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黃芩素誘導細胞自噬及其機制研究

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Effects and Mechanisms of Baicalein-induced Autophagy in Cancer
Cells and Non-cancer cells

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摘要

黃芩素 (baicalein, BA) 是一種從中藥黃芩 (*Scutellariae Radix*) 中提取出的黃酮類化合物, 是黃芩的主要活性成分之一, 具有抗菌、抗病毒、抗炎、抗氧化、神經保護等藥理活性。

自噬 (autophagy) 是一種細胞防禦和應激的調控系統, 可以將細胞中受損蛋白和細胞器隔離並運送到細胞溶酶體中降解。而在腫瘤發生發展的過程中, 自噬的異常和腫瘤的存活、能量代謝以及耐藥等方面有著緊密的聯繫, 同時細胞自噬在肝臟疾病的發展中具有重要作用。瞭解自噬有望為腫瘤及肝病的治療及預防提供新方向。

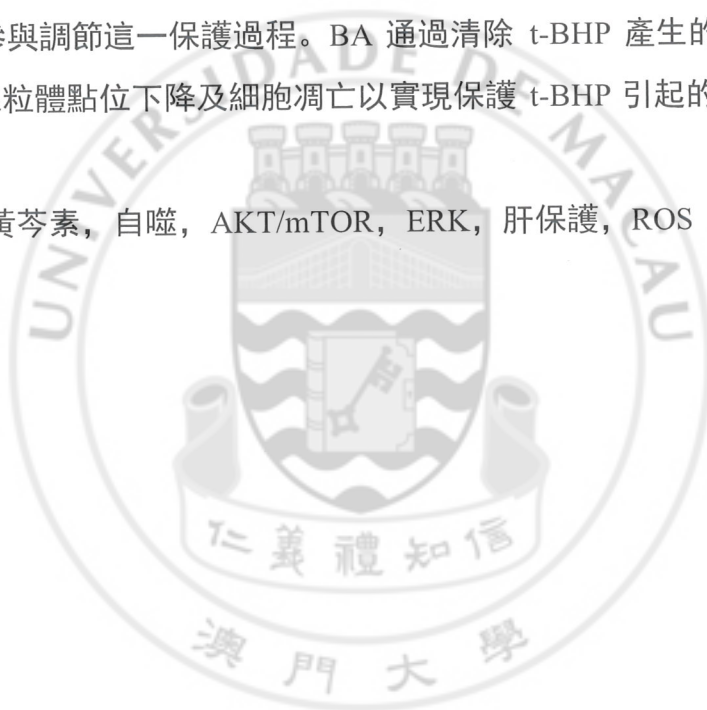
此文旨在探討 BA 誘導腫瘤細胞自噬的作用及機制, 並研究 BA 誘導的自噬對 t-BHP 引發的肝損傷的影響。MTT 法和細胞克隆形成實驗結果顯示 BA 能夠抑制肝癌 HepG2 細胞和卵巢癌 HEY 細胞的增殖。通過 Western blot 檢測微管相關蛋白 1 輕鏈 3 (microtubule-associated protein 1 light chain 3, LC3) 的表達、單丹磺醯戊二胺 (monodansylcadaverine, MDC) 染色法、電鏡實驗等, 發現 BA 可在腫瘤細胞中誘導自噬。與自噬抑制劑氯喹 (chloroquin, CQ) 合用, 可增強 BA 對腫瘤細胞的增殖抑制作用、促進凋亡, 表明 BA 在腫瘤細胞中誘導保護性自噬。AKT/mTOR 通路是細胞自噬的重要通路, 在自噬誘導過程發揮負調節作用。實驗表明 BA 能抑制肝癌 HepG2 細胞內 p-AKT (Ser473), 及 mTOR 下游蛋白 ULK1 (Ser757)、p-4EBP1 (Thr37) 和 p-4EBP1 (Ser65) 的表達。有意思的是, BA 在卵巢癌 HEY 細胞中誘導的自噬機制與 HepG2 不同, 蛋白 p-AKT (Ser473) 和 p-ULK1 (Ser757) 在 BA 的作用下表達量明顯增加, 表明 BA 並不通過 AKT/mTOR 通路介導 HEY 細胞的自噬。進一步研究顯示 BA 可以啟動 HEY 細胞的胞外信號調節蛋白激酶 (extracellular signal-regulated kinases, ERK) 通路。使用 ERK 抑制劑 U0126 或者敲除基因 ERK 都可下調 BA 誘導的卵巢癌 HEY 細胞自噬標記蛋白 LC3II 的表達, 表明 BA 可通過 ERK 通路介導 HEY 細胞自噬。

前期研究表明, BA 能夠在肝癌細胞中誘導保護性自噬, 於是我們進一步研究 BA 誘導的自噬能否對叔丁基過氧化氫 (tert-butyl hydroperoxide, t-BHP) 導致的肝細胞損傷起到保護作用。MTT 和 LDH 的實驗結果表明, BA 能保護 t-BHP 引起的肝損傷, 同時 BA 可上調 LO2 細胞 LC3II 的表達, 表明 BA 能誘導

LO2 細胞發生自噬。然而，使用自噬抑制劑 CQ 和自噬激動劑雷帕黴素 (rapamycin, RAPA) 並沒有對 BA 的保護作用產生影響，說明自噬沒有參與調節 BA 對 t-BHP 引起的肝損傷的保護過程。進一步研究顯示 BA 能夠通過清除 t-BHP 產生的活性氧 (reactive oxygen species, ROS)，減弱線粒體膜電位下降及肝細胞凋亡，保護 t-BHP 引起的肝損傷。

綜上所述，我們的研究首次證明 BA 可誘導肝癌 HepG2 細胞和卵巢癌 HEY 細胞發生自噬，而與自噬抑制劑 CQ 聯用可增加 BA 對腫瘤細胞的殺傷作用。BA 可抑制 AKT/mTOR 通路調節 HepG2 細胞自噬，啟動 ERK 通路調節 BA 介導的 HEY 細胞自噬。此外，BA 可保護 t-BHP 引起的非腫瘤肝細胞 LO2 損傷，並且自噬沒有參與調節這一保護過程。BA 通過清除 t-BHP 產生的 ROS，抑制 t-BHP 引起的線粒體膜電位下降及細胞凋亡以實現保護 t-BHP 引起的 LO2 細胞損傷的。

關鍵字：黃芩素，自噬，AKT/mTOR，ERK，肝保護，ROS



Abstract

Baicalein (BA), one of the major compounds isolated from *Scutellariae Radix*, exhibits anti-oxidant, anti-inflammatory, and anti-cancer effects. Autophagy is a conserved cellular self-consumption process in which there is an elimination of damaged organelles or proteins in the cytoplasm. It plays critical roles in a number of physiological and pathological functions and diseases.

In this study, we found that BA reduced cell viability in hepatocellular carcinoma HepG2 cells and ovarian cancer HEY cells. Treatment of cells with BA remarkably induced the formation of autophagosomes determined by immunofluorescence with monodansylcadaverine (MDC) staining as well as transmission electron microscopy, respectively. Moreover, BA obviously up-regulated the expression of microtubule-associated protein 1A/1B-light chain 3-II (LC3-II). Combined treatment with chloroquine and BA obviously reduced cell viability, colony formation and increased cleavage of poly (ADPribose) polymerase (PARP), indicating that BA induces a protective autophagy in cancer cells. Our further study showed that BA both concentration- and time-dependently decreased the expression of p-AKT (Ser473), p-ULK1 (Ser757) and p-4EBP1 (Thr37 and Ser65), suggesting that the AKT/mammalian target of rapamycin (mTOR) pathway, might be involved in BA-induced autophagy in HepG2 cells. However, we found an increase in the phosphorylation of AKT (Ser473) and ULK1 (Ser757) which are different of that in HepG2 cells. In addition, we found an increase in the phosphorylation of extracellular signal-regulated kinase (ERK, Thr202/Thr204) after BA treatment, and inhibition of ERK activation by pharmacological inhibitor U0126 or ERK siRNA blocked BA-induced autophagy. Meanwhile, knockdown of Beclin 1 by siRNA remarkably decreased BA-induced LC3-II lipidation. Taken together, these results suggest that BA induces Beclin 1- and ERK-dependent autophagy in ovarian cancer cells.

Our previous study indicated BA induced a protective autophagy in cancer cells, therefore we detected whether the cytoprotective autophagy induced by BA can prevent cell damage in non-cancer cells. We found that BA possesses protective properties against t-BHP injury in liver LO2 cells. While combined treatment with chloroquine and rapamycin did not change the hepatoprotective properties, indicating that the protective effects of BA-induced autophagy is not related to autophagy. Moreover, Moreover, BA significantly prevented t-BHP-induced depolarization of mitochondrial membrane potential (MMP), decreased the percentage of apoptotic cell,

and significantly prevented intracellular reactive oxygen species (ROS) generation in LO2 cells.

In conclusion, our study demonstrated that BA induced autophagy in HepG2 and HEY cells. AKT/mTOR pathways and ERK are involved in BA-induced autophagy in HepG2 cells and HEY cells, respectively. Furthermore, BA possesses hepatoprotective properties against t-BHP-triggered injury in LO2 cells, BA induced autophagy while autophagy is not involved in the protective effect of BA against t-BHP-induced LO2 cells injury. BA may attenuate damages caused by t-BHP in LO2 cells by reducing intracellular production of ROS and inhibiting apoptosis.

Keywords: baicalein, autophagy, AKT/mTOR, ERK, hepatoprotection, ROS



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