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.