The metabolic profile of drug-induced autophagy in cancer

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Background Autophagy is a response to stress and starvation whereby cellular organelles and proteins are sequestered and targeted for lysosomal degradation as an alternate energy source, which allows cancer cells to prolong their survival in hostile environments or during chemotherapy [1]. The underlying genetic regulation of autophagy is gradually becoming understood, but there is little information available on the downstream metabolic adaptations in cells undergoing drug-induced autophagy.

Aim To evaluate the metabolic changes of drug-induced autophagy in cancer cells by ¹H NMR.

Methods Autophagy was induced in a prostate (PC3) and three colon carcinoma cell lines, HT29, HCT116 wild type (HCT116-WT) and Bax-deficient (HCT116-KO), by exposure to dichloroacetate (DCA, 75 mM, 24h) or the class-I PI3 kinase inhibitor (PI103 20 μ M, 24h). Apoptosis was also induced in HCT116WT cells treated with TNF-related apoptosis-inducing ligand (TRAIL 30ng/ml, 24h). The HCT116-KO cells were found to be resistant to the same dose of TRAIL treatment, thus were used as a non-responsive control. After the dual phase cell extraction, water-soluble metabolites were lyophilised, re-suspended in D₂O and neutralized, and TSP (3-trimethylsilyl-[2,2,3,3-2H4]-propionic acid) added for chemical shift calibration and quantification. High-resolution $^{\rm l}$ H NMR spectroscopy was performed on the cell extracts using a 500MHz Bruker MR system. We confirmed the cellular status of autophagy and apoptosis by using electron microscopy, western blotting, and Annexin V/propidium iodide (PI) flow cytometry analysis.

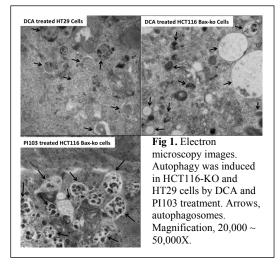
Results: Induction of autophagy was found in all cell lines treated with DCA or PI-103, as confirmed by western blotting (increased LC3BII expression) and electron microscopy (presence of autophagosomes, Fig 1), with minimal apoptosis or necrosis present, as shown by Annexin V/PI flow cytometry analysis and western blots (absence of cleaved PARP and cleaved caspase-3). ¹H NMR measurement revealed a similar pattern of metabolic alterations when autophagy was induced by different treatments in different cell lines (Fig 2 and 3). There were at least 2-fold increases in amino acids (leucine, isoleucine, valine, histidine, tyrosine, phenylalanine, alanine glycine, glutamine and glutamate) in all cell lines undergoing autophagy when compared to controls. Intracellular glucose levels were found to increase in autophagy, whereas the cellular lactate varied among different treatment. The NAD(H) pool remained largely unchanged. Regarding the cellular bioenergetics, we found the ADP/ATP levels were unchanged or decreased. Interestingly, a strikingly elevated level of glycerophosphocholine (GPC) and reduced level of phosphocholine (PC) were observed in all the DCA treated cells. This metabolic profile of autophagy was found to be different from the apoptotic and the non-responsive groups.

Discussion Our ¹H NMR study indicates the presence of a distinct metabolic profile of autophagy across various cell lines and treatments, which reflects its underlying celluar mechanism. Increased levels of amino acids may arise from degradation of cellular organelles and proteins in autophagosomes. These amino acids are used as an energy resource in the TCA cycle to maintain cellular energy homeostasis. These changes are consistent with a recent published report of autophagy, which shows glutamine/glutamate metabolism has a primary role in fuelling the TCA cycle [2]. Reduced PC and increased GPC levels indicate a change in membrane metabolism, which might relate to the rapid formation of double-membrane autophagosomes and degradation after their fusion with lysosome [1].

Conclusion Metabolomic analysis of autophagy provides a distinct metabolic profile, which might have potential for use as a non-invasive surrogate biomarker of autophagy.

References [1] Mathew R et al., Cancer Nat Rev 2007;7:961-7. [2] Eng et al., Sci Signal 2010;27;119ra31:1-9.

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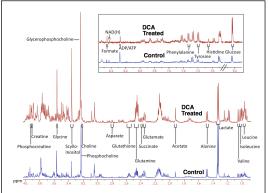


Fig 2. Representative ¹H NMR spectra of water-soluble metabolites. Autophagy was induced in HCT116KO cells by dichloroacetate (DCA) treatment.

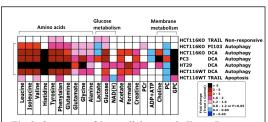


Fig 3. Summary of intracellular metabolites. Data was presented color-coded as fold of changes of in different treated groups compared with their controls. GPC, glycerolphosphocholine; PCr, phosphocreatine; PC, phosphocholine.