breast cancer cells as they metastasize. Adipocytes at the invasive edge of human breast tumours exhibit a modified phenotype, and consequently these adipocytes have been named Cancer Associated Adipocytes (CAA). Previous *in vitro* research has shown that co-culture with CAA promotes breast cancer cell resistance to radiation and some therapeutic agents, as well as breast cancer cell invasiveness both *in vitro* and *in vivo*. The aim of the current research was to identify cellular proteins that are differentially regulated in breast cancer cells co-cultured with CAA.

Material and methods This study collected human breast adipose tissue samples, from which pre-adipocytes were isolated, differentiated into mature adipocytes, and co-cultured with hormone receptor positive (MCF-7) and negative (MDA-MB-231) human breast cancer cells for 3 days in a transwell co-culture system. The proteomes of co-cultured and control breast cancer cells were compared quantitatively using iTRAQ labelling and LC-coupled LTQ-Orbitrap tandem mass spectrometry. Validation of iTRAQ results was performed by Western blotting.

Results and discussions This study identified and quantified a total of 1126 and 1218 proteins expressed in MCF-7 and MDA-MB-231 cells, respectively. Of these, 85 in MCF-7 and 63 in MDA-MB-231 had a fold change >1.5 between co-culture and control breast cancer cell samples. Overall, MCF-7 cells had more proteins downregulated (n=53) than upregulated (n=32) after co-culture, whereas, more proteins were upregulated (n=51) than downregulated (n=12) in MDA-MB-231 cells. Enrichment analysis revealed an upregulation of proteins involved in metabolic pathways, namely TCA cycle proteins in MCF-7 cells and glycolysis proteins in MDA-MB-231 cells. The metabolic enzyme PGK1 was upregulated in response to co-culture with CAA in both cell lines, and is associated with poorer overall survival and resistance to the chemotherapy agent paclitaxel in breast cancer. Western blotting results validated mass spectrometry ratios for three candidate proteins.

Conclusion Overall, this study describes for the first time the local effect CAA have on the expression of proteins in breast cancer cells *in vitro*, and provides a comprehensive platform for further research investigating local interactions between breast cancer cells and CAA.

PO-235 FENOFIBRATE OVERCOMES THE DRUG-RESISTANCE OF HUMAN PROSTATE CANCER CELLS

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Introduction Microevolution of drug-resistant cancer cell populations is **a serious obstacle for** currently available cancer therapies. Reports on the inhibitory effects of fenofibrate (FF) on the growth, survival and invasiveness of prostate cancer cells suggest its potential for metronomic strategies of prostate cancer therapy. Therefore, we assessed the interference of FF with the drug-resistance of prostate cancer cells.

Material and methods Additive effects of DCX/FF on the propagation, invasiveness and drug-resistance of native prostate cancer cells and of their invasive DCX-resistant variants (DU145_DCX20 and DU145_DCX50) were estimated with time-lapse and fluorescence microscopy. Flow-cytometric and cytofluorimetric tests were performed to assess the interference of FF with ATP production, pro-apoptotic and pro-autophagic pathways; and with the activity of ABC transporters.

Results and discussions When administered alone, 2.5 nM DCX significantly attenuated the proliferation of native DU145 cells, but exerted no effect on the viability of DU145_DCX20 and DU145_DCX50 cells. FF (25 mM) sensitised these cells to DCX through PPARa/ROS-independent interference with intracellular ATP production and P-gp activity, as demonstrated by control assays with elacridar. Concomitantly, DCX/FF treatment considerably reduced neoplastic and invasive potential of drug-resistant DU145 cells via the activation of mTOR-sensitive suicidal autophagy signalling(s).

Conclusion Our observations suggest that FF can be applied to reduce the effective doses of chemotherapeutic drugs, to attenuate their adverse effects and to inhibit the microevolution of drug-resistant cells induced by chemotherapy. Thus, it can be considered as an metronomic agent that can enhance the efficiency of long-term palliative prostate cancer treatment.

Poster Presentation: Cancer Cell Biology

PO-237 THE PRO-ONCOGENIC TRANSCRIPTION FACTOR STAT3 REGULATES CA2 +RELEASE AND APOPTOSIS FROM THE ENDOPLASMIC RETICULUM VIA INTERACTION WITH THE CA2 +CHANNEL IP3R3

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Introduction Signal Transducer and Activator of Transcription (STAT) 3 is an oncogenic transcription factor found constitutively activated in several tumours, where it exerts its functions both as a canonical transcription factor and as a non-canonical regulator of energy metabolism and mitochondrial functions. These two activities rely on different post-translational activating events; the phosphorylation on Y705 is involved in nuclear activities, while that on S727 is relevant for mitochondrial functions. Mitochondrial STAT3 increases aerobic glycolysis and decreases ROS production, partly by interacting with the Electron Transfer Complexes (ETC).

Material and methods By means of cell fractionations, we tested STAT3 localization to the Endoplasmic Reticulum (ER) in breast cancer cell lines dependent or not on STAT3 activity. We then measured Ca^{2+} release and apoptotic response in the same cells. The physical interaction between inositol 1,4,5-trisphosphate receptor type 3 (IP3R3) and STAT3 was demonstrated by co-IP either of the endogenous proteins or of their truncated/mutated forms, while STAT3 role in the degradation of IP3R3 was tested by serum starvation and refeeding experiments, followed by WB.

Results and discussions We describe here the previously undetected abundant localization of STAT3 also to the ER. In this cellular compartment IP3R3, a Ca^{2+} channel that allows Ca^{2+} release from the ER and the mitochondrial associated membranes (MAMs) in response to IP3, regulates the balance

between mitochondrial activation and Ca^{2+}-triggered apoptosis. We observed that STAT3 within the ER physically interacts with IP3R3 and, via its phosphorylation on S727, it downregulates Ca^{2+} release and apoptosis. Indeed, STAT3 silencing enhances both ER Ca^{2+} release and sensitivity to apoptosis following oxidative stress in STAT3-dependent mammary tumour cells, correlating with increased IP3R3 levels. In line with this, basal-like breast tumours, which frequently display constitutively active STAT3, show an inverse correlation between IP3R3 and STAT3 protein levels.

Conclusion Our results indicate that S727-phosphorylated STAT3 contribute to mammary tumour aggressiveness, also by localising to the ER and regulating Ca^{2+} fluxes. STAT3-mediated enhanced IP3R3 degradation leads to decreased Ca^{2+} release and thus to resistance to apoptosis. This new non-canonical STAT3 role appears to be particularly relevant in basal-like breast cancers, adding a new mechanisms through which STAT3 exerts its well established pro-oncogenic anti-apoptotic role.

PO-238 DICHLOROACETATE (DCA) TREATMENT AFFECTS MITOCHONDRIAL ACTIVITY AND STEMNESS IN PANCREATIC CANCER (PC) CELL LINES

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Introduction Targeting metabolism represents a new approach to treat cancer, expecially when conventional chemoterapy fails. In this study, we tested a metabolic approach to treat PC, investigating, *in vitro* and *in vivo*, its response to DCA treatment.

Material and methods Two PC cell lines, BXPC3 and PANC1, were treated with DCA 4 and 10 mM for 24 hour. Cell viability and proliferation were assessed by MTS assay and xCELLigence, apoptosis and ROS by flow-cytometry; $pPDH^{Ser293}$/tot PDH, LC3B, DRP1, MFN1, MNF2, OPA1 and TOMM20 protein expression was evaluated by western blotting, lin28 gene expression by qPCR. The oxygen consumption rate (OCR) and extra-cellular acidification rate (ECAR) were measured by Seahorse Technology. Ultra-low attachment plates were used to form spheroids. *In vivo*, DCA was administered to BXPC3-luc tumor-bearing nude mice. After measuring bioluminescence signalling, the tumour masses were harvested, photographed and weighed.

Results and discussions DCA treatment reduced cell proliferation, decreasing cell survival with an increase in ROS production and apoptosis in both cell lines. Despite PDH activation by dephosphorylation, DCA did not restore bioenergetic profile but decreased OCR, a measure of oxidative phosphorylation efficiency. ECAR was not affected, suggesting that the glicolytic capacity was not modified by DCA treatment. These observations led us to explore mitophagy, whose activation was confirmed by LC3B protein overexpression and TOMM20 downregulation, and mitochondrial dynamics also altered following DCA treatment as shown by the downregulation of MFN1, MFN2, OPA1, key proteins of mitochondrial fusion. Interestingly, DCA was able to negatively affect the cancer stem cell (CSCs) fraction in both cell lines, reducing the expression of stemness genes such as Lin28 and inhibiting spheroid formation. When added to 3D cultures already formed, it was able to downregulate stemness genes expression, leading to a significant size reduction and affecting spheroid viability. Finally, DCA efficacy was confirmed in a xenograft pancreatic cancer mouse model in which DCA treatment displayed a significant retarded progression of PC, reducing diameter of the tumour mass.

Conclusion Our data suggest that DCA is able to strongly affect PC cells metabolism counteracting mitochondrial activity. This effect is not related to PDH activity stimulation. In addition, the ability of DCA to hit CSCs offers a further rationale to candidate this drug for PC treatment, trying to reach a complete tumour eradication.

PO-239 BENZO(A)PYRENE, AN ACTIVE PRODUCT OF CIGARETTE SMOKE, ROLE IN PLA2 ISOFORMS ACTIVATION IN COLON CANCER

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Introduction One of the active combustion product of cigarette smoke, Benzo[a]pyrenes, role in pulmonary cancer is clearly understood. However, its role in gastrointestinal cancer including colon cancer is not clearly understood.

Material and methods In this study, benzo(a)pyrene's was treated to colon cells to evaluate its role in cell viability, cellular ROS, and gene expression of various PLA_2 isoforms was evaluated by FACS and PCR. The identified PLA_2 was silenced at the gene level to evaluate its role in cell viability and ROS generation.

Results and discussions B(a)P treatment at 1 µg/ml for 48 hour to HCT-15 male colon cells significantly reduced the cell viability without affecting HT-29 female colon cells. Higher doses and longer treatment duration with B(a)P showed that female colon cells were highly sensitive than male colon cells. Annexin-V/PI staining for pre-apoptotic detection showed that B(a)P treatment increased the apoptosis in both the cell types in a concentration and time-dependent manner. The cytosolic ROS (cROS) and superoxide radical (SOR) formation in the female colon cells was significantly higher than male colon cells unlike the mitochondrial ROS (mtROS) production which was significantly higher in male colon cells. Treatment with B(a)P significantly upregulated the IID and IVA PLA_2 isoform groups in HCT-15 male colon cells, whereas IB was upregulated in HT-29 female colon cells among the various PLA2 isozyme gene studied (IB, IID, III, IVA, IVB, IVC, VI, X, $aiPLA_2$ and $iPLA_2$). Gene silencing experiments targeting PLA_2 IID and IVA in the HCT-15 male colon cells and IB in HT-29 female colon cells showed no effect with B(a)P treatment on the cell proliferation, apoptosis, membrane integrity and free radicals (ROS, mtROS, and SOR) generation.

Conclusion Targeting specific PLA_2 isozymes in a cell specific manner abolished the B(a)P-induced PLA_2 mediated oxidative damage related signalling pathways.