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

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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 exam the lipid profiles of drug-induced autophagy in cancer cells using ^{1}H NMR.

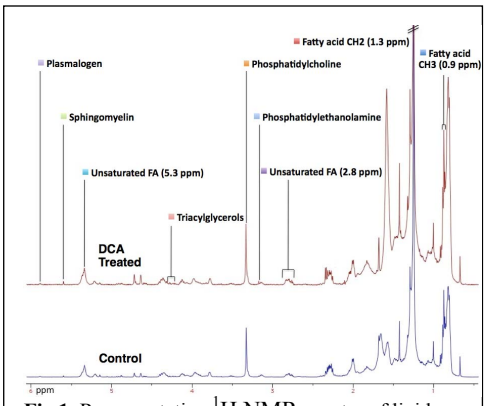

Fig 1. Representative ^{1}H NMR spectra of lipid metabolites. Autophagy was induced in HCT116-KO cells by dichloroacetate (DCA) treatment.

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 TNF-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 dried and reconstituted in deuterochloroform, with tetramethylsilane (TMS) added for chemical shift calibration. High-resolution ^{1}H 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.

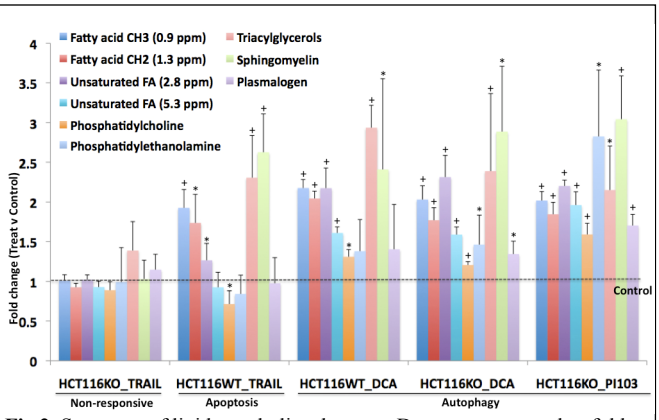

Fig 2. Summary of lipid metabolite changes. Data was presented as fold change of in different treated group when compared with their controls. *, $p < 0.05$; +, $p < 0.001$.

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 autophagosome), 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 a G2 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 ^{1}H-NMR (Fig 1 and 2) . 1.5- to 3-fold increases in triacylglycerol, sphingomyelin and phosphatidylcholine were also observed. An increase of phosphatidylethanolamine was found in DCA and PI-103 treatment groups. Nile Red confocal microscopy images showed increases in lipid droplets in DCA- or PI-103-treated cells (Fig 3). Apoptosis was seen in TRAIL-treated HCT116-WT cells, as confirmed by western blotting (cleaved caspase-3) and electron microscopy (nuclear condensation and membrane blebbing), with a significantly increased apoptotic population shown by Annexin V/PI flow cytometry analysis. In this group, similar increases in fatty acid peaks at 0.9 and 1.3 ppm (1.5 fold), triacylglycerol and sphingomyelin (2.5 fold) were observed. However, no significant change in the unsaturated fatty acid peak at 5.3ppm was found in the apoptosis group. Interestingly, a significant 30% decrease in phosphatidylcholine was seen in the apoptosis group, in contrast to the increases observed in the autophagy group. No significant changes in any lipid metabolites were found in the non-responsive group (TRAIL-treated HCT116-KO cells).

Discussion: ^{1}H-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 5.3ppm) 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].

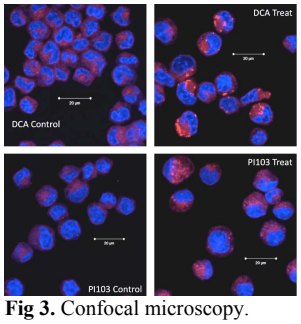

Fig 3. Confocal microscopy. Autophagy was induced in HCT116-KO cells by DCA and PI103 treatment. Red, Nile Red; Blue, Hoechst 33258.

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

References [1] Singh R et al., Nature 2009;458:1131-5. [2] Mathew R et al., Cancer Nat Rev 2007;7:961-7. [3] Blankenberg FG et al., Blood 1997;89:3778-86. [4] Hakumäki JM et al., Nat Med 1999;5:1323-7.

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