CIP2A-MEDIATED REGULATION OF SENESCENCE IN PROTEIN DISULFIDE ISOMERASE A4 REGULATES CANCER DEVELOPMENT VIA CANCER STROMAL CELLS

**Introduction**
Basal-like breast cancer (BLBC) is an aggressive breast cancer molecular subtype, and it lacks efficient therapy options. Expression levels of oncoprotein CIP2A are increased in BLBC as compared to other breast cancer subtypes. Our results demonstrate that high CIP2A expression significantly predicts poor patient survival in triple negative breast cancer, that constitutes a major subset of BLBC. CIP2A inhibition also significantly impairs tumor growth of human BLBC xenografts. Previously, we have shown that CIP2A promotes breast cancer by inhibiting senescent growth arrest (Laine et al., Cancer Discov 2013). Induction of senescence and senescence associated secretome (SASP) has been shown to alter function of tumour infiltrating immune cells. Based on this we hypothesise that in addition to effects of CIP2A-mediated senescence inhibition to tumour cell-intrinsic mechanisms, it may impact infiltrating immune cells and thus therapy response.

**Material and methods**
To investigate the role of CIP2A in BLBC a carcinogen DMBA-induced tumour formation approach in Cip2a-/- mouse model was applied. In order to test our hypothesis about the effect of CIP2A-targeted senescence in immune cells we have set up mouse mammary tumour cell and organoid cultures from a spontaneous BLBC genetically engineered mouse model K14Cre;Brca1fl/fl;Tps3fl/fl. These cultures are genetically manipulated by CRISPR/Cas9 system to knock out CIP2A both prior to transplantation into recipient mice and in established tumours. To validate the in vivo findings we are using several different human breast cancer patient cohorts.

**Results and discussions**
Our results demonstrate that CIP2A-deficient mice are severely impaired in a formation of DMBA-induced basal-like mammary tumours. Together with the clinical data, these results suggest that CIP2A is a novel driver oncoprotein, and a potential therapeutic target, in human BLBC. In addition, we have validated the tumour cell-intrinsic role of CIP2A in K14Cre;Brca1fl/fl;Tps3fl/fl BLBC mouse model as loss of CIP2A induces senescent growth arrest in mammary gland tumour cells in vivo. Currently, we are studying the interplay between the immune system and CIP2A-inhibited tumours in vitro and in vivo.

**Conclusion**
Overall, this study will address feasibility of CIP2A targeting as a novel approach to combat basal-like breast cancer. Results of this project will also enhance our general understanding of cross-talk between senescent cancer cells and the tumour environment in this clinically challenging human cancer type.
DECONVOLUTION OF THE TUMOUR MICROENVIRONMENT OF PRIMARY HUMAN PANCREATIC DUCTAL ADENOCARCINOMA AND NORMAL PANCREAS REVEALS SPECIFIC DEREGLUTATED SIGNALLING NODES

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Introduction Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive disease with dismal prognosis. Despite promising results using in vitro systems or genetically engineered mouse models, new drug candidates have not yet made significant impact on PDAC patients. This highlights the urgent need for a better understanding of the complex signalling cascades taking place within human tumours in situ. However, due to the extensive presence of desmoplastic stroma within PDAC tumour mass, expression analyses of bulk tumour samples are difficult to interpret and remain uninformative with respect to the complex cellular and molecular architecture of these tumours. Thus, the detailed analysis of the tumor-stroma interactions occurring in primary human PDACs seems a critical missing link for a better understanding of this complex disease, what is critically important to develop novel innovative therapies.

Material and methods We have isolated all major cell types present within primary human PDAC tumours (cancer associated fibroblasts (CAFs), immune, endothelial and epithelial cancer cells) by FACS sorting more than 20 fresh PDACs and supplemented these with the same cell populations isolated from 7 tumor-free adjacent (normal) pancreas samples. From these we have generated transcriptomic data by RNA-Seq and performed whole genome bisulfite sequencing (epithelial cancer/normal cells only) to determine their methyloyme. We have analysed and integrated these data using various computational analysis tools.

Results and discussions We found that PDIA4 is up-regulated in cancer stromal cells as well as tumour cells, as shown by Real-time PCR and immunoblot. Consistently, oncomine database showed that PDIA4 expression level is positively correlated to the prognosis and metastasis in patients with different types of cancer. Knockout of host PDIA4 in tumor-bearing mice reduced tumour development, metastasis and angiogenesis. This reduction was relevant to the composition and function of cancer stromal populations. We are on the process of identify the cellular and molecular mechanism of PDIA4 in tumour microenvironment.

Conclusion This study demonstrates that PDIA4 can not only work on tumour cells but also regulate tumour microenvironment. The data suggest that PDIA4 is a potential therapeutic target for cancer.