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REVIEW ARTICLE

Molecular Insight in the Multifunctional Effects of Oridonin

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Abstract Oridonin has attracted considerable attention in the last decade because of its anti-cancer pharmacological properties. This ent-kaurane diterpenoid, isolated from the Chinese herb *Rabdosia rubescens* and some related species, has demonstrated great potential in the treatment profile of many diseases by exerting anti-tumor, anti-inflammatory, pro-apoptotic, and neurological effects. Unfortunately, the mechanisms via which oridonin exerts these effects remain poorly understood. This review provides an overview of the multifunctional effects of oridonin as well as the reasons for its potential for investigations in the treatment of many diseases other than cancer.

Key points

Oridonin has attracted considerable attention in the last decade because of its effects against cancer.

Several molecular targets of Oridonin have been identified, suggesting that oridonin may be effective in the treatment of many other disorders in addition to cancer (Alzheimer disease, inflammation, immunomodulation).

Due to its multitarget activity, oridonin has potential to be a promising compound for future drug development.

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1 Introduction

Oridonin is a famous diterpenoid isolated from the Chinese medicinal herb *Rabdosia rubescens*, also known as dong ling cao (Fig. 1). This member of the *Lamiaceae* family is widely distributed in China and Japan. It is traditionally used in Chinese medicine as an anti-tumor, anti-microbial, anti-inflammatory, and anti-oxidant compound [1, 2]. It is also used as a supplement in the treatment of many cancers, such as esophagus, mammary gland, liver, and prostate cancers and has even been a folk remedy for carcinomas of the heart and esophagus in Hunan province [3]. Oridonin was identified for the first time in 1967 and synthesized in 1973 [4]. It is currently one of the most important compounds isolated from traditional Chinese herbal medicines [5]. Indeed, oridonin is an ent-kaurane diterpene that has been shown to have multiple biological activities. Among them, the anti-cancer activity, which is reported to occur through different cellular signaling pathways, has been repeatedly reported. For instance, the chemopreventive and anti-tumor effects of oridonin have been related to its ability to interfere with several pathways involved in cell proliferation, cell cycle arrest, and apoptosis [6].

To characterize the bioactive compounds in Rabdosia, oridonin was first isolated from the plant *Isodon japonicus* (Burm. f.) H. Hara, the common name of which in Japanese is *orido*, hence the chemical name [7]. The compound has drawn attention for its remarkable apoptosis and autophagy-inducing activity in cancer therapy. However, the results of many prominent studies have proven that oridonin possesses many other therapeutic effects, such as anti-inflammatory, neuroprotective, anti-bacterial, and anti-neoplastic effects [7, 8]. For instance, oridonin attenuates β-amyloid deposition, plaque-associated amyloid precursor protein (APP)

expression, and microglial activation in the brain of transgenic mice [9]. These observations reveal the promising therapeutic effects of oridonin, suggesting that it may be a potent alternative drug for human neurodegenerative and other inflammation-related diseases. Oridonin could also induce apoptosis through the generation of reactive oxygen species (ROS) in human hepatoma HepG2 cells [10]. Moreover, it has been suggested that oridonin is able to inactivate many signaling pathways such as protein kinase B (Akt) and ERK and activate p38 mitogen-activated protein kinase (MAPK) and c-Jun N-terminal kinase (JNK) signaling pathways [11, 12]. Oridonin can also trigger cell cycle arrest, apoptosis, and autophagy in different neoplastic cell lines [13]. Despite the large number of studies reporting the therapeutic effects of this diterpene, the entire molecular mechanism underlying its multi-targeted effects remains to be elucidated. This review summarizes the considerable knowledge about the action mechanisms of oridonin, which has been studied in recent years. We also focus on highlighting the updated data related to multi-functional effects of oridonin in many diseases, as well as its potential application in preclinical trials.

2 Oridonin: Isolation and Structure

Oridonin is a diterpenoid (Fig. 1) isolated from the traditional Chinese herb *Rabdosia rubescens*. However, many reports also mention the isolation of oridonin from other plants and from different countries (e.g. *Isodon japonica* leaves) (Table 1). Oridonin has been reported in 12 of 74 species of isodon, and phylogeny results indicate that its production has arisen at least three times in the genus [14]. The production source and harvesting region have an important impact on the quality and quantity of oridonin produced. Therefore, *Isodon rubescens* or *Isodon japonicus* from Hunan province are considered to provide the best source of oridonin [14, 15].

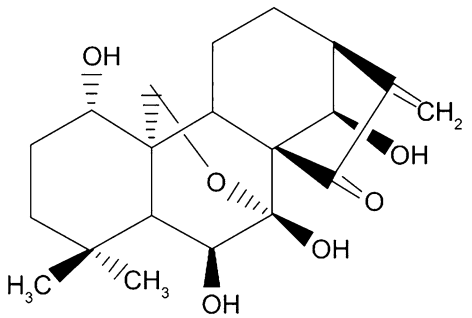

Fig. 1 Structure of oridonin

3 Molecular Targets of Oridonin

Interest is increasing in the study of oridonin activities and its potential effect in the treatment of many diseases [16]. Several of these studies have reported anti-proliferative, anti-inflammatory, and anti-neoplastic effects as well as effects on the immune system [17, 18]. The exact mechanisms by which oridonin exerts these activities are not well understood. Several proteins and receptors with which oridonin can directly or indirectly interact have been reported. Among the targets of oridonin, proteases, transcription factors, and kinases have been characterized, and the most important of them are discussed in this review.

3.1 Structural Proteins Targeted by Oridonin

Oridonin is reported to act on different cellular targets. The compound regulates the protein expression of MAP-LC3 and Beclin 1 in HeLa cells [19]. Oridonin was also reported to regulate p53 and p21 protein expressions in oridonin-treated MCF-7 human breast cancer cells [20]. The molecular chaperone heat shock protein (HSP)-70 1A was identified as an oridonin target in Jurkat cells, where HSP70 inhibition by oridonin might result in the impairment of some client proteins, thus in turn affecting several molecular pathways [6]. The expression of the anti-apoptotic protein BCL-2 was reported to decrease in apoptotic MCF-7 breast cancer cells treated with an oridonin nanosuspension [21]. Experimental data showed that oridonin significantly reduced c-Myc protein levels in vitro

Table 1 Isolation of oridonin from different plant sources

Plant source	Part of the plant	Compounds isolated	References
Rabdosia rubescens	Leaves	16, 17-exo-epoxide oridonin and 11,15-0,0-diacetylrabdoternins	[94]
Rabdosia nervosa	Stem and leaves	Lasiakaurin, ponicidin, oridonin	[99]
Rabdosia japonica (Labiatae)	Aerial part	Oridonin, nodosin	[95, 100]
Isodon japonica	Leaves	Epinodosin, oridonin, epinodosinol, lasiokaurin, rabdoternin A	[101]
Isodon rubescens	Leaves	Oridonin, ponicidin, lasiokaurin, enmenol	[101, 102]
Isodon serra	Leaves	Oridonin, lasiodonin	[55]
Isodon leucophyllus	Aerial parts	Macrocalin B, oridonin, rosthorin, rabdoternin A	[103]
Tibetan medicine Caryoteris toroetii	–	Oridonin, nodosin	[104]

and in vivo and that this reduction was mediated by the ubiquitin-proteasome system [22]. The compound was also reported to increase the ratio of BAX/BCL-2 protein expression in murine L929 cells [23]. A downregulation of the levels of Mcl-1 and BCL-x(L) cells, but not of Bcl-2 protein, was observed in both MT-1 and RPMI8226, which are adult T-cell leukemia and multiple myeloma cells, respectively [24]. The downregulation of activator protein (AP)-1 gene expression is considered to be an initial event in the oridonin-mediated inhibition of colorectal cancer, and AP-1 downregulation is also considered to be the initial response to treatment with oridonin [25]. Oridonin treatment downregulated the expression of the inhibitor of apoptosis protein (IAP) in osteosarcoma cells, and also induced the release of cytochrome c accompanied by activation of caspase-9, caspase-3, and cleavage of poly (ADP-ribose) polymerase (PARP) [26]. Oridonin induced apoptosis in A431 cells via an upregulation of the ratio of mitochondrial proteins, BAX/BCL-2 (Table 2). In addition, the total tyrosine kinase activity of cellular mitochondrial proteins and expression of epidermal growth factor receptor (EGFR) were markedly reduced after oridonin treatment [27]. The changes in BCL-2 and BAX protein levels play an important role in the mechanism of action of oridonin [5]. Oridonin has significant anti-proliferation effects on HPB-ALL cells via induction of apoptosis as well as directly causing cell necrosis by activation of caspase 3 as well as downregulation of anti-apoptotic protein BCL-2, BCL-XL, and upregulation of pro-apoptotic proteins BAX and BID, indicating that oridonin may serve as a potential anti-leukemia agent [28]. A proteomic identification of proteins involved in the anticancer activities of oridonin showed that the upregulation of Hsp70, serine/threonine kinase receptor-associated protein (STRAP), translationally controlled tumour protein (TCTP), Sti1, and protein phosphatase (PPase), as well as the downregulation of heterogeneous ribonucleoprotein (hnRNP)-E1 could be responsible for the apoptotic and G2/M-arresting effects of oridonin [29]. Oridonin also simultaneously induced apoptosis and autophagy of human multiple myeloma RPMI 8266 cells via regulation of SIRT1 nuclear protein [30]. Finally, oridonin was reported to induce HeLa cells apoptosis by altering balance of BCL-2 and BAX protein expression [31]. As represented in Table 3, experimental evidence exists of the interaction between oridonin and many protein targets. This interaction in the cell may be responsible for the different activities observed with this compound. Moreover, as

Table 2 Effect of oidonin on cellular signaling pathways

Signaling pathway	Cell line	Effect	References
MAPK-p38	BxPC-3	Enhances anti-tumor activity of gemcitabine	[58]
ASK1 and JNK1	HuH-6 cells	Activated apoptosis signal	[33]
NF kappaB and p38	Inhibition	Induction of apoptosis	[72]
ERK-p53	Murine fibrosarcoma L929 cells	Activation	[35]
PTK-Ras-Raf-JNK	Murine fibrosarcoma L929 cells	Inhibition	[35]
Ras, JNK, and p38	Human cervical carcinoma HeLa cells	Regulation	[19]
LYN/mTOR	Ph+ acute lymphoblastic leukemia cells	Inhibition of activation	[52]
P65 or p50 forms of NF-kappa and its upstream regulator I-kappa	Human breast cancer cells MCF-10A	Decrease of expression	[39]
PI3K/Akt	Cervical carcinoma HeLa cell line	Induction of apoptosis	[73]
AP-1, NF-kappa B, and p38	Colorectal cancer cell lines Lovo and SW480	Dowregulation	[67]
Akt and MAPK	Human osteosarcoma cells	Induction of apoptosis	[68]
P38 and JNK	Human osteosarcoma cells	Activation	[68]
Ras/Raf/ERK	A431 cells	Blockage	[76]
Fas/FasL signaling	U937 cells	Regulation	[74]
ERK	U937 cells	Induction of cell apoptosis	[75]
Insulin-like growth factor 1 receptor signaling	Human melanoma A375-S2 cells	Induction of cell death	[76]
Caspase 9	A375-S2 cells	Induction of apoptosis	[77]
P53 and ERK	A375-S2 cells	Activation	[43]

Akt protein kinase B, *AP* activator protein, *ERK* extracellular signal-regulated kinase, *JNK* c-Jun N-terminal kinase, *MAPK* mitogen-activated protein kinase, *mTOR* mammalian target of rapamycin, *NF* nuclear factor, *PI3K* phosphatidylinositol-3-kinase

Table 3 Effect of oridonin on receptors, enzymes, and protein expression

Receptor/enzyme/proteins	Cellular model	Effect	References
P53, Bax	LNCap cells	Upregulation	[34]
Bcl-2	LNCap cells	Downregulation	[34]
Androgen receptor	Carcinoma A431	Downregulation	[78]
Bax proteins	LNCap cells	Induction	[79]
Bax/Bcl-2 ratio and cytochrome c	HeLa cells	Induction	[80]
HSP 70	Jurkat cells	Inhibition	[81]
Nrf2	UROtsa cells	Activation	[37]
P16, p21, p27 and c-myc	Colorectal cancer cells	Regulation	[38]
HO-1	Rat splenic lymphocytes	Induction	[18]
c-Myc	Cancer cells	Reduction of protein levels	[22]
Pro-TNFalpha expression and IkB phosphorylation	L929 fibrosarcoma cells	Induction	[23]
P53, Bcl-2	L929 cells	Promotes phosphorylation of p53 and increased Bax expression	[82]
AKT, FOXO transcription factor and GSK3	Human osteosarcoma cells	Dephosphorylation	[26]
EGFR	Human laryngeal cancer cells	Augmentation of apoptosis induction	[83]
Fatty acid synthase	Human colorectal cancer cells	Suppression	[84]
Tyrosine kinase and EGFR	Human epidermoid carcinoma A431 cells	Decrease in tyrosine kinase activity and blockage of EGFR	[85]
Telomerase	K562 cells	Inhibition	[86]
Bcl-2	Lung cancer cell line SPC-A-1	Downregulation	[5]
IL2, IFN gamma, IL12p40, and TNF alpha	Murine splenic lymphocytes	Inhibition	[55]
Bax	Lung cancer cell line SPC A-1	Upregulation	[5]
Caspase-3-zymogen protein, Bcl-2, and Bcl-XL	HPB-ALL cells	Downregulation	[28]
Telomerase, Bcl-2	K562 cells	Downregulation	[54]
Bax	K562 cells	Upregulation	[54]
Telomerase	HL-60 cells	Downregulation	[87]
hTERT mRNA, and telomerase	HL-60 cells	Dowregulation	[88]
Bcl-2/Bax ratio	A549 cells	Induction of ratio decrease	[56]
PI3K, PKC, and ERK	Macrophage-like U937 cells	Activation	[40]
TNF alpha, IL-1 beta	Macrophage-like U937 cells	Release	[89]
Apaf-1, cytochrome c, and caspase-3	Gastric cancer cell line HGC-27	Induction	[90]
p-JNK, p-p38, p-p53, and p21	HepG2 cells	Incretion	[42]
B1/p-Cdc2 (Tyr15) complex	HepG2 cells	Increase	[42]
Hsp70.1, STRAP, TCTP,Sti1 and PPase	HepG2 cells	Upregulation	[29]
hnRNP-E1	HepG2 cells	Downregulation	[29]
Bcl-2/Bax ratio, caspase-8, NF-kappa B(p65), IKKalpha, phospho-mTOR	Human breast cancer cells	Reduction	[57]
Cleaved PARP, Fas, and PPAR gamma	Human breast cancer cells	Increase	[57]
NO, TNF alpha, IL6	LPS-activated microglia	Inhibition	[45]
Nucleoporin 88 and 214	OCIM2 acute erythroleukemia cells	Protection from apoptosis	[91]
SIRT1	Human multiple myeloma cells	Regulation	[30]
Raf-1, JNK, and p-JNK	HeLa cells	Induction	[92]
BAFF	mice	Inhibition	[93]
BIM	Melanoma OCM-1 and MUM2B cell lines	Upregulation	[110]

Table 3 continued

Receptor/enzyme/proteins	Cellular model	Effect	References
Fatty acid synthase	Melanoma OCM-1 and MUM2B cell lines	Downregulation	[110]
PARP	BXPC-3 cells	Induction	[111]

AKT protein kinase B, *BAFF* B-cell activating factor, *Bcl* B-cell lymphoma, *EGFR* epidermal growth factor receptor, *FOXO* forkhead box O, *GSK3* glycogen synthase kinase 3, *hnRNP* heterogeneous ribonucleoprotein, *HSP* heat shock protein, *hTERT* human telomerase reverse transcriptase, *IFN* interferon, *IL* interleukin, *mRNA* messenger RNA, *mTOR* mammalian target of rapamycin, *NO* nitric oxide, *PARP* poly (ADP-ribose) polymerase, *PI3* phosphatidylinositol-3, *PKC* protein kinase 3, *PPAR* peroxisome proliferator-activated receptor, *PPase* protein phosphatase, *STRAP* serine/threonine kinase receptor-associated protein, *TCTP* translationally controlled tumour protein, *TNF* tumor necrosis factor

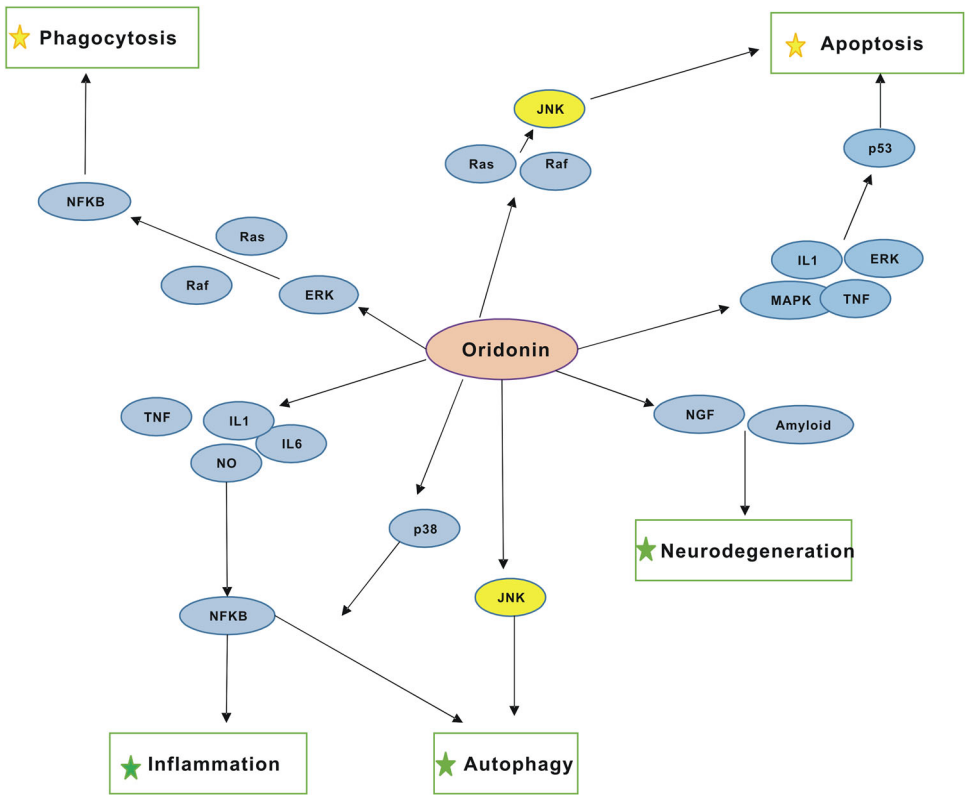

Fig. 2 An overview of the molecular targets of oridonin and potential therapeutic effects. The interaction of oridonin with target proteins and signaling pathways regulates several cellular responses, including apoptosis, autophagy, inflammation, and neuroinflammation, either positively (indicated by *green star*) or negatively (indicated by *yellow star*). Thereby, a potential therapeutic effect of oridonin in the treatment of many diseases can be emphasized

represented in Fig. 2, the connections between the different proteins affected by oridonin are crucial for the comprehension of its mechanism of action.

3.2 Regulation of Transcription Factors and Signaling Pathways by Oridonin

The activation of many transcription factors and signaling pathways is reported to be crucial in the mechanism of action of oridonin. Oridonin induced growth inhibition and apoptosis in cultured HuH-6 cells through ASK1/JNK1 signaling pathways [32], which enhances the understanding of the molecular mechanisms of oridonin in hepatoblastoma management [33]. Oridonin upregulated p53 and BAX and downregulated BCL-2 expression in a dose-dependent manner in human prostate cell lines DU-145 [34]. Oridonin induction of apoptosis in L929 cells is regulated by ROS-mediated signaling pathways, and oridonin-induced autophagy may block apoptosis by upregulating p38 and nuclear factor (NF)-kappa B activation [35]. The autophagy induced by oridonin in HeLa cells was also investigated; results showed that the oridonin-induced autophagic process was negatively regulated by Ras but positively regulated by P38 and JNK MAPKs [19]. Oridonin also downregulated the phosphorylation of ERK, whereas those of JNK and P38 were upregulated in human breast cancer MCF-7 cells [36]. The transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2) is a

critical regulator of the cellular antioxidant response and xenobiotic metabolism. Oridonin activates the Nrf2 signaling pathway at a low subtoxic dose and is able to stabilize Nrf2 by blocking Nrf2 ubiquitination and degradation, leading to accumulation of the Nrf2 protein and activation of the Nrf2-dependent cytoprotective response [37]. Oridonin induced apoptosis and senescence in colorectal cancer cells by increasing histone hyperacetylation and regulation of p16, p21, p27, and c-myc [38]. The NF-kappa B-inhibiting capacity of oridonin has been studied in several different cell types. For instance, decreases in p6 or p50 forms of NF-kappa B and its upstream regulator I-kappa B were found in oridonin-treated human cancer cells MCF-7 [39]. Oridonin also inhibits the proliferation of cells from lymphoid malignancies in association with blockade of the NF-kappa B signaling pathways [24]. NF-kappa B, AP-1, and P38 were all found to be downregulated in vivo after 4 weeks of treatment with oridonin in the colorectal cancer cell line Lovo, indicating that the downregulation of AP-1 could be an initial response to treatment by oridonin and this regulation in turn affects the expression of NF-kappa B and MAPK pathways, thereby inhibiting tumor growth [25]. The enhancement of phagocytosis of apoptotic bodies in human macrophage-like U937 cells by oridonin has been reported to occur through an activation of phosphatidylinositol-3 kinase (PI3K), protein kinase 3 (PKC), and ERK-dependent signaling pathways [40]. Another study on the phagocytic activity demonstrated that Ras/Raf1/ERK signaling pathway-dependent I-kappa B-alpha degradation, resulting in NF-kappa B activation, participates in regulation of oridonin-enhanced phagocytosis [41]. Oridonin was reported to induce G2/M cell cycle arrest and apoptosis by increasing the expression levels of p-JNK, p-p38, p-p53, and p21 and elevating the level of cyclin B1/p-Cdc2 (Tyr15) complex in HepG2 cells [42]. Oridonin induced A375-S2 cell apoptosis by activating in parallel p53 and ERK pathways, simultaneously promoting the release of cytochrome c into the cytosol, resulting in apoptotic cell death [43].

4 Immunomodulatory Effects

Among the diverse activities of oridonin, its effect on the immune system and on pro-inflammatory mediators is one of the most important. Oridonin was reported to promote differentiation of cluster of differentiation (CD)-4^+/$CD25^+$ T-regulatory (Treg) cells, and to modulate the T-helper (Th)-1/Th2 balance in rat splenic lymphocytes [18]. An investigation of the effect of oridonin on intracellular tumor necrosis factor (TNF)-alpha expression revealed that the compound augments endogenous pro-TNF alpha expression and its upstream protein IkB phosphorylation [23]. Oridonin also blocked TNF-alpha and lipopolysaccharide-stimulated NF-kappa activity in Jurkat cells as well as in RAW264.7 murine macrophages [24]. Oridonin was reported to enhance phagocytosis of apoptotic bodies by activating PI3K-, PKC-, and ERK-dependent pathways in human macrophage-like U937 cells [40]. Another study demonstrated that Ras/Raf/ERK signaling pathway-dependent Ikappa-Balpha degradation, resulting in NF-kappa B activation, participates in regulation of oridonin-enhanced phagocytosis, and that one of its effector functions is to induce the synthesis of interleukin (IL)-1 beta, which partially contributes to its phagocytic activity [41]. Oridonin facilitated the phagocytic activity against apoptotic cells through TNF alpha and IL-1 beta release, thereby contributing to its anti-tumor activities [44]. Moreover, oridonin pretreatment inhibits the release of pro-inflammatory mediators, including nitric oxide, TNF alpha, IL-1 beta, and IL-6, resulting in the inhibition of the DNA-binding activity of NF-kappa B [45]. Oridonin is also reported to be a potential modulator for trinitrobenzene sulfonic acid-induced colitis and other Th1/Th17-mediated inflammatory diseases [108].

5 The Role of Reactive Oxygen Species in Oridonin Activity

ROS are important signaling molecules involved in immune defenses, cell proliferation, and cell repair. Oridonin has been shown to increase intracellular hydrogen peroxide levels and reduce the glutathione content in colorectal cancer cells (Table 4). These effects were important in oridonin-induced apoptosis and senescence in these cells [46]. Another study showed that oridonin stimulates mitochondrial transmembrane permeabilization in a ROS-dependent manner, and these ROS produced are responsible for the oridonin-induced HepG2 apoptosis through p53, MAPK, and mitochondrial signaling pathways [47]. The growth inhibitory activity of oridonin was studied in L929 cells, where a rapid generation of ROS was noted as being triggered by oridonin, and subsequently upregulation of phospho-p53 expression and increased expression ratio of BAX/BCL-2 was observed. In this study, it was demonstrated that oridonin induced cell death in murine fibrosarcoma L929 by enhancing ROS generation [48]. It was also demonstrated that the hydroxyl radical OH(·) plays a pivotal role in oridonin-induced apoptosis and autophagy in human epidermoid carcinoma A431 cells [49]. Oridonin was reported to cause a modest level of ROS generation in U937 cells, with hydrogen peroxide (H_2O_2) and hydrogen free radical OH(·) as the major types. These two types of ROS were positive regulators involved in oridonin-enhanced engulfment of apoptotic cells. H_2O_2 and

Table 4 Effect of oridonin on cellular models

Effect	Cell type/model	References
Cytotoxic effect	Human Hep G2, COLO 205, MCF-7 and HL-60 cancer cells	[94]
Cytotoxicity	HL-60, HO-8910, and A-549 human tumor cells	[95]
Anti-proliferative activity	Human cell lines derived from prostate (DU-145, LNCaP), breast (MCF-7), and ovarian (A2780 and PTX10) cancers	[34]
Induction of apoptosis and autophagy	Murine fibrosarcoma L929 cells	[72]
Intracellular ROS generation, lipid peroxidation as well as decrease in SOD and glutathione activities	Murine fibrosarcoma L929 cells	[72]
Induces G2/M arrest and apoptosis	Murine fibrosarcoma L929 cells	[35]
Induction of apoptosis and autophagy	HeLa cells	[80]
Confers protection against arsenic-induced toxicity	UROtsa cells	[37]
Growth inhibition and inhibition of apoptosis	MCF-/breast cancer cells	[21]
Induce apoptosis and senescence and increase histone hyper acetylation	Colorectal cancer cells	[38]
Induces apoptosis and senescence by increasing H_2O_2 and glutathione depletion	Colorectal cancer cells	[46]
Inhibition of proliferation	MKN45 cells	[96]
Induced S/G2M arrest and G1/S block	MCF-7 cells	[39]
Promotes differentiation	$CD4^+/CD25^+$ T-reg	[18]
Induced TGF-beta and IL-10	Rat splenic lymphocytes	[18]
Inhibited IL-2 and IFN-gamma		
Induction of G(2)M phase arrest and apoptosis	Human laryngeal carcinoma cells	[97]
Enhance phagocytosis of apoptotic bodies	Human macrophage-like U937 cells	[40]
Induction of cell motility	Murine melanoma K1735M2 cells	[98]
Induction of G2/M cell cycle arrest	HepG2 cells	[42]

CD cluster of differentation, *IFN* interferon, *IL* interleukin, *ROS* reactive oxygen species, *SOD* superoxide dismutase, *TGF* transforming growth factor, *T-reg* T-regulatory cells

OH generation also activated PI3K-Akt and phospholipase C gamma-protein kinase C (PLC gamma)-Ras-Raf-ERK signaling pathways, which are essential for oridonin-induced apoptosis [50]. Nitric oxide was reported to augment oridonin-induced efferocytosis (phagocytosis of apoptotic cells) in human histocytic lymphoma U937 cells via autophagy and the NF-kappa B-cyclooxygenase-2-IL-1beta pathway [51]. Oridonin was reported as inducing apoptosis and autophagy of human multiple myeloma RPMI8266 cells via the regulation of intracellular ROS generation [30].

6 Oridonin: Top Interacting Genes

Oridonin is considered to be a safe and multi-targeted compound and has been reported to interact with many genes. We used the Comparative Toxicogenomics Database to find the top interacting genes, and observed that *HMOX1*, *BLC2*, *CASP3*, *IFNG*, *IL10*, *PARP1*, and *TGFB1* are the most important (Table 5). HMOX1 (heme oxygenase 1, EC 1.14.99.3) is an essential enzyme in heme catabolism that cleaves heme to form biliverdin. Oridonin is reported to increase the activity of HMOX1 messenger RNA (mRNA) and protein in rat splenic lymphocytes [18]. Oridonin is also reported to downregulate anti-apoptotic protein BCL-2 expression in many cancer cells [5, 24, 28, 34, 52–54]. Oridonin also inhibits/decreases the expression of IL2 protein in rat splenic lymphocytes and in BALB/c mouse splenic lymphocytes [18, 55]. Oridonin decreased the expression of IFN gamma (IFNG) and increased the expression of IL10 protein [18]. Oridonin increases the cleavage of caspase-3 protein [26, 56].

PARP1 is an enzyme that in humans is encoded by the *PARP1* gene, which is involved in differentiation, proliferation, and tumor transformation. Oridonin was reported to increase the cleavage of PARP1 protein in highly metastatic human breast cancer cells [57]. Transforming growth factor beta 1 (TGFB1) is a polypeptide member of the TGFB superfamily of secreted cytokines that perform many cellular functions, including apoptosis, cell differentiation, and cell proliferation. In humans, TGFB1 is encoded by the *TGFB1* gene, and oridonin is reported to increase expression of the TGFB1 protein [18].

Table 5 Oridonin top interacting genes

Interacting gene	Interaction	References
IL10	Increases expression of the protein	[18]
IL2	Increases expression of the protein	[18]
IFNG	Decreases expression of the protein	[55]
HMOX1	Increases mRNA expression	[18]
HMOX1	Increases expression of the protein	[18]
CASP3	Increases cleavage of the protein	[105]
BCL2	Decreases expression of the protein	[106]
PARP1	Increases cleavage of the protein	[20]
TGFB1	Increases expression of the protein	[107]

BCL B-cell lymphoma, *HMOX* heme oxygenase, *IFNG* interferon gamma, *IL* interleukin, *mRNA* messenger RNA, *PARP* poly (ADP-ribose) polymerase, *TGFB* transforming growth factor B

7 Adjuvant/Synergistic Effects

In order to increase the effect of oridonin, it has been combined with other compounds with reported therapeutic effects. Oridonin could potentiate the effects of gemcitabine in PaCa pancreatic cancer cells through the MAPK-p38 signaling pathway [58]. An oridonin and arsenic trioxide combination were reported to enhance tumor-suppression activity in a human hepatocellular carcinoma (HCC) model compared with single agent treatment in vivo, demonstrating that oridonin can sensitize HCC cells to As2O3 treatment and therefore facilitate the optimization of As2O3 therapy for HCC patients [59]. Oridonin in combination with imatinib revealed a synergetic anti-leukemia effect in Ph+ acute lymphoblastic leukemia cells in vitro by inhibiting the activation of the LYN/mammalian target of rapamycin (mTOR) signaling pathway [52]. A promising combined activity of oridonin and wogonin was reported in advanced-stage ovarian cancer cells [60]. The synergistic effect of oridonin and cisplatin on cytotoxicity and DNA cross-link against mouse sarcoma S180 cells in culture was reported to increase after combining the two drugs [61].

8 Oridonin Formulation and Bioavailability

Despite the promising effects of oridonin, its clinical development has been hampered by its limited aqueous solubility and bioavailability [62]. Moreover, the oridonin solution is not stable [63]. The development and application of oridonin is also reported to be limited by its rapid plasma clearance. To solve this problem, many different approaches have been developed to increase the solubility and bioavailability of oridonin. PEGylated oridonin linked with succinic acid (SA) as a spacer moiety (PEG-SA-ORI) was synthesized [64]. All polymeric conjugates obtained showed satisfactory aqueous solubility, and in vitro studies showed that the drug solubility increased, suggesting that PEGylation could be a promising method to increase the efficacy of oridonin. Oridonin nanosuspension was reported to be more effective than free oridonin on G(2)/M cell cycle arrest and apoptosis in the human pancreatic cancer PANC-1 cell line [65]. The drug carrier monomethoxy poly(ethylene glycol)-poly(epsilon-caprolactone) (MPEG-PCL) was used to increase the water solubility of oridonin and the prepared oridonin micelles obtained presented great potential for direct intravascular administration [66]. A self-microemulsifying drug-delivery system (SMEDDS) was developed to enhance the bioavailability of oridonin. The absorption of oridonin from SMEDDS showed a 2.2-fold increase in relative bioavailability compared with that of the suspension, demonstrating the promising use of SMEDDS for the oral delivery of oridonin [67]. Oridonin-loaded nanoparticles coated with galactosylated chitosan (ORI-GC-NP) was reported as a promising intravenous drug-delivery system for oridonin that could be developed as an alternative to the conventional oridonin preparations [68]. In a recent study, a novel biotin-modified nanostructured lipid carrier was developed to enhance the bioavailability of oridonin [109].

9 Oridonin as a Therapeutic Agent in Many Diseases

Oridonin could be associated with the treatment of many diseases via interaction with many inference networks. The Comparative Toxicogenomics Database reports 232 different diseases that could be directly or indirectly associated with oridonin and the different inference networks involved. Based on these observations, the compound really needs to be investigated for the treatment of many other diseases aside from cancer. For instance, a recent publication showed oridonin was able to ameliorate neuropathological changes and behavioral deficits in a mouse model of cerebral amyloidosis by attenuating b-amyloid deposition, plaque-associated APP expression, and microglial activation in the brain of transgenic mice [9]. Oridonin was shown to lower pulmonary artery pressure in rats [68]. It is therefore presented as a new research target for future and promising investigations.

10 Structure/Activity Relationship

The chemical structure of oridonin has been modified in some studies to increase its therapeutic effects. Therefore, overcoming synthetic challenges of oridonin A-ring structural diversification is attracting high levels of interest. Regio and stereoselective installation of azides and 1, 2, 3-triazoles at the C-1, C-2, or C-3 position was shown to

improve the effect of oridonin [69]. New nitrogen-enriched oridonin analogs obtained by rationally modifying the structure of oridonin with thiazole-fused in the A-ring through an efficient protecting group-free synthetic strategy were shown to enhance the anti-cancer profile, as well as to improve the water solubility [62].

Oridonin was reported to exhibit moderate effects against highly aggressive cancers. Therefore, efficient and regioselective enone construction strategies have been established. With oridonin ring A-based diverse constructions of enone functionality, novel dienone analogs were produced and reported effective against highly aggressive breast cancer by inducing apoptosis [70]. The structure-activity relationship of six ent-kaurane diterpenoids, including oridonin, on cytotoxicity and DNA damage potential against three human tumor HepG2, GLC-82, and HL-60 cell lines was reported. It was found that exo-methylene cyclopentanone in the molecular structure is important in maintaining the cytotoxicity and DNA damage potential of oridonin. With an OH group at position C1, oridonin exhibited a higher cytotoxicity and DNA damage potential on the tumor cells than lasiokaurin (OAc group at position C1) [71].

11 Conclusion

Oridonin has shown promising effects in the modulation of molecular pathways in different cell models. Several molecular targets have already been identified, which can partially explain the broad range of in vitro biological effects of the compound. The compound has also shown promising effects in the treatment of diseases other than cancer. Therefore, further studies aiming at investigating the effect of oridonin in many other disease treatments could be promising.

Compliance with Ethical Standards

Conflicts of interest No funding was received in the preparation of this review. Brice Ayissi Owona and Herman J Schluesener have no conflicts of interest to declare.

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