DOCTORAL THESIS

Autophagy/mitophagy Inhibitors and Magnolol Synergistically Promote Apoptosis and Antitumor Efficacy

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Abstract

As one of the most important organelles, mitochondria provide cells with energy and act as a signalling platform to play crucial roles in deciding cell fate. It is therefore not surprising that mitochondrial dysfunctions are implicated in the pathogenesis of many diseases, including cancer, neurodegenerative diseases, aging, heart failure, and metabolic disorders. Thus, it is crucial to remove dysfunctional mitochondria to keep a healthy mitochondrial network. Many classic anticancer agents are known to trigger cell death through induction of mitochondrial damage. Mitophagy is a key mitochondrial quality control process that effectively removes damaged mitochondria through autophagic pathways. In addition to its well-known implications in the pathogenesis of neurodegenerative diseases, the roles of mitophagy in tumorigenesis and cancer drug resistance have caught substantial attention. However, at present, the precise roles of mitophagy in tumorigenesis and anticancer agents' treatment remain largely unclear. One key question remains: Can targeting mitophagy improve cancer therapy?

Magnolol, a natural compound isolated from *Magnolia officinalis*, displays potent anticancer activities in various types of cancers. Due to few side effects and the property of passing through the blood-brain barrier, magnolol is possibly an ideal anticancer drug, especially for neuroblastoma and glioblastoma. It has been reported that magnolol can induce autophagy in various of cancer cells and magnolol can target multiple mitochondrial proteins. However, at present, magnolol's effects on mitophagy have not been investigated.

In this study, we aimed to explore the molecular mechanism and the functional implication of mitophagy in the anticancer properties of magnolol. First, we have

found that magnolol induces mitochondrial depolarization, causes excessive mitochondrial fragmentation and increases mitochondrial ROS. Second, magnolol induces PTEN-induced putative kinase protein 1 (PINK1)-Parkin-mediated mitophagy through the regulation of two positive feedforward amplification loops. Third, magnolol triggers cancer cell death and inhibits neuroblastoma tumor growth via the intrinsic apoptosis pathway. Furthermore, magnolol can prolong the survival time of tumor-bearing mice. Last but not least, inhibition of mitophagy by PINK1/Parkin knockdown or using inhibitors targeting different autophagy/mitophagy stages significantly promotes magnolol-induced cell death and enhances magnolol's anticancer efficacy both *in vitro* and *in vivo*.

In conclusion, our study strongly demonstrates that magnolol can induce apoptosis and mitochondria damage which might activate autophagy/mitophagy pathway for cancer cell to adapt cell stress, while blockage of autophagy/mitophagy pathway is able to remarkably increase cell death in cancer cells and enhances the anticancer efficacy of magnolol. These results indicates that: (1) magnolol causes mitochondrial dysfunction and induces intrinsic apoptosis; (2) magnolol induces mitophagy in a PINK1- and Parkin-dependent manner, which antagonizes magnolol's cytotoxicity; and (3) genetic and pharmacological inhibition of mitophagy dramatically enhances magnolol's anticancer efficacy, both *in vitro* and *in vivo*. These findings not only suggests that natural compound such as magnolol can be a novel clinic strategy for cancer therapy, but also clue that combined mitophagy/autophagy inhibitors with these natural compound (s) may be a promising strategy to overcome resistance of chemotherapy, especially in the condition that those compounds trigger cell death through induction of mitochondrial damage.