Autophagy alters lipid metabolism in human colon carcinoma cells evaluated using 1H NMR

Gigin Lin1, Dow-Mu Koh1, Simon P Robinson1, Martin O Leach1, and Yuen-Li Chung1
1CRUK and EPSRC Cancer Imaging Centre, The Institute of Cancer Research, Sutton, Surrey, United Kingdom

Background: The intracellular storage and utilization of lipids can play an essential role in maintaining energy homeostasis when normal sources of energy are restricted, as can occur in starvation or stress, leading to autophagy [1]. During autophagy, intracellular proteins and organelles are sequestered in double-membrane vesicles (autophagosomes), and degraded by fused lysosomes to produce energy. This process allows cancer cells to prolong survival in hostile environments and aids recovery once the stress was removed. There is increased interest in studying this effect as a cellular reaction to cancer therapeutics [2]. However, the detailed mechanisms and intracellular lipid changes in drug-induced autophagy are still unknown.

Aim: To examine the lipid profiles of drug-induced autophagy in cancer cells using 1H NMR.

Methods: Autophagy was induced in HCT116 wild type (HCT116-WT) and Bax-deficient (HCT116 KO) colon carcinoma cells by dichloroacetate (DCA, 75 mM for 24h) or PI103 (a class-I PI3 kinase inhibitor, 20 μM for 24h). Apoptosis was also induced in HCT116-WT cells by treatment with TNFR-related apoptosis-inducing ligand (TRAIL, 30ng/ml for 24h). The HCT116-KO cells were found to be resistant to the same dose of TRAIL and its lipid profile was used as a non-responsive control. After dual-phase cell extraction of the adherent cells, the lipid extracts were derivatized and reconstructed in deuterochloroform, with tetramethylsilane (TMS) added for chemical shift calibration. High-resolution 1H NMR spectroscopy was performed on the lipid cell extracts using a 500MHz Bruker MR system. Ratios of lipid integrals relative to TMS were calculated and standardised to cell number. We confirmed the cellular status of autophagy and apoptosis by using electron microscopy, western blotting, and Annexin V/propidium iodide (PI) flow cytometry. Cell cycle analysis and confocal microscopy with Nile Red stain were also performed and related to the lipid profile changes.

Results: Autophagy was shown in cells treated with either DCA or PI-103 treatments, as confirmed by western blotting (increased LC3BII expression) and electron microscopy (presence of autophagosomes), with minimal apoptosis and necrosis (as shown by Annexin V/PI flow cytometry analysis (<10% cell population) and western blots (absence of cleaved PARP and cleaved caspase-3). Cell cycle analysis showed an S2 and a G1 arrest following DCA and PI103 treatment, respectively. About a 2-fold increase in the fatty acid signals at 0.9 and 1.3 ppm, and unsaturated fatty acids peaks at 2.8 ppm and 5.3 ppm were found in lipid extracts by 1H-NMR (Fig 1 and 2). 1.5- to 3-fold increases in lipid profile following autophagy induced by two different treatments has been used as a tool to assess apoptosis both in vitro [3] and in vivo [4]. In this study, we observed a unique change in lipid profile following autophagy induced by two different agents in a pair of cell lines. In the HCT116 cell lines, the lipid profile of autophagy shares some common changes with apoptosis, such as increased fatty acid resonances at 0.9 and 1.3 ppm, triacylglycerol and sphingomyelin, which differentiate them from the non-responsive group. The increased unsaturated fatty acids (at 5.3 ppm) and phosphatidylcholine were mostly prominent in autophagic cells and not in apoptotic cells. We did not observe the unsaturated fatty acids increase (at 5.3 ppm) in our apoptotic cells as reported in literature [4], this may be due to early apoptosis was induced in our study and the level of unsaturated fatty acids is not yet changed in these cells. Our NMR findings were supported by the increased number of lipid droplets observed under confocal microscopy and also supported by the recent report on the roles of autophagy in lipid metabolism [1].

Discussion: 1H-NMR analysis of lipid metabolites has been used as a tool to assess apoptosis both in vitro [3] and in vivo [4]. In this study, we observed a unique change in lipid profile following autophagy induced by two different agents in a pair of cell lines. In the HCT116 cell lines, the lipid profile of autophagy shares some common changes with apoptosis, such as increased fatty acid resonances at 0.9 and 1.3 ppm, triacylglycerol and sphingomyelin, which differentiate them from the non-responsive group. The increased unsaturated fatty acids (at 5.3 ppm) and phosphatidylcholine were mostly prominent in autophagic cells and not in apoptotic cells. We did not observe the unsaturated fatty acids increase (at 5.3 ppm) in our apoptotic cells as reported in literature [4], this may be due to early apoptosis was induced in our study and the level of unsaturated fatty acids is not yet changed in these cells. Our NMR findings were supported by the increased number of lipid droplets observed under confocal microscopy and also supported by the recent report on the roles of autophagy in lipid metabolism [1].

Conclusion: Different lipid profiles were found in the autophagic cells when compared with early apoptotic and non-responsive cells in colon carcinoma HCT116 cells.


Acknowledgement: We acknowledge the support received for the CRUK and EPSRC Cancer Imaging Centre in association with the MRC and Department of Health (England) grant C1060/A10334, NHS funding to the NIHR Biomedical Research Centre, The Royal Society, and Chang Gung Memorial Hospital (Taiwan) grant CMRPG370441. We would like to thank Dr. Paul Clarke for generously provided us with the paired HCT116 cell lines.