

RAPID MITOCHONDRIAL REACTIVE OXYGEN SPECIES (ROS) GENERATION INDUCED BY CURCUMIN LEADS AUTOPHAGIC CELL DEATH IN HUMAN PAPILLARY THYROID CARCINOMA BCPAP CELLS

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Background/Purpose: We previously found that curcumin could induce autophagy in thyroid cancer cells. However, the mechanism of autophagy induced by curcumin is still not clear.

Methods: Mitochondrial succinate dehydrogenase (SDH) activity was determined by MTT reduction assay. Expression of LC3 was detected by western blot. Cells treated with curcumin for various time-intervals were stained with dihydroethidium and analyzed by flow cytometry for ROS production.

Results: The amount of MTT formazan produced by BCPAP cells increased with curcumin incubation (12.5 to 50 μ M) within 1 h, indicating curcumin could elevate mitochondrial SDH activity rapidly. When BCPAP cells were treated with curcumin at 50 μ M, the fluorescent intensity increased to the maximum level at 5 min (from 0.5% at 0 min to 71.81% at 5 min), indicating a rapid increase in intracellular ROS production, after which the fluorescence intensity steadily reduced within 1 h (38.02% at 15 min; 26.67% at 30 min; 11.87% at 60 min). 3-nitropropionic acid (3-NP), a specific SDH inhibitor, blocked curcumin induced ROS production in every indicated time-interval. Especially at 5 min point, the intracellular ROS production decreased dramatically from 71.8% to 0.91% by 3-NP treatment as compared to the treatment of curcumin alone. Furthermore, 3-NP reversed the increasing LC3-II to LC3-I ratio, which is the hallmark of autophagosome formation and dose-dependently prevented curcumin-induced cell death by MTT assay.

Discussion & Conclusion: These results provide strong evidences for the involvement of mitochondrially-generated ROS production in the induction of autophagy by curcumin in human papillary thyroid BCPAP cancer cells.