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Review

Triptolide, A Potential Autophagy Modulator*

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ABSTRACT As a major active component extracted from traditional Chinese herb *Tripterygium wilfordii* Hook F, triptolide exhibits multiple pharmacological effects. Autophagy is an evolutionary conserved intracellular catabolic process involved in cytoplasmic materials degradation. Autophagic dysfunction contributes to the pathologies of many human diseases, which makes it a promising therapeutic target. Recent studies have shown that triptolide exerts neuroprotection, anti-tumor activities, organ toxicity, and podocyte protection by modulating autophagy. This article highlights the current information on triptolide-modulated autophagy, analyzes the possible pathways involved, and describes the crosstalk between autophagy and apoptosis modulated by triptolide, in hope of providing implications for the roles of autophagy in pharmacological effects of triptolide and expanding its novel usage as an autophagy modulator.

KEYWORDS triptolide, autophagy, apoptosis, *Tripterygium wilfordii* Hook F

Autophagy is a highly conserved and tightly regulated process that transports cellular substrates to lysosomes for bulk degradation. There are 3 major types of autophagy, including macroautophagy, microautophagy and chaperone-mediated autophagy. Although differs on the substrates and mechanisms of sequestration, all 3 types eventually converge on the delivery of cargos to the lysosome for degradation and recycling. In this article, we will focus on macroautophagy (hereafter referred to as autophagy for simplicity). Autophagy plays a wide variety of physiological roles; disordered autophagy is implicated in many kinds of human diseases.[1-3] For instance, monoallelically deletion of the essential autophagy gene Beclin1 and decreased levels of the protein presented in human breast, ovarian and prostate cancers;[4] altered expressions of other autophagy related proteins and elevated microtubule-associated protein 1 light chain 3 (LC3) puncta have been observed in human cancer specimens.[5,6] Autophagic vacuoles abnormally accumulated in the brains of patients with diverse neurodegenerative diseases, which might represent induction of autophagy or blockage of autophagic flux.[7] Autophagy deficiency in mice led to behavioral defects, progressive deficits in motor function, and the accumulation of polyubiquitinated cytoplasmic inclusion bodies in neurons.[8,9] Therefore, the autophagic pathway has been an ideal target for the prevention or treatment of these autophagy-relavent disorders, and pharmacologic autophagy modulators may be regarded as promising drug candidates.

Since Chinese herbs are long-term applied usually with relatively defined pharmacological actions, toxicities or side effects, they have been important sources for screening autophagy modulators. Indeed, many natural components from Chinese herbs exert autophagic-regulatory effects in various pathological conditions, suggesting their potential novel therapeutic effects via modulating autophagy.[10,11] Triptolide, a diterpene triepoxide, is a major active component extracted from Chinese herb *Tripterygium wilfordii* Hook F, which exhibits multiple pharmacological effects, including anti-inflammatory, anti-oxidant, immune modulation, neuroprotection, and anti-tumor activity. And triptolide has been widely used to treat autoimmune, inflammatory diseases and different types of tumors.[12,13] The pharmacological effects of triptolide involve multiple signal pathways and several cellular targets have been demonstrated,

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such as Hsp70, nuclear factor-kappa B, vascular endothelial growth factor and RNA polymerase Ⅱ.[14] Recent studies have revealed that triptolide is capable of modulating autophagic pathway in several cell lines and tissues.[12,15-29] Herein we present recent advances in triptolide-modulated autophagy, summarize the molecular mechanisms underlying it, discuss the interconnection between autophagy and apoptosis modulated by triptolide, hopefully providing information for better understanding the mechanism of pharmacological action and developing the novel potential application of triptolide as an autophagy modulator.

Overview of Autophagy

Autophagy consists of several sequential steps: it initiates from the formation of isolation membranes (also known as phagophore), which sequester cytoplasmic constituents. The membrane structures elongate resulting in the double-membraned autophagosome. Subsequently, matured autophagosome fuses with the lysosome, thereby forming an autolysosome, where the materials contained within it and the inner membrane of the autophagosome are breakdowned by lysosomal hydrolases, and the resulting degradation products are transported back to the cytosol for reuse.[7] As a major degradation system, autophagy occurs constitutively at low basal levels in all eukaryotes for the turnover of long-lived proteins and organelles under normal conditions, acts as the quality-control machinery for cytoplasmic components, and functions in maintaining cellular homeostasis.[30] Autophagy can be induced in response to various extra- or intracellular stresses and signals such as nutrient limitation, hypoxia, heat, endoplasmic reticulum (ER) stress, and pathogen infection.[31] In *Sacharomyces cerevisiae*, autophagy plays a significant role in maintaining viability during starvation. In mammals, except as a cellular response to stress conditions, autophagy is also important in development, differentiation and tissue remodeling, maintaining genome integrity and longevity. Moreover, it can function in innate and adaptive immunity.[31] In addition, autophagy may participate in type Ⅱ programmed cell death that is distinct from apoptosis.[32]

A series of highly conserved AuTophaGy-related genes (ATG) have been identified that they are essential for autophagic functions. Four functional subgroups constitute the core molecular machinery that are required for autophagosome formation and maturation: the ATG1/unc-51-like kinase (ULK) complex; two ubiquitin-like protein conjugation systems; phosphatidylinositol 3 kinase class Ⅲ (PI3KⅢ)/Vps34 complex; and two transmembrane proteins, ATG9/mATG9 and vacuole membrane protein 1 (VMP1).[33] And autophagic process is strictly controlled by several signaling pathways, such as Ras-Raf1-mitogen-activated protein kinase kinase 1/2 (MEK1/2)-extracellular regulated protein kinases 1/2 (ERK1/2) pathway, Beclin1-PI3KⅢ pathway, PI3KI-Akt-mammalian target of rapamycin complex 1 (mTORC1) pathway and adenosine 5'-monophosphate (AMP)-activated protein kinase (AMPK) mediated signaling pathway.[31]

Triptolide Modulates Autophagy

Up-to-date, it is demonstrated that triptolide functions in neuroprotecion, anti-tumorigenic effects through autophagic pathways in *in vitro* and *in vivo* models. Autophagy also involves in hepatotoxicity and cardiotoxicity of triptolide. Moreover, triptolide can protect podocyte via modulation of autophagy. As described in more detail in Tables 1 and 2, we list the studies supporting triptolide as an autophagy modulator.

Triptolide Regulates Autophagy in Cancer Cells

Triptolide possesses anti-tumor activity in various tumor models,[13,34] while autophagic pathway has been found to be one of its action targets and is closely associated with triptolide-induced cytotoxic effects. Triptolide induced cell death through stimulating autophagy in neuroblastoma, leukemia, pancreatic cancer, lung cancer, and colon cancer cell lines.[12,20-22,24,26,27] And increased autophagic activity was detected in triptolide-treated pancreatic cancer cell orthotopic tumor sample in nude mouse.[21] However, in prostate cancer and cervical cancer cells, autophagy protected against triptolide-induced cell death.[23,25] In *in vivo* studies, compared with triptolide treatment group, autophagy inhibitor and triptolide combined treatment further prevented prostate cancer growth in nude mouse, further proving autophagy played cytoprotective roles in triptolide-induced prostate cancer cell death.[23] Indeed, autophagy has dual roles in treatment of cancer which is context dependent.[35] On one hand, autophagy negatively regulates tumor development, acts as a tumor suppressor. On the other hand, autophagy may be a survival mechanism

Table 1. Triptolide Modulates Autophagy *in vitro*

Target cell line	Doses and incubation time	Regulation of autophagy	Role of autophagy	Related biological effect
Human pancreatic cancer cells S2-VP10 and S2-013[21,22]	25–200 nmol/L, 24 h	Induction	Pro-death	LC3 Ⅱ protein ↑; LC3 puncta ↑; acidic autophagosome ↑; inhibition of Akt/mTOR/p70s6k pathway; upregulation of ERK1/2 pathway; cytoplasmic calcium ↑; chronic ER stress
Human pancreatic cancer cell Hs766T[21]	100–200 nmol/L, 24 h	Induction	Pro-death	LC3 Ⅱ protein ↑; LC3 puncta ↑; acidic autophagosomes ↑
Mouse leukemia cells WEHI-3[12]	60 nmol/L, 24 h	Induction	Pro-death	Ultrastructural characterization of autophagyic vesicles ↑; acidic vesicular organelles and autophagolysosomes ↑; LC3 Ⅱ protein ↑; ATG5, ATG7, ATG12 and Beclin1 proteins ↑
Human neuroblastoma cell SH-SY5Y[20]	50–100 nmol/L, 6 h	Induction	Pro-death	LC3 Ⅱ protein ↑; autophagosome formation ↑; cytoplasmic calcium ↑
Human prostate cancer cells PC-3, lncap and C4-2[23]	10–100 nmol/L, 24 h	Induction	Pro-survival	LC3 Ⅱ protein ↑; p62 protein ↓; acidic autophagosomes ↑; autophagic vesicles ↑; autophagic flux ↑; activation of CaMKK β -AMPK signaling pathway; cytoplasmic calcium ↑; inhibition of the mTORC1 complex; activation of both the ULK and PI3K Ⅲ complexes
Human lung cancer cells A549[24]	100 nmol/L, 48 h	Induction	Pro-death	Acidic vesicular organelles ↑; GFP-LC3 dot ↑; LC3 Ⅱ protein ↑; p-ERK protein ↑
Human cervical cancer cell Hela[25]	50 nmol/L, 24 h	Induction	Pro-survival	LC3 puncta ↑; LC3 Ⅱ protein ↑, p62 protein ↓; autophagic flux ↑
Mouse colon cancer cell CT26[26]	50 nmol/L, 24 h	Induction	Pro-death	LC3 Ⅱ/LC3 Ⅰ ratio ↑; p62 protein ↓; autophagic flux ↑
Human colorectal cancer cell HCT116[27]	40 nmol/L, 48 h	Induction	Pro-death	LC3 Ⅱ protein ↑; acid autophagic vacuoles ↑
Differentiated rat pheochromocytoma cell PC12[18]	0.1 nmol/L, 24 h	Inhibition	Pro-death	Acidic vesicular organelles ↓; LC3 staining intensity and bright fluorescent particles of LC3 ↓; LC3 Ⅱ/LC3 Ⅰ ratio ↓
Mouse dopaminergic cell MN9D[19]	50 nmol/L, 24 h	Induction	Pro-survival	LC3 Ⅱ protein ↑; p62 protein ↓; GFP-LC3 puncta ↑; ultrastructural characterization of autophagic vesicles ↑
Rat primary cortical neuron[19]	50 nmol/L, 24 h	Induction	Not mentioned	LC3 Ⅱ protein ↑
Human liver cell HepG2[28]	5 μmol/L, 24 h	Induction	Not mentioned	LC3 Ⅱ/LC3 Ⅰ ratio ↑; autophagic flux ↑
Rat cardiomyocyte H9c2[15,16]	40–640 nmol/L, 1.5–12 h	Induction	Pro-survival (40–160 nmol/L, 1.5–6 h); Pro-death (40–160 nmol/L 6–12 h; 320–640 nmol/L, 1.5–12 h)	Beclin1 mRNA and protein ↑; LC3 Ⅱ protein ↑; GFP-LC3 dots ↑; lysosomal activity ↑
Primary neonatal rat cardiomyocyte[15]	40–640 nmol/L, 1.5–12 h	Induction	Pro-survival (40–160 nmol/L, 1.5–6 h); Pro-death (40–160 nmol/L, 6–12 h; 320–640 nmol/L, 1.5–12 h)	Beclin1 protein ↑; LC3 Ⅱ protein ↑
Mouse podocyte MPC5[17]	10 ng/mL, 4 h	Induction	Pro-survival	Inhibition of mTOR-ULK1 pathway; p-mTOR, p-ULK1 and p-Akt proteins ↓; LC3 Ⅱ and Beclin1 proteins ↑

Notes: ↑: increase; ↓: decrease; LC3: microtubule-associated protein 1 light chain 3; ERK: extracellular regulated protein kinase; ER: endoplasmic reticulum; ATG: autophagy-related gene; mTORC1: mammalian target of rapamycin complex 1; ULK: unc-51-like kinase; PI3K Ⅲ: phosphatidylinositol 3 kinase class Ⅲ; GFP: green fluorescent protein; AMPK: adenosine 5'-monophosphate-activated protein kinase; CaMKK β: calcium/calmodulin-dependent protein kinase kinase β

Table 2. Triptolide Modulates Autophagy *in vivo*

Target tissue	Doses and incubation time	Regulation of autophagy	Role of autophagy	Relative biological effect
Nude mouse S2-013 or HS766T orthotopic tumor sample[21]	0.4 mg/(kg•d), 21 d	Induction	Not mentioned	LC3 Ⅱ protein ↑
Nude mouse with PC3 cell tumor xenografts[23]	0.15 mg/(kg•2d), 18 d	Induction	Pro-survival	LC3B protein ↑
Rat brain tissue[29]	0.2 mg/kg, 12 h	Induction	Pro-survival	Beclin1 protein ↑; mTOR protein ↓
Mouse liver tissue[28]	0.5 mg/kg, 48 h	Induction	Not mentioned	LC3 protein ↑
Balb/c mouse cardiac tissue[15]	1.2 mg/kg, 24 h	Induction	Pro-survival	Beclin1 protein ↑; LC3 Ⅱ protein ↑

Notes: ↑: increase; ↓: decrease; LC3: microtubule-associated protein 1 light chain 3; mTOR: mammalian target of rapamycin

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for tumor cells under stress conditions. Tripchlorolide, a derivative of triptolide, suppressed the viability of lung cancer cells as well as increased autophagosome formation, whereas inhibition of the autophagosome formation dramatically decreased the mortality of lung cancer cells,[36] suggesting tripchlorolide inhibited the lung cancer cells growth mainly by activating autophagy. Moreover, triptolide increased lysosomal enzymatic activity, destroyed lysosomal integrity, promoted the linkage of lysosomal hydrolases into cytosol, and triggered atypical apoptosis in breast cancer cells.[37] So, triptolide can modulate autophagy that has pro-death or pro-survival effects in different cancer cells.

Triptolide Regulates Autophagy of Neuronal Cell Lines

Triptolide has been proven to possess significant neuroprotective properties on central nervous system diseases,[38] and recent studies suggest autophagy function in it. Autophagy facilitates the clearance of aggregate-prone mutant proteins associated with different neurodegenerative diseases, such as α-synuclein in Parkinson's disease.[39] Triptolide promoted the clearance of α-synuclein via induction of autophagy and protected against α-synuclein-induced cytotoxicity in neuronal cells,[19] suggesting triptolide has the potential beneficial effects against aggregrate-prone protein causing neurodegeneration including Parkinson's disease through upregulation of autophagic activity. In a pre middle cerebral artery occlusion (MACO) model, a significant increase in infarction area was observed and autophagy was inhibited, while triptolide administration reduced the infraction area and attenuated cerebral ischemia through moderately upregulating autophagy, leading to a neuroprotective effect.[29] The abnormal accumulation of amyloid-beta-peptide$_{25-35}$ (Aβ_{25-35}) in plaque in brain tissue plays a key role in Alzheimer's disease. In differentiated pheochromocytoma cell line, Aβ_{25-35} treatment resulted in a decrease in cell viability and an increase in the apoptotic rates. Meanwhile, Aβ_{25-35} treatment activated autophagy, suggesting a role of autophagy in Aβ_{25-35}-mediated toxicity.[18] Indeed, alteration of autophagy in brain is a well-established characteristic of Alzheimer's disease.[40] Triptolide inhibited Aβ_{25-35}-induced cytotoxicity and suppressed the elevated autophagy, while using autophagy inhibitor or activator regulated autophagic activities further confirmed triptolide protected differentiated pheochromocytoma cell line from Aβ_{25-35}-induced cytotoxicity by inhibiting autophagic activity.[18] In together, these results show that upregulated or downregulated autophagy involves in the neuroprotective effects of triptolide.

Triptolide Regulates Hepatic and Myocardial Autophagy

Triptolide exhibits multiple pharmacological effects as well as serious multi-organ toxicity, such as hepatotoxicity and cardiotoxicity. Many mechanisms that are responsible for triptolide-mediated liver and heart injury have been explored,[13] and recent studies have revealed triptolide could modulate autophagy to regulate hepatic and myocardial injury. Triptolide administration led to obvious liver injury in mice, increased the expression of the tail vein injected LC3-green fluorescent protein (GFP) plasmid in liver tissue; *in vitro* experiments showed triptolide induced autophagic flux of liver cells.[28] Similarly, triptolide exerted toxic effects both in cultured cardiomyocytes (H9c2 cells and primary neonatal rat cardiomyocytes) and the *in vivo* mouse heart accompanied by induction of autophagy. Pharmacological enhancement or inhibition of autophagy attenuated or augmented triptolide-induced cardiomyocyte injury, respectively; up-regulated autophagy by pretreatment with rapamycin alleviated triptolide-induced myocardial damage in mice heart tissues.[15] Thus, these results indicate that autophagy contributes to liver and heart injury, and modulation of autophagy could be a novel therapeutic target to attenuate triptolide-induced hepatotoxicity and cardiotoxicity.

Triptolide Regulates Podocyte Autophagy

Podocytes injury occurs in several forms of human glomerular disease.[41] Autophagic activity was much higher in podocytes than in other renal cells, and autophagy deficiency in podocyte exhibited increased susceptibility to glomerular disease.[42] In an *in vitro* podocyte injury model, puromycin amino nucleotide (PAN) decreased the autophagy level, and injured conditionally immortalized mouse podocyte cells (MPC5). Triptolide incubation reduced podocyte injury by increasing the levels of autophagy.[17,41] These results suggest podocyte injury caused by PAN involves changed autophagic activity, and triptolide-induced autophagy have a protective effect on damaged podocytes.

Possible Mechanisms of Triptolide-Modulated Autophagy

Several molecular mechanisms participate in

triptolide-modulated autophagy, and oxidative stress is a major contributor to it. Indeed, a genome-wide microarray analysis has demonstrated that triptolide altered the expression of various genes involved in oxidative stress pathway.[43] Triptolide reduced anti-oxidant enzymes activity, abnormally increased intracellular reactive oxygen species (ROS) levels, led to mitochondria damage and oxidative stress, and finally caused injury in cardiomyocytes or heart tissues.[44] Autophagy can be triggered by oxidative stress to degrade toxic cellular proteins and damaged organelles.[45] In triptolide-treated cardiomyocytes, damaged mitochondria, as a major source of ROS, were selectively sequestered into acidic lysosomal vacuoles for degradation.[15] So, triptolide-modulated autophagy is thought to be induced by oxidative stress, resulting in clearance of the damaged mitochondria. And induction of autophagy by rapamycin protected cardiomyocytes against triptolide-induced mitochondrial dysfunction and thus attenuated oxidative stress. Moreover, triptolide stimulated autophagy by increasing the production of ROS and promoted leukemia cells death.[12]

Besides this, cytoplasmic calcium, as a potent inducer of autophagy,[46] also plays an important role in triptolide-modulated autophagy in leukemia cells,[12] likewise in other cell lines. Triptolide elevated intracellular calcium levels and subsequently activated autophagy in neuroblastoma cell line,[20] while in differentiated pheochromocytoma cell line, triptolide inbibited upregulated intracellular calcium and ROS levels caused by $A\beta_{25-35}$, thereby weakening autophagy.[18] Triptolide-induced autophagy in pancreatic cancer cells was associated with an elevation of intracellular calcium concentration, and this process was also mediated by inhibition of Akt/mTOR/p70S6K pathway and activation of ERK signaling pathway,[21] as well as chronic ER stress.[22] ER stress was caused by triptolide in prostate cancer cells, which subsequently released free calcium into cytoplasm. Calcium further activated calcium/calmodulin-dependent protein kinase kinase β (CaMKKβ)-AMPK signaling pathway, resulted in inhibition of mTOR and activation of ULK1 and PI3KⅢ complexes, and ultimately induced autophagy.[23] Triptolide inhibited mTOR-ULK1 pathway, decreased the expression of p-mTOR, p-ULK1 and p-Akt, and upregulated autophagy in podocytes.[17]

Other mechanisms have been explored in triptolide-modulated autophagy. Triptolide-induced autophagy in lung cancer cells may be correlated with phosphorylation of ERK protein.[24] In Hela cells, triptolide induced autophagy by both increasing the nuclear localization of p53, which in turn upregulated the expression of lysosomal protein DNA damage-regulated autophagy modulator (DRAM), and downregulation of mTOR.[25] Triptolide attenuated the upregulated expression level of mTOR caused by cerebral ischemia, leading to an increased autophagic activity in rat brain.[29] In addition, lysosome could be a specific action site for triptolide in breast cancer cells and cardiomyocyte.[16,37] So, triptolide probably modulates autophagy through targeting multiple machineries or signal pathways (Figure 1).

Crosstalk between Autophagy and Apoptosis Modulated by Triptolide

Although autophagy and apoptosis are distinct cellular processes that even result in opposite consequences, several regulatory elements of both autophagy and apoptosis interact together, and the signaling pathways are closely interconnected.[32] For example, Beclin1 was originally identified as a novel Bcl2 interacting protein, and binding of Bcl2 inhibited autophagy,[47] whereas caspases-mediated cleavage of Beclin1 inactivated autophagy and enhanced apoptosis.[48] Triptolide-induced apoptosis has been extensively studied.[13,34] As expected, there exists an intimate crosstalk between triptolide-modulated autophagy and apoptosis.[12,15,20,21,23,25-27,29] In different types of neuroblastoma or pancreatic cancer cell line, triptolide treatment resulted in caspase-dependent apoptotic or caspase-independent autophagic cell death, respectively,[20,21] although a significant increase in cytoplasmic calcium, an effector of both autophagy and apoptosis, was commonly observed. In the presence of triptolide, inhibition of autophagy caused apoptotic cell death in pancreatic cancer cells which normally underwent autophagic cell death.[21] So, triptolide might prefer to kill one cell type by autophagy and apoptosis in the other, and it is also assumed that apoptosis could appear when autophagy is inhibited as autophagy antagonizes apoptosis. Additionally, in the same type of leukemia cell,[12] prostate cancer cell,[23] cardiomyocyte,[15] colorectal cancer cell,[26,27] and cervical cancer cell,[25] triptolide induced apoptosis as well as autophagy. Moreover, *in vivo* studies also proved triptolide influenced autophagy and apoptosis simultaneously.[15,29] Through intervening triptolide-modulated autophagic process, it is indicated that autophagy involves in triptolide-induced

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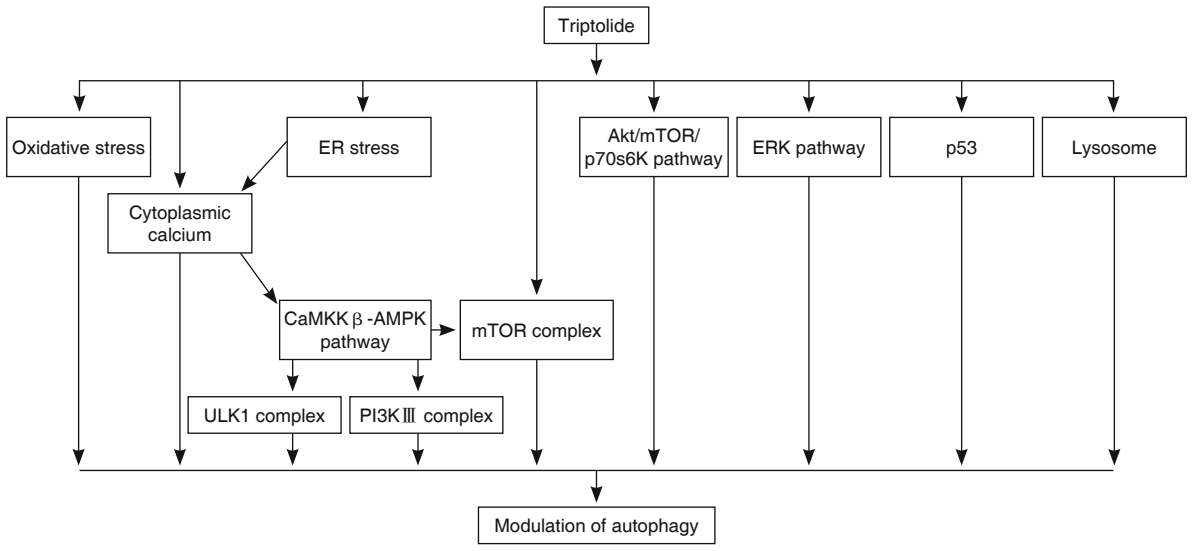

Figure 1. Machineries or Signal Pathways Related with Triptolide-Modulated Autophagy

Notes: ER: endoplasmic reticulum; mTOR: mammalian target of rapamycin; ERK: extracellular regulated protein kinase; CaMKKβ: calcium/calmodulin-dependent protein kinase kinase β; AMPK: adenosine 5'-monophosphate-activated protein kinase; ULK1: unc-51-like kinase 1; PI3K: phosphatidylinositol 3 kinase

apoptosis. Upregulation of autophagy ameliorated triptolide-mediated apoptosis in cardiomyocytes,[15] or enhanced triptolide-mediated apoptosis in colorectal cancer cells,[26,27] whereas downregulation of autophagy also promoted triptolide-mediated apoptosis in prostate cancer cell and cervical cancer cell.[23,25] Interestingly, there is a time- and concentration-dependent relationship between autophagy and apoptosis induced by triptolide in cardiomyocytes.[15] At early time point, lower concentration of triptolide-induced autophagy might attenuate apoptosis, while high concentration of triptolide-mediated autophagy induced apoptosis, so did at later time point lower concentration of triptolide-mediated autophagy. It is consistent with the controversial role of autophagy which can be adaptive or maladaptive.[2] These results also show that triptolide-induced autophagy has a pro-survival or pro-death effect which might be dependent on the cell lines, triptolide incubation time or dosages.

With the mechanisms underlying the interplay between triptolide-modulated autophagy and apoptosis, altered autophagy or apoptosis proteins may play critical roles in it. For example, triptolide increased the expressions of several specific autophagy proteins in leukemia cells, such as ATG5, ATG7, ATG12 and Beclin1,[12] while these proteins regulated apoptotic pathway directly.[32] Conversely, the altered apoptosis protein or activated caspases may involve in the regulation of autophagy.[12,15,21,23,25,29] In addition, p53 is a vital player in the triptolide-modulated autophagy and apoptosis crosstalk, as inhibition of p53-dependent transcription partly inactivated triptolide-modulated autophagy and apoptosis in human cervical cancer cell.[25] So, the crosstalk between triptolide-modulated autophagy and apoptosis indicates they may share the common regulators or signaling pathways, and each can modify the activity of the other. However, more details about the crosstalk deserve a deep study.

Conclusions and Perspectives

The active components or fractions from Chinese herbs have attracted increasing attention for high-throughput screening of autophagy modulators,[11,49,50] and triptolide, a monomeric compound isolated from *Tripterygium wilfordii* Hook F, has been shown to exert potent autophagic-regulatory effect via multiple mechanisms. Dependent upon the known observations, triptolide induces autophagy in most *in vitro* and *in vivo* models with the exception that triptolide inhibits autophagy in differentiated rat pheochromocytoma cell line. It could be a cell line-specific response to triptolide, and also be possibly a common one considering a relatively lower concentration of triptolide used (0.1 nmol/L).[18] However, one major limitation of these studies is that they have addressed the triptolide-modulated autophagy in some *in vitro* models and not with direct comparison to *in vivo* models. Until now, the processes or targets through which triptolide influence the intricate autophagy machinery are not understood well. Further experiments are still needed to clarify the molecular signaling mechanisms involve it. In addition,

since autophagy plays key roles in normal physiological functions, a noticeable question is whether triptolide-modulated autophagy will be sufficiently selective to pathological tissues while sparing normal tissues from the possible deleterious consequences, and it is worthy to check the effects of triptolide in normal cells or tissues when using it to modulate autophagy. Lastly, the current studies mainly focus the regulatory effects of triptolide on macroautophagy and future investigations are necessary to explore its regulation on the other two types of autophagy.

In conclusion, autophagy could be a common pathway that triptolide exerts its multiple pharmacological activities. With a comprehensive understanding of the mechanisms and functions implicated in triptolide-mediated autophagy, it is highly reasonable that triptolide might be used as a novel autophagy modulator either alone or in combination with other treatments for diseases therapeutic in future.

Conflict of Interest

The authors have no conflict of interest.

Author Contributions

Wei YM conceived the work and drafted the manuscript; Wang YH and Xue HQ performed the data analyses; Luan ZH, Liu BW and Ren JH revised the manuscript and approved the final version.

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