INHIBITION OF PROSTATE CANCER GROWTH BY DEFERIPRONE

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INTRODUCTION: Activation of mitochondrial aconitase (m-Acon) is an early biochemical change during prostate cancer (PCa) development, leading to a shift from citrate-producing to a citrate-oxidizing malignant phenotype (1). Deferiprone (DFP), an iron chelator used in the clinic, e.g. Thalassemia (2), has been shown to impair aconitase activity, by removing iron from the mitochondria, and also to inhibit cell growth (3). Since m-Acon is a potential target for PCa therapy, here we study the effects of DFP on TRAMP (transgenic adenocarcinoma of the mouse prostate) C2 cells (4).

PURPOSE: To investigate the effects of TRAMP C2 cell exposure to DFP in terms of cell growth and metabolism – by perfusing live cells with ^{13}C-labeld glucose – and monitoring their oxygen consumption rates.

METHODS: TRAMP C2 cells cultured *in vitro* in regular growth conditions (25 mM glucose, 4 mM glutamine, 21% O_2) were exposed to DFP at: (a) different concentrations, 0-1500 μM; and (b) different incubation periods, 3-14 days. Growth response to the drug was determined by cell counting (ViaCount Assay, Guava Technologies). TRAMP C2 cells were also grown on microcarriers (Plastic Plus, SoloHill) and studied in our MR-compatible cell perfusion system (1.0±0.2 x10^8 cells), as described previously (5). The MR experiments were carried out on a Bruker 500 MHz spectrometer while perfusing the cells for 32h with 99% 1-^{13}C-glucose under regular growth conditions. The ^{13}C-glucose-derived metabolites were detected by ^{13}C-MRS, while energy metabolism was observed by ^{31}P-MRS. Each experiment was repeated 3 times. Spectral analyses of peak areas were carried out with AMARES (jMRUI v4.0). Cellular mitochondrial function was assessed with an XF96 Analyzer (Seahorse Bioscience, Billerica, MA) (5), measuring the changes in oxygen consumption rates (OCR) induced by specific inhibitors of the respiratory chain: oligomycin (ATP Synthase), FCCP (uncoupler: H^+ ionopher), antimycin A (Complex III) and rotenone (Complex I). Changes in OCR induced by different concentrations of DFP (0-500 μM) were also measured in TRAMP C2 cells. Expression levels of m-Acon (Cell Signaling) relative to beta-actin (Abcam) were assessed by Western Blot in TRAMP C2 cells a exposed for 24h to 0 μM and 100 μM DFP, essentially as in (1).

RESULTS: The doubling time of TRAMP C2 cells was ~31h. DFP induced cell growth inhibition after 3 day exposure (IC_{50}= 49 μM) and the minimum dose tested that generated maximum effect at 3-14 days exposure was 100 μM (Fig. 1-A). Earlier effects of exposure to 100 μM DFP were observed with the cell perfusion system (Fig. 1-B). ^{31}P-MRS showed changes in choline metabolism (increase in glycerol-phosphocholine to phospho-choline ratio: GPC/PC) after 10h exposure, while changes in energy metabolism (decrease of phospho-creatine and ATP: PCr and β-NTP, respectively) were detected after ~20h. Marked changes in glucose (Glc) metabolism were detected by ^{13}C-MRS (ongoing quantifications): DFP quickly induced a 2-fold decrease in Glc consumption rate, and a 9-fold decrease in the synthesis rate of glycerol-3-phosphate (G3P), essential for phospholipid synthesis. Lactate (Lac) synthesis rate was not affected but less Glc was used for alanine (Ala) synthesis. The synthesis rate of glutamate (Glu) from Glc decreased 23-fold, which agrees with inhibition of m-Acon, and therefore the TCA cycle, and could partially explain the decrease in synthesis of Ala. Oxygen consumption is coupled to ATP production in TRAMP C2 cells (data not shown). When exposed to DFP for 26h, TRAMP C2 cells significantly decrease their OCR, with maximum effect observed at 100 μM (Fig. 1-C), consistent with the lower expression of m-Acon detected by Western Blot (Fig. 1-D) and consequent decrease in oxidate phosphorylation.

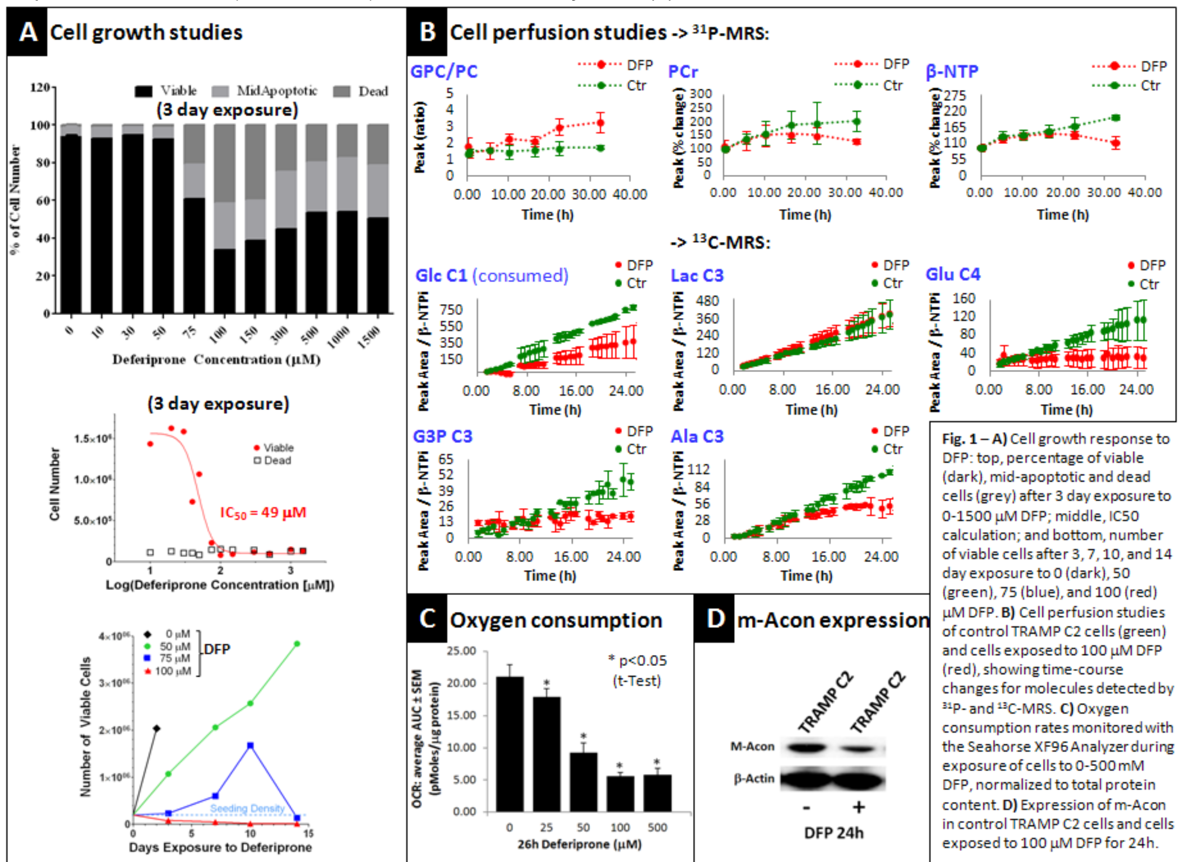

Fig. 1 – A) Cell growth response to DFP: top, percentage of viable (dark), mid-apoptotic and dead cells (grey) after 3 day exposure to 0-1500 μM DFP; middle, IC50 calculation; and bottom, number of viable cells after 3, 7, 10, and 14 day exposure to 0 (dark), 50 (green), 75 (blue), and 100 (red) μM DFP. **B)** Cell perfusion studies of control TRAMP C2 cells (green) and cells exposed to 100 μM DFP (red), showing time-course changes for molecules detected by ^{31}P- and ^{13}C-MRS. **C)** Oxygen consumption rates monitored with the Seahorse XF96 Analyzer during exposure of cells to 0-500 mM DFP, normalized to total protein content. **D)** Expression of m-Acon in control TRAMP C2 cells and cells exposed to 100 μM DFP for 24h.

CONCLUSION: Our results show the potential of DFP to inhibit PCa growth at clinically relevant doses. We are investigating this further in other PCa cell lines, and also studying the effects of DFP on cell cycle (flux cytometry) and migration (Scratch assay). Besides m-Acon, other mitochondrial targets of DFP cannot be discarded and are also being investigated.

ACKNOWLEDGMENTS: Work supported by NIH grants PO1 CA115675 and NCI P30 CA0874 (Cancer Center Support Grant).

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