The apoptotic effect of 1'S-1'-Acetoxychavicol Acetate (ACA) enhanced by inhibition of non-canonical autophagy in human non-small cell lung cancer cells

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Abstract

Autophagy plays a role in deciding the fate of cells by inducing either survival or death. 1'S-1-acetoxychavicol acetate (ACA) is a phenylpropanoid isolated from rhizomes of Alpinia conchigera and has been reported previously on its apoptotic effects on various cancers. However, the effect of ACA on autophagy remains ambiguous. The aims of this study were to investigate the autophagy-inducing ability of ACA in human non-small cell lung cancer (NSCLC), and to determine its role as pro-survival or pro-death mechanism. Cell viability assay was conducted using MTT. The effect of autophagy was assessed by acridine orange staining, GFP-LC3 punctate formation assay, and protein level were analysed using western blot. Annexin V-FITC/PI staining was performed to detect percentage of cells undergoing apoptosis by using flow cytometry. ACA inhibits the cell viability and induced formation of cytoplasmic vacuoles in NSCLC cells. Acidic vesicular organelles and GFP-LC3 punctate formation were increased in response to ACA exposure in A549 and SK-LU-1 cell lines; implying occurrence of autophagy. In western blot, accumulation of LC3-II accompanied by degradation of p62 was observed, which further confirmed the full flux of autophagy induction by ACA. Beclin-1 was downregulated upon ACA treatment indicating the Beclin-1-independent autophagy pathway. An early autophagy inhibitor, 3-methyladenine (3-MA), failed to suppress the autophagy triggered by ACA; validating the existence of Beclin-1-independent autophagy. Silencing of LC3-II using short interfering RNA (siRNA) abolished the autophagy effects, enhancing the cytotoxicity of ACA through apoptosis. This proposed ACA triggered a pro-survival autophagy in NSCLC cells. Consistently, co-treatment with lysosomal inhibitor, chloroquine (CQ), exerted a synergistic effect resulting in apoptosis. Our findings suggested ACA induced pro-survival autophagy through Beclin-1-independent pathway in NSCLC. Hence, targeting autophagy pathway using autophagy inhibitor such as CQ