

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

Zhang, Li<sup>1</sup>; Yu, Huixin<sup>1</sup>; Tan, Chen<sup>1</sup>; Wu, Jing<sup>1</sup>; Guan, Haixia<sup>2</sup>; Bao, Jiandong<sup>1</sup>

<sup>1</sup>Jiangsu Institute of Nuclear Medicine, Key Laboratory of Nuclear Medicine, Ministry of Health, Jiangsu Key Laboratory of Molecular Nuclear Medicine, Wuxi, Jiangsu, China; <sup>2</sup>Department of Endocrinology and Metabolism and Institute of Endocrinology, The First Affiliated Hospital of China Medical University, Shenyang, Liaoning, China

**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  $\mu$ M) within 1 h, indicating curcumin could elevate mitochondrial SDH activity rapidly. When BCPAP cells were treated with curcumin at 50  $\mu$ 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.