| Ovarian cancer A278/DDP and SKOV3/DDP cell lines | Induction of apoptosis, increase of cells in G0/G1 phase, downregulation of Bcl-2, upregulation of Bax, and decrease of MPP-2 and MMP-9 | 114 |
| Leukemia K562/ADR cell line | Upregulation of BIM-S by diminishing miRNA-7 and miRNA-20a | 118 |
| AML MV4-11/DDP and MOLM-13/DDP cell lines | Induction of apoptosis, inhibition of MPP-2 and MMP-9 | 115 |
| Renal cell carcinoma 786-O cell line | Induction of death via activation of HSF-1 | 120 |
| Colorectal cancer HCT-15 and HCT-15/SFU-R cell line | Upregulation of ROS/JNK/c-Jun axis | 121 |
| Gastric cancer SGC7901/DDP cell line | Downregulation of P-gp, MRP1 and cyclin D1 | 110 |
| Pancreatic cancer PANC-1 (PANC-1/Gem) gemcitabine-resistant cell line | Inhibition of GST pi and LRP1/ERK/JNK signaling | 112 |
| NSCLC H1975/gefitinib-resistant cell line | Suppression of EGFR/ERK/MPP-12 and CIP2A/PP2A/Akt signaling pathways | 123 |
| Leukemia Ph⁺ (K562, KU812 and SUP-B15) cell lines | Depletion of BCR-ABL through activating HSF-1 for chaperone-mediated degradation | 126 |
| Leukemia imatinib-sensitive (K562–S) and imatinib-resistant (K562–R) cell lines | Downregulation of p-Lyn and inhibition of mTOR and Bcl-2 | 127 |

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3. Wang et al, 2023
4. Li et al, 2023
Necroptosis induced by oridonin was accomplished through depleting GSH and enhancing ROS generation via regulation of the ROS/JNK/c-Jun axis in colorectal cancer cells.\textsuperscript{121} Thus, ROS production may be a rate-limiting factor in cells in order for oridonin to induce apoptosis, necrosis, or both. An additional benefit of combining oridonin with 5-FU or other chemotherapeutic agents is to reduce doses of chemotherapeutic drugs, leading to less toxicity and side effects. These studies demonstrate that induction of programmed cell death is one of its major mechanisms of action for oridonin.

MDR-associated gene overexpression and/or activation essentially contribute to chemoresistance in many cancer types. Human DDP-resistant cell line, SGC7901/DDP, showed upregulated P-gp, MRPI, and cyclin D1. Administration of oridonin at a 10 \textmu M concentration effectively reduced the expression of those proteins by at least 1.25-fold, and combination of oridonin and DDP showed a synergistic effect in decreasing the IC\textsubscript{50} of DDP, achieved via suppression of the CIP2A/PP2A/Akt signaling pathway.\textsuperscript{110} Other than modulating P-gp and MRPI, oridonin overcomes gemcitabine resistance by regulating GST, which is involved in MDR and LRP/1 ERK/JNK signaling in PANC-1/Gem cells.\textsuperscript{122} In addition, oridonin is also effective against gefitinib-resistant NSCLC cells by modulating the CIP2A/PP2A/Akt signaling pathway.\textsuperscript{123} By using silico-based technology to validate potential targets of oridonin in various drug-resistant tumor cells, one study showed that oridonin interacts with signaling molecules of the Akt/EGFR pathway. Accumulating evidence and our unpublished data suggest that Akt and STAT3 signaling are critically involved in oridonin’s effect against chemoresistance.\textsuperscript{124}

The \textit{BCR-ABL} gene encodes tyrosine kinase which is a hot therapeutic target for leukemia, and gefitinib and imatinib are frequently used tyrosine kinase inhibitors (TKIs). It was reported that \textit{BCR-ABL} gene amplification or point mutations in the kinase domain leads to imatinib-associated resistance.\textsuperscript{125} Oridonin was reported to target \textit{BCR-ABL} by binding to its cysteine-153 residue in the HSF1 domain, which regulates the expression of HSPO70 and ubiquitin proteins, UBB and UBC, to downregulate \textit{BCR-ABL} in leukemia cells.\textsuperscript{126} Further, oridonin was shown to inhibit K562 cells with imatinib-resistance via downregulation of Bcl-2 and p-Lyn, which is a member of Src family of protein tyrosine kinases thought to be involved in developing resistance to imatinib via the mTOR pathway.\textsuperscript{127} These reports suggest that oridonin reverses resistance raised from targeted therapies including \textit{BCR-ABL}, and this effect may be non-specific towards any tyrosine kinase inhibitors.

Oridonin was reported to dose-dependently decrease the levels of MMP-2 and MMP-9, which are related to invasion and metastasis.\textsuperscript{114,115} Oridonin also inhibits migration, invasion, and adhesion of gefitinib-resistant NSCLC cell line H1975 \textit{in vitro} and \textit{in vivo} through EGFR/ERK/MAPK-12 pathway.\textsuperscript{123} Thus, oridonin may suppress cancer metastasis and serve as a therapy for late-stage/metastatic cancers.

**Targets of oridonin and its derivative compounds**

As mentioned earlier, oridonin and its various derivative compounds can suppress cancer cell proliferation \textit{in vitro} and tumor growth \textit{in vivo}. Oridonin combined with other chemical agents enhances the anticancer effect and decreases adverse side effects. In terms of potency and toxicity, many oridonin derivatives demonstrated improved efficacy, higher bioavailability, and less toxicity, justifying the previous and ongoing efforts to overcome the noticeable disadvantages of oridonin. Accumulating evidence suggests that oridonin and its analogs, alone or combination with other drugs, trigger apoptosis, autophagy, cell cycle arrest, and EMT through differential molecular pathways and mechanisms. However, deconvolution of targets for oridonin and its derivatives has been a significant challenge. Here we summarized the reported signaling pathways involved in the mechanism of action of oridonin in an attempt to facilitate future endeavors to identify targets directly and/or indirectly interacting with oridonin associated with chemoresistance or other disease settings (Fig. 2 and Table S2).

Extrinsic and intrinsic apoptotic pathways have been demonstrated as the primary mechanisms of action involved in oridonin and its derivatives’ anti-cancer activities. The caspase family, Bcl-2 family, p53, MDM2, NF-\textit{kB}, PI3K/Akt/mTOR, MAPK-p38, and JAK/STAT have been shown to contribute to oridonin and its derivatives’ effects. Among them, the NF-\textit{kB} signaling pathway plays a key role in anti-tumor, anti-fibrosis, anti-inflammatory, and immune regulation. As shown in Fig. 2 and Table S2, multiple pathways and potential targets of oridonin were reported, however, direct targets are not fully determined. Previous studies suggest that two oridonin derivative compounds developed by our team, CYD0617 and CYD0618, may target \textit{\beta}-catenin and PDPK1, and STAT3 signaling, respectively.\textsuperscript{11,72,79} Huang H. et al found that c-Myc, a helix-loop-helix leucine zipper transcription factor, is one of the oridonin targets in the K562 cell model, through comparing the miRNA expression with oridonin exposure.\textsuperscript{126} Oridonin was shown to directly bind to the cysteine-153 residue of HSF1 to activate HSF1 and enhance the expression of HSPO70, eventually inducing \textit{BCR-ABL} degradation in leukemia cells.\textsuperscript{126} Involvement of miRNA such as miRNA-7 and miRNA-20a may help deconvolute downstream targets of oridonin and its derivative compounds. These studies suggest that oridonin and its derivative compounds directly and indirectly target multiple pathways and molecules to exert their various anti-cancer efficacy. Further research is therefore warranted to identify the \textit{bona fide} and the key indirect targets of oridonin and oridonalog for better understanding of their mechanisms of action.

**Perspectives and conclusions**

Oridonin as a natural product with a relatively safe profile has displayed potent pharmacological activities, such as anti-tumor, anti-inflammatory and immune regulation. It targets many genes or proteins important in various signaling pathways involved in human diseases including cancers. In the past decade, oridonin has been vastly investigated, alone or in combination, for synergistic effects with other therapeutic agents. With its moderate efficacy and relatively low bioavailability, over one hundred oridonin-based structurally diversified derivative
compounds were synthesized and evaluated in various in vitro and in vivo systems. Combination therapy with oridonin, not only enhances chemotherapy, but also reduces commonly seen adverse side effects. Extended implication for oridonin and its oridonalogs is to overcome therapeutic resistance developed during or after cancer treatment. Beyond induction of apoptosis as the primary mechanism of action, oridonin also has yet-to-elucidate functions in inflammation and immunity regulation. Further investigation of the putative targets for oridonin and its novel analogs is therefore warranted in order to obtain more supporting evidence. With improved overall drug-like properties including aqueous solubility and bioavailability, oridonin-based new scaffolds may provide promising drug candidates with potential to be advanced into future human clinical trials.

Authors contribution

X. Liu, J. Zhou and Q. Shen conceived the concept. X. Liu conducted literature search and summarization. X. Liu wrote the manuscript and J. Xu contributed in part to Table 1. X. Liu, J. Xu, J. Zhou and Q. Shen revised the manuscript. All authors reviewed manuscript and granted approval for submission.

Conflict of Interests

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2020.06.010.

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