PO-153 SOX9 REGULATES EPITHELIAL TO MESENCHYMAL TRANSITION AND SELF-RENEWAL IN PANCREATIC ADENOCARCINOMA PROMOTING METASTASIS AND CHEMORESISTANCE

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Introduction Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive malignancy characterised by dismal prognosis due to early metastasis and chemoresistance. However, the molecular basis underlying these features remains poorly understood. SOX9 is a developmental transcription factor that regulates the stem cell biology in the embryo and also in mature organs, including the pancreas. As SOX9 is required for the maintenance of the pancreatic ductal identity and is involved in the initiation of pancreatic cancer, we aimed at investigating its putative roles in pancreatic tumour cell plasticity, dissemination and resistance to therapies.
Material and methods To address our objectives we have analysed human PDAC samples, patient-derived xenografts (PDXs) and animal models, and we have performed functional studies of gain and loss of SOX9 function in human PDAC cell lines.
Results and discussions SOX9 expression was analysed in 198 cases of human PDAC and also in 24 PDXs. Our results revealed that SOX9 protein was highly expressed in pancreatic cancer, being the expression particularly high in metastatic cases. SOX9 expression was correlated with high vimentin expression ($p=0.0006$) and low E-cadherin expression ($p=0.003$), reflecting a process of epithelial–mesenchymal transition (EMT). Gain and loss of SOX9 analysis in primary and metastatic PDAC cells, respectively, determined that SOX9 induction increases migration, invasion and metastatic dissemination of primary tumour cells, whilst SOX9 silencing in metastatic cells impaired the metastatic colonisation potential, along with a reversion to epithelial features and loss of self-renewal and tumour maintenance ability. Additionally, we determined that high SOX9 expression confers resistance to gemcitabine. Mechanistically, we identified that the transcriptional repressor BMI-1 is a mediator in the oncogenic activity exerted by SOX9 in PDAC.
Conclusion We conclude that in PDAC SOX9 is critical for tumour maintenance, dissemination and metastatic colonisation through its action on EMT, self-renewal and proliferation.

PO-154 PROGESTERONE THROUGH PROGESTERONE RECEPTOR-B INHIBITS INVASION OF HUMAN BREAST CANCER CELLS BY TARGETING CYTOPLASMIC CYCLIN D1

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Introduction Progesterone Receptor (PR) positivity is associated with a good prognosis and better response to breast cancer treatment. Conversely, cyclin D1 (CD1) is retained a marker of poor outcome since it has been associated with breast cancer metastasis in clinical studies.
Material and methods 17-Hydroxyprogesterone (OHPg) was from Sigma-Aldrich. Antibodies and Protein A/GPLUS-Agarose were from Santa Cruz Biotechnology. T47-D, MCF-7 and MDA-MB-231 human breast cancer cells from the American Type Culture Collection; Total real-time RT-PCR assay; Western blotting and immunoprecipitation; Transfections and luciferase assays; Lipid-Mediated Transfection of siRNA Duplexes; Chromatin immunoprecipitation (ChIP) assays and realtime ChIP; Wound-healing assays; Transmigration assays; Cell invasion assay; Phalloidin staining.
Results and discussions Herein we provide evidences that OHPg through PR-B isoform, reduces motility and invasion of T47-D and MCF-7 breast cancer cells, by targeting the cytoplasmic CD1. Specifically, OHPg reduces CD1 expression through a transcriptional mechanism due to the occupancy of CD1 promoter at a canonical half progesterone responsive element by PR-B. This allows the recruitment of HDAC1 influencing a less permissive chromatin conformation for gene transcription and the release of RNA Pol II. CD1 has an active role in the control of cell migration and metastasis through the interaction with key components of focal adhesion such as Paxillin (Pxn). In untreated T47-D and MCF-7 cells a specific co-immunoprecipitation of endogenous cytoplasmic CD1 with Pxn was detected. Interestingly, OHPg exposure reduced the interaction between these proteins although total Pxn expression was substantially unaffected. Moreover a concomitant reduction of p-Pxn levels was observed and these effects were required for OHPg/PR-B dependent delay in cell invasion, as evidenced by assays carried out with the phoshomimetic mutants of Pxn.
Conclusion Collectively these findings support the importance of PR-B expression in breast cancer cells behaviour, suggesting potentiating of PR-B signalling as a prospective useful strategy to restrict breast tumour cells invasion and metastasis.

PO-155 STUDY OF THE EFFECT OF PENTRAXIN 3 ON THE METASTASIS-RELATED PROTEIN OF HUMAN OSTEOSARCOMA CELL LINES

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Introduction Metastasis of cancer cells, a primary cause of cancer death and a multiple and intricate processes, may complicate the clinical management and lead to a poor prognosis for cancer patients and has tremendous physical or economic impact to patients or communities. Pentraxin 3 (PTX3) plays an important role in innate immune responses and in inflammatory diseases. Over the past decades, extensive efforts have been made to find the role of PTX3 in tumour progression. Moreover, PTX3 expression also has been identified as a new diagnostic or prognostic biomarker of various types of cancers. However, little evidence of a direct relationship between PTX3 expressions and osteosarcoma.
Material and methods Boyden chamber assay was used to evaluate the effects of cell migration and invasion. RT-PCR, QPCR, western blot and luciferase analysis were used to evaluate the expression of different gene, including silencing of PTX3 by short hairpin RNAi, on PTX3-LGMN-S100A16 associated metastasis in osteosarcoma cells.
Results and discussions We compared the protein expression of Pentraxin-3 (PTX3) in four osteosarcoma cell lines (U2OS, HOS, MG-63 and Saos-2). We established the PTX3 siRNA