Bax-deficiency reduces glycolysis and alters metabolic profile in human colorectal carcinoma cells

G. Lin[1], D-M. Koh[1], S. P. Robinson[1], P. Clarke[2], M. O. Leach[1], and Y-L. Chung[1]
[1]Cancer Research UK and EPSRC Cancer Imaging Centre, Institute of cancer research and Royal Marsden Hospital, Sutton, Surrey, United Kingdom, [2]Cancer Research UK Centre for Cancer Therapeutics, Institute of cancer research and Royal Marsden Hospital, Sutton, Surrey, United Kingdom

Background Metabolomics is a quantitative measurement of the dynamic multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modification. Bax, a Bcl-2 family protein, plays a central role in apoptosis pathways, which may alter the fate of cells treated with cancer therapeutics. 4% of human colorectal carcinoma Hct116 cells are Bax-deficient and are known to be resistant to chemotherapy and TRAIL-induced apoptosis [1]. However, there is little information available on the metabolic effect of Bax-deficiency on colorectal carcinoma cells. In this study, a ^{1}H NMR based metabolomics study was performed on isogenic wild type and Bax-deficient Hct116 colorectal carcinoma cells. The aim was to examine the metabolic effects of Bax-deficiency in cancer cells.

Methods Isogenic Hct116 wild type (WT, n=5) and Bax-deficient cells (KO, n=5) were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and antibiotics in semi-confluent condition for 24 hours. After standard dual phase cell extraction, the water-soluble metabolites were lyophilised, re-suspended in D_2O and neutralised, and TSP (3-trimethylsilyl-[2,2,3,3-2H4]-propionic acid) was added as internal reference standard. Culture media samples were also collected and analysed. High resolution ^{1}H NMR spectroscopy was performed on the cell extracts and media samples using a 500MHz Bruker MR system. Bax status in both cell lines was verified by Western blotting.

Results and Discussion Depletion of Bax in KO cells was confirmed by western blots (Fig. A). The cellular metabolite ratios (KO/WT) are shown in Fig.B and only the metabolites that are significantly different between KO and WT cells are illustrated. A reduced level of glucose was found in KO cells (Fig B), and this was due to a 30% reduction of glucose uptake in the KO cells when compared to WT cells ($p=0.002$), as shown by the data from the media samples. Similar cellular lactate and alanine levels were found in KO and WT cells, whereas, a 19% reduction in lactate production was found in the media of the KO cells when compared with media from the WT cells ($p<0.01$). A higher level of lactate production than the level of glucose uptake was found in the KO cells, suggesting that this lactate arises from other sources, as well as glycolysis. Lower glutamine was found in the KO cells, whilst the glutamine uptake was not significantly different between the two groups, indicating a higher level of glutamine metabolisation by the KO cells. The growth rate and the ATP and ADP level were similar between the two cell lines. Taken together, these findings suggested that glycolysis was down-regulated in the KO cells and it is consistent with findings in a recently published proteomic analysis of this same pair of cell lines, showing down-regulation of some glycolytic enzymes in the KO cells [2]. Glutamine could be metabolized by the KO cells via various pathways that support bioenergetics and biosynthesis, to compensate the down-regulated glycolytic pathway [3].

A reduced cellular level of glycine, formate, phosphocholine, phosphocreatine, acetate, glutathione, UTP and UDP were found in KO cells and this may indicate a less active serine/purine synthesis and metabolism. These observations are consistent with the down-regulation of phosphoserine aminotransferase and inosine-5-monophosphate dehydrogenase [2]. Essential amino acids (leucine, isoleucine, valine, phenylalanine, tyrosine) in the cells and their uptakes from the culture media were also found to be lower in the KO cells when compared with WT, suggesting a reduction in protein synthesis.

Conclusion Many metabolic adaptations were found in Bax KO cells when compared with WT cells. Further studies are required to elucidate the underlying mechanisms and the effects of these metabolic adaptations on the cells' response to drug treatment.

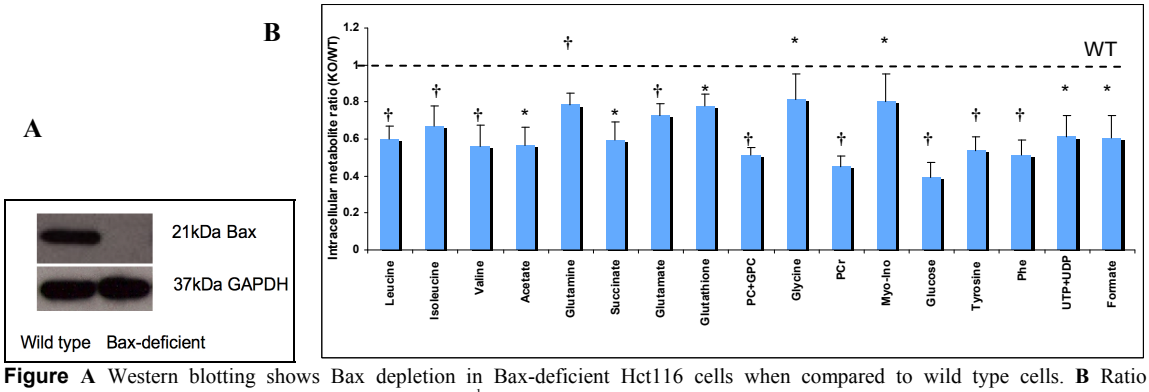

Figure A Western blotting shows Bax depletion in Bax-deficient Hct116 cells when compared to wild type cells. **B** Ratio of intracellular metabolites (KO/WT cells) measured by ^{1}H NMR spectroscopy. Only the metabolites that are significantly different are shown. Data are expressed as mean ± SD. GPC, glycerophosphocholine; Myo-Ino, myo-Inositol; PC, phosphocholine; PCr, phosphocreatine; Phe, phenylalanine; UDP, uridine diphosphate; UTP, uridine triphosphate. *, $p<0.05$; †, $p<0.001$, unpaired t-test was used.

References 1. Zhang L *et al.* Science, 290, 989-92 (2000). 2. Wang P *et al.* J Proteome Res, 8, 3403-14 (2009). 3. DeBerardinis RJ et al. Proc Natl Acad Sci USA, 104, 19345–50 (2007).

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