PO-039 SOPHORIDINE INDUCES APOPTOSIS AND S PHASE ARREST VIA ROS-DEPENDENT JNK AND ERK ACTIVATION IN HUMAN PANCREATIC CANCER CELLS

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Introduction Pancreatic cancer is generally acknowledged as the most common primary malignant tumour, and it is known to be resistant to conventional chemotherapy. Novel, selective antitumor agents are pressingly needed.

Material and methods CCK-8 and colony formation assay were used to investigate the cell growth. Flow cytometry analysis was used to evaluate the cell cycle and cell apoptosis. The peroxide-sensitive fluorescent probe DCFH-DA was used to measure the intracellular ROS levels. Western blot assay was used to detect the levels of cell cycle and apoptosis related proteins. Xenografts in nude mice were used to evaluate the effect of Sophoridine on pancreatic cancer cell in vivo.

Results and discussions Sophoridine killed cancer cells but had low cytotoxicity to normal cells. Pancreatic cancer cells were particularly sensitive. Sophoridine inhibited the proliferation of pancreatic cancer cells and induced cell cycle arrest at S phase and mitochondrial-related apoptosis. Moreover, Sophoridine induced a sustained activation of the phosphorylation of ERK and JNK. In addition, Sophoridine provoked the generation of reactive oxygen species (ROS) in pancreatic cancer cells. Finally, in vivo, Sophoridine suppressed tumour growth in mouse xenograft models.

Conclusion These findings suggest Sophoridine is promising to be a novel, potent and selective antitumor drug candidate for pancreatic cancer.

PO-040 CHARACTERISATION OF CDK12 KNOCKED OUT OVARIAN CANCER CELL LINES

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Introduction While cyclin-dependent kinases (CDKs) have a key role in promoting/controlling transition between the different phases of the cell cycle, transcriptional kinases, like CDK12, are mainly involved in gene transcription. CDK12 has been shown to regulate the expression of genes involved in DNA damage and to maintain genomic stability. Impairment of CDK12 activity is synergic with PARP inhibitor and cisplatin treatments in different cellular systems. We here aimed to generate ovarian cancer cell lines knocked out (KO) for CDK12 to understand its role in ovarian cancer and in response to chemotherapy.

Material and methods A2780 and SKOV3 CDK12 KO clones were generated with CRISPR/Cas9 technology. Cell cycle analysis was evaluated by standard flow cytometric methods and DNA repair genes levels by Real Time PCR. Caspase 3 activity was measured to detect apoptosis with a luminescence-based assay. Cytotoxicity experiments were performed treating cells with different drug concentrations and evaluating cell survival after 72 hours by MTS assay. For in vivo studies 7.5 millions of cells were transplanted subcutaneously in nude mice and animals were monitored for tumour appearance and growth.

Results and discussions We obtained 2 CDK12 KO ovarian cancer clones, A2780 KO and SKOV3 KO, out of more than 300 clones screened. The cell growth of both A2780 KO and SKOV3 KO cells is slower than the wild type (WT) cells, they have a less clonogenic ability and a tetraploid DNA content. Both CDK12 KO clones have a higher basal caspase activity than the WT cell lines, indicative of higher basal induction of apoptosis, while no increase in autophagy or senescence is observed. Both CDK12 KO clones show a decreased expression in BRCA1 and FANCD2 DNA repair genes than the WT cells. Cytotoxic experiments with anticancer agents with different mechanism of action show that both KO clones are less sensitive to ATM, CHK1 and WEE1 inhibitors treatment as compared to WT cells, while platinum and PARP inhibitors show similar cytotoxic activity in KO and WT cells. Interestingly enough, when KO clones were transplanted in nude mice, no tumour take was observed.

Conclusion We were able to obtain CDK12 KO cells. We think that these models could help in disclosing new roles of CDK12 in ovarian carcinona and may represent a useful tool to study new combination therapies for tumours with CDK12 mutations.
to identify the potassium channels that could be implicated in the response to TNF.

**Material and methods** We analysed the effects of TNF on two CRC cell lines, HCT116 that is KRAS mutated and HT29 that is KRAS wildtype. Cell lines viability and migration were determined by MTT assay and scratch assay, respectively. Cell cycle was examined by propidium iodide DNA staining. Gene expression of TNF pathway and potassium channels was measured by RTqPCR and Western blot.

**Results and discussions** Our work shows that TNF increased the migration of HT29 cells while reducing that of HCT116. In addition, TNF reduced the viability of HCT116 cells and their colony formation capacity. Moreover, cell cycle analyses showed an increase in the proportion of sub-G1 phase in HCT116 cells after TNF treatment, with no effect on HT29 cells. We also demonstrated an increased expression and phosphorylation of STAT3 protein in HT29 cells, contrary to HCT116 cells that showed reduced phosphorylation following TNF treatment. Interestingly, exogenous TNF increased the transcriptional expression of TNF in both cell lines and this result was associated with an increased expression of its receptor, TNFR2, only in HT29 whereas it was abrogated in HCT116 cells. Furthermore, TNF caused a global decrease in the expression of potassium channels coding genes in HCT116 cells, while this effect was less pronounced in HT29 cells.

**Conclusion** Taken together, our results suggest that the modulation of TNF pathway could be associated to KRAS status. Furthermore, potassium channels could be implicated in CRC cells response to TNF.

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**PO-043**

**TARGETING THE THIOL OXIDOREDUCTASES ERP57 AND PDI HITS CANCER CELLS ON MULTIPLE FRONTS: PROLIFERATION, RADIORESISTANCE AND ER STRESS RESPONSE (UPR)**

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**Introduction** Fast-growing tumour cells show enhanced protein synthesis and therefore depend on efficient folding for nascent export proteins in the endoplasmic reticulum (ER). Herein, the two most prominent ER resident thiol oxidoreductases ERP57 and PDI play important parts in formation of disulfide bonds in client proteins. This and the finding that both proteins fulfill various roles also in other compartments (i.e. cytoplasm, nucleus, cell membrane) encouraged us to investigate the impact of their depletion on colorectal cancer cells.

**Material and methods** Using an inducible knockdown (KD) system we tested ERP57 and PDI deficiency in long term survival assays in normoxia and hypoxia combined with irradiation.

**Results and discussions** KD of ERP57 or PDI triggered a severe attenuation of proliferation, but only ERP57 deficiency led to activation of the PERK-dependent UPR and apoptosis. When combined with an ERP57 KD, irradiation displayed the most dramatic growth reduction even under 1% oxygen. The absence of ERP57 reduced expression of cellular proliferation factors like c-Myc, PLK-1, AKT, PDK1, ERK1,2 and others.

Further, we demonstrated for the first time that PDI is an essential activator of the ER stress sensor PERK that enforces cancer cell survival under global ER stress in hypoxia. In the absence of ER stress, ERP57 functions as a reductase for PDI that keeps PERK in an inactive state.

**Conclusion** Our data identified ERP57 and PDI as promising new targets for a mono- and combination anti-cancer therapy due to multiple cellular points of attack.