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The Targeting of Autophagy and Endoplasmic Reticulum Stress Mechanisms by Honokiol Therapy

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ABSTRACT

In recent decades, special attention has been paid to cell death mechanisms, with the exception of apoptosis. This could be due to the resistance of cells, particularly cancer cells, to apoptosis. Among novel pathways, autophagy and endoplasmic reticulum (ER) stress have attracted the attention of researchers. A large number of antitumor drugs have been developed based on their modulatory effects on autophagy and ER stress. On the other hand, ER stress could stimulate autophagy and apoptosis, which is indicative of the dual role of this pathway. Therefore, the monitoring of these pathways could contribute to the treatment of pathological conditions. Among the multiple synthetic and natural modulators of autophagy and ER stress, natural agents are used more extensively owing to their few side-effects, valuable biological activities, and cost-efficiency. Honokiol as a lignin extracted from the bark of magnolia tree. This compound has been reported to have antioxidant, anti-inflammatory, anti-diabetic, and antitumor effects. The present study aimed to first introduce honokiol, autophagy, and ER stress and assess the modulatory effects of honokiol on the autophagy and ER stress mechanisms so as to demonstrate the therapeutic efficacy of this natural compound.

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Introduction

There is an absolute need for organisms to maintain their homeostasis in physiological and pathological conditions. Regulated cell death (RCD) is involved in this homeostasis through the degradation of impaired or potentially toxic organelles and macromolecules. Furthermore, RCD is observed in physiological and pathological conditions in a large number of organisms, such as eukaryotes and some prokaryotes (1-13). In general, cell death is classified into three distinct categories, including apoptosis, autophagy, and necrosis (14).

Apoptosis has not exhibited a promising profile in cancer therapy. In an experiment performed by Roumane et al. (15), the results indicated that the effective treatment of cancer requires the proper understanding cell death mechanisms rather than apoptosis, which is mainly due to the proliferative effects of apoptosis on the surrounding surviving cells; this is referred to as apoptosis-induced proliferation. From an organelle standpoint, endoplasmic reticulum (ER) also plays a key role

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in homeostasis. As such, the proper targeting of these pathways and organelles could be beneficial in the treatment of the pathological conditions associated with the impairment of these pathways. It is of paramount importance to find compounds with modulatory effects on these mechanisms as a promising strategy in the management of pathological conditions. It is also notable that synthetic drugs have been reported to have numerous side-effects, whereas plant-derived products have shown favorable biological activities (16-27).

The present study aimed to describe the beneficial effects of honokiol as a natural compound on the targeting of autophagy and ER stress.

Literature Review

Molecular Mechanisms of Autophagy

Autophagy is a catabolic process, which is responsible for the monitoring and conservation of cellular energetic balance during the degradation of proteins and organelles in lysosomes (28-37). Autophagy is stimulated by starvation and other stress conditions so as to provide adequate energy and maintain survival (38). If the energy level is insufficient (nutrient deprivation), autophagy is activated to partly decompose its deposits for the provision of energy. The periodic incidence of autophagy occurs at least once during the day; for instance, autophagy is induced between meals in organs such as the liver in order to preserve its primary functions, while providing amino acids and energy as well (39-44). As an evolutionary process, autophagy is also observed in yeasts, and its main function is to respond to starvation.

Based on mechanisms and functions, autophagy is classified into three distinct types, including micro-autophagy, macro-autophagy (autophagy), and chaperone-mediated autophagy (CMA). In micro-autophagy, cytoplasmic structures are surrounded by the pre-existing lysosomes or vacuole membranes. In CMA, the guidance of cargos toward the lysosome is mediated by chaperone proteins, such as HSPA8 (HSC70). In macro-autophagy (autophagy), proteins and lipids are recruited from some intracellular membranes so as to form autophagosomes (double-membrane vesicles). Following that, they are infused with lysosomes to degrade their content (45-49).

Based on the procedures of cargo delivery to the lysosome, autophagy is divided into the selective and non-selective types. Seemingly, CMA is more selective and specific compared to micro- and macro-autophagy (50,51). Furthermore, a basal level of autophagy is required to maintain homeostasis and prevent the accumulation of impaired macromolecules and organelles in normal conditions. Therefore, the strict regulation of autophagy mechanisms is considered essential (39,40,52).

According to the literature, the PI3K/Akt/mTOR pathway is the most important regulator of autophagy. In normal conditions (i.e., balanced energy level), the mammalian target of rapamycin complex 1 (mTORC1) suppresses the autophagy process through the phosphorylation of Unc-51-like kinase 1/2 (ULK1/2) and autophagy-related gene 13 (ATG13). In addition, class-I PI3K (phosphatidylinositol-3-phosphate kinase) and class-III PI3K are considered to be the inhibitors and stimulators of autophagy, respectively (53,54).

Under stress conditions (e.g., starvation), ATG1 activates autophagy (55,56). After activation, ATG1 produces the phagophore (cup-shaped structure) that surrounds the cargo. Afterwards, this complex phosphorylates the Beclin-1 at Ser14 residues in order to accelerate the formation of autophagosome through the activation of other ATGs on the membranes of the phagophore (57). Following that, the signal of the triggering autophagy is induced via the ubiquitination and degradation of ULK-1 by Cul3-KLHL20 ligase complex (58). At this phase, autophagosome has been formed and is in the nucleation stage, where the VPS34-Beclin-1 complex plays a remarkable role (59,60). Finally, the elongation of the autophagosome occurs, in which two ubiquitin-like conjugation systems (ATG5-ATG12 and light chain 3 complexes [LC3]) are involved (61).

Molecular Mechanisms of Endoplasmic Reticulum Stress

The regulation of protein homeostasis is referred to as proteostasis, which is a mechanism investigating the synthesis, folding, assembly, translocation, and decomposition of proteins. Endoplasmic reticulum (ER) is an organized, double-membrane compartment that is involved in the proper folding of a large number of eukaryotic cell proteins (proteostasis), Ca^{2+} storage and release, and lipid and carbohydrate metabolism (62-64). In this regard, it is of utmost importance to consider the key role of these proteins as some must lodge in ER, Golgi apparatus, lysosomes, and plasma membrane, while the others are involved in intracellular signaling pathways, which confirms their pivotal role in cellular processes as well. Therefore, it is crucial to maintain ER homeostasis, which results in normal organismal physiology. The quality control mechanisms in ER guarantee the proper folding and assembly of proteins, and disturbances in ER homeostasis are mainly caused by redox imbalance, protein folding defects, infections, hypoxia, and impairments in Ca2+ homeostasis, which in turn lead to ER stress. In order to restore normal ER function, the quality

control mechanisms of ER are activated, including the unfolding of the protein response (UPR) and ER-associated degradation (ERAD) (65-71).

Three important pathways are available to preserve ER homeostasis. Inositol-requiring protein-1 (IRE1) is a membrane-localized kinase/endoribonuclease pathway, which affects ER homeostasis through alterations in mRNA, so that IRE1 converts the transcription factor X-box binding protein 1 (XBP1) into its active form through eliminating a specific intron from the mRNA of XBP1. Following that, XBP1 activates the transcription of the genes that are associated with protein degradation and folding (72-74). In addition, IRE1 uses regulated IRE1-dependent decay, which is a process for the decomposition of ER-localized mRNAs, in order to decrease the synthesis of proteins. The subsequent reduction in protein synthesis is associated with diminution in the required folding in ER, while the quality control mechanisms of ER achieve this time to degrade and re-fold the misfolded and unfolded proteins (75). Following the mentioned strategy of IRE1 for the stimulation of the alterations in the transcription factor, c-Jun N-terminal kinase (JNA) signaling pathway are affected (76).

Protein kinase RNA-like ER (PERK) is another major pathway, which is involved in the reduction of the protein load into ER through phosphorylation, which in turn leads to the suppression of the eukaryotic translation initiation factor 2α (elF2α) (77). During ER stress, stimulating transcription factor 6 (ATF6) is transferred to the Golgi apparatus, where it is converted into its active form by cleavage. Afterwards, ATF6 is translocated to the nucleus, positively affecting the expression of the genes associated with protein and lipid synthesis. ATF6 and PERK have been reported to stimulate the expression of a pro-apoptotic factor, known as CCAAT/enhancerbinding protein homologous protein (CHOP) (78,79).

Association of ER Stress with Autophagy

Previous findings have confirmed the induction of autophagy by ER stress. However, it is essential to determine whether the autophagy induction under ER stress is associated with cell death or cell survival. In a study, Song et al. investigated the role of intermittent-hypoxia (IH)-induced autophagy on pancreatic cells (80), reporting that IH could enhance the autophagy level and increase the concentrations of the proteins associated with ER stress (e.g., CHOP, PERK, p-elF2, and ATF4). The inhibition of these signaling pathways resulted in the inhibition of autophagy, indicating that ER stress could stimulate autophagy. In the mentioned study, rapamycin and chloroquine were used as the stimulator and inhibitor of autophagy, respectively in order to investigate the effects of autophagy on cell viability. Interestingly, the increased level of autophagy by rapamycin decreased the cell death caused by IH, and the reduced autophagy by chloroquine was reported to increase cell death in pancreatic cells. Therefore, it was concluded that autophagy induction by IH through ER stress plays a key role in the protection of pancreatic cells against cell death.

In this regard, the findings of Li et al. were inconsistent with the mentioned research (81). According to the latter, sodium fluoride could increase the level of ER stress in MC3T3-E1 osteoblastic cells, demonstrating that the increased level of ER stress is associated with higher autophagy and apoptosis in osteoblastic cells, which confirms the adverse effects of ER stress-induced autophagy. In fact, Li et al. claimed that the ameliorative or adverse effects of autophagy depend on the excitation level of autophagy, so that a specific level of autophagy induction could alleviate ER stress, while high levels of autophagy induction are associated with the self-digestion of the cell and apoptosis.

Honokiol

Honokiol is a lignan, which is extracted from the bark of magnolia tree. It is a small molecule with the molecular weight of 266 and molecular formula of C18H18O2. In recent decades, multiple derivatives of honokiol with antitumor activities have been developed, including 3/-formyl-honokiol, 5-formyl-honokiol, and 3,5/-diformyl-honokiol (82). Honokiol has remarkable biological properties, including anti-inflammatory (83,84), antimicrobial (85), antioxidant (86), hepatoprotective (87, 88), neuroprotective (81,89), and protective effects against thrombosis (90) and angiopathy (91-95).

According to the literature, honokiol was extensively used in Chinese traditional medicine for the treatment of some diseases, such as thrombotic stroke, gastrointestinal disorders, anxiety, and nervous system impairment (96,97). Moreover, honokiol is a popular compound in Japan with extensive usage (98,99).

Recently, Cen et al. examined the effects of honokiol on lung squamous cell carcinoma (100), reporting that honokiol exerted inhibitory effects on the FGF2-FGFR1 signaling pathway, thereby stimulating apoptosis in lung squamous cell carcinoma and decreasing the viability and proliferation of cancer cells.

Honokiol is also beneficial in the prevention of type II diabetes (101). Previous findings have indicated that the consumption of honokiol is associated with the reduction of the abnormal alterations

of hepatic cytochrome P450 and transporter mRNA expression (e.g., hepatic Oat2 and Oatp2b1) in rats with type II diabetes induced by a high-fat diet and streptozotocin, which are involved in the incidence and development of diabetes. Furthermore, honokiol has been reported to ameliorate insulin resistance, nonalcoholic steatosis, and liver dysfunction (102-104). Considering its anti-inflammatory properties, honokiol could significantly inhibit the expression of cyclooxygenase-2 and activation of nuclear factor-kappa B (105).

Honokiol and Autophagy

Several studies have confirmed the protective and antitumor effects of honokiol through autophagy (Figure 1). For instance, XQ et al. examined the beneficial effects of honokiol on anti-b1-adrenergic receptor autoantibody-induced myocardial dysfunction (106), reporting that honokiol treatment could inhibit b1-AAB-induced effects, conserving the myocardial tissues against dysfunction. Moreover, honokiol could enhance the contractile ability of the heart and remarkably decrease the activity of lactate dehydrogenase.

In another study, the potential antitumor activity of honokiol in human thyroid cancer cells was evaluated (107), and the obtained results indicated that honokiol could stimulate autophagy in human thyroid cancer cells via the Akt/mTOR signaling pathway, thereby reducing the viability and proliferation of these cancer cells.

Table 1. Studies Confirming Autophagy Modulatory Properties of Honokiol

Reference	In-vitro/In-vivo	Cell Type/Animal Model	Major Outcomes
(110)	In-vitro	U87-MG, GL261, and U87-MR-R9 (glioma cells)	Induction of autophagy and subsequent apoptosis in glioma cells
(111)	In-vitro and In-vivo	Human Osteosarcoma Cells	Decreased tumor growth by stimulation of autophagy and apoptosis through ROS/ERK1/2 signaling pathway
(112)	In-vitro and In-vivo	Oral Squamous Cell Carcinoma	Inhibition of MAPK pathway and regulation of Akt/mTOR or AMPk pathways for induction of autophagy in oral squamous cell carcinoma
(64)	In-vitro	Osteosarcoma Cells	Increased level of LC3II protein and decreased levels of PI3K, p-Akt, and p-mTOR, resulting in autophagy induction in osteosarcoma cells
(113)	In-vitro	Human Glioblastoma Cells	Decreased levels of Akt and PI3K and subsequent stimulation of autophagy and exerting antitumor effects on human glioblastomas
(93)	In-vitro	Neuroblastoma Cells	Inhibition of migration of cancer cells by activation of autophagy through PI3K/Akt/mTOR pathway
(114)	In-vitro	Human Non-small-cell Lung Cancer	Decreased tumor growth through induction of cell death and inhibition of autophagy
(115)	In-vitro	Human Prostate Cancer Cells	Increased level of LC3II protein and induction of autophagy in prostate cancer cells
(116)	In-vitro	Glioblastoma Multiforme Cells	Increased levels of Beclin-1 and LC3-II, autophagy induction, and subsequent decreased cancer cell viability

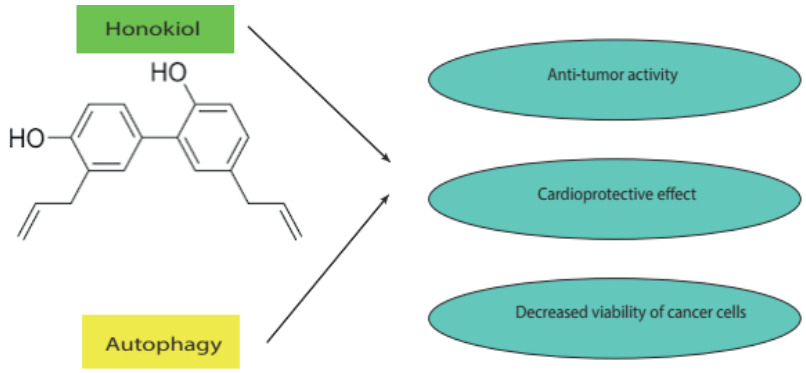

Figure 1. Modulatory Effects of Honokiol on Autophagy Pathway

Honokiol and ER Stress

In general, honokiol uses the ER stress pathway to exert its protective or antitumor effects (Figure 2). In a study in this regard, Chiu et al. applied honokiol to suppress tumor growth and metastasis (117). According to the obtained results, honokiol stimulated ER stress through CHOP activation, thereby significantly decreasing the viability of melanoma cells and suppressing the epithelial mesenchymal transition of these cancer cells. Similarly, Jangra et al. investigated the neuroprotective effects of honokiol mediated by ER stress (118), reporting that honokiol significantly reduced the CHOP level in the hippocampus of stressed mice in order to inhibit ER stress, which in turn results in the inhibition of cognitive impairment and depressive behaviors in the stressed mice. Other findings in this regard have demonstrated that honokiol exerts stimulatory effects on ER stress through enhancing the level of glucose-regulated protein 78, leading to the induction of autophagy and reduced migration of neuroblastoma cells (93).

Table 2. Studies Confirming Modulatory Effects of Honokiol on ER Stress

Reference	In-vitro/In-vivo	Cell Type/Animal Model	Major Outcomes
(119)	In-vitro	Highly Metastatic Gastric Cancer Cell Lines	Stimulation of ER stress and subsequent inhibited gastric tumor growth and peritoneal dissemination
(120)	In-vivo	Torsion-/Detorsion-induced Testicular Injury in Rats	Inhibition of ER stress-related apoptosis and amelioration of testicular injury
(121)	In-vivo	Mouse Model with Acute Pancreatitis and Associated Acute Long Injury	Increased levels of ER stress-related proteins (e.g., elF2α and CHOP) and alleviation of intensity of acute pancreatitis and associated lung injury
(122)	In-vitro and In-vivo	Human Chondrosarcoma Cells	Decreased viability and tumor growth by apoptosis stimulation via mitochondrial dysfunction and ER stress

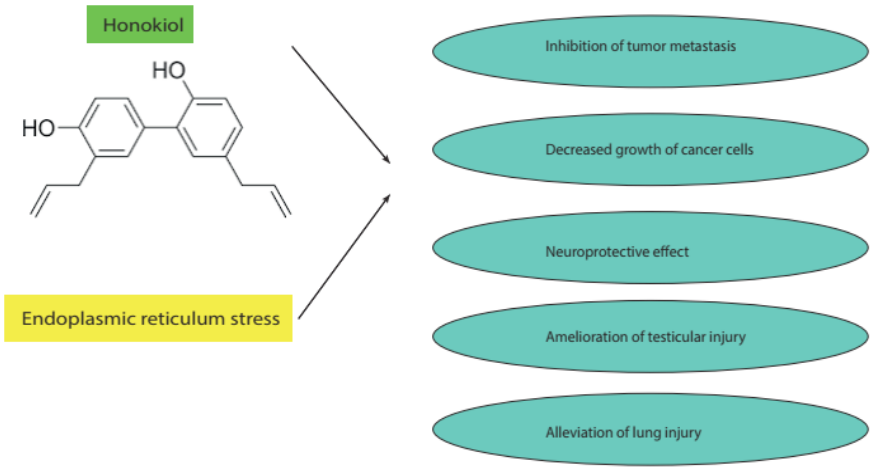

Figure 2. Modulatory Effects of Honokiol on ER Stress Pathway

Conclusion

ER stress is involved in the pathogenesis of some disorders, such as neurological disorders, pulmonary fibrosis, and cancer. In these pathological conditions, UPR is activated to partly restore the homeostasis of ER by the proper folding of proteins and through the degradation of unfolded and misfolded proteins in severe conditions. In addition, autophagy is associated with pathological conditions; for instance, in Alzheimer's disease, the low level or lack of autophagy contributes to the aggregation of amyloid-α. Therefore, the targeting of these mechanisms could be beneficial in the treatment of various pathological conditions. According to the information in Table 2, honokiol has stimulatory and inhibitory effects on ER stress in two modes depending on the condition. As such, it plays a key role in maintaining homeostasis and proteostasis. According to the information in Table 1, in some conditions, honokiol could stimulate autophagy, while in other conditions, it inhibits autophagy. In both its modes, honokiol exerts protective and antitumor effects. Further investigations are required in order to clarify the association of honokiol with ER stress and auto-

phagy, with the findings of this review laying the groundwork in this regard.

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Conflict of Interest

The authors declare no conflict of interest.

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