Inhibition of the mTORC1-pathway can feedback-activate H-ras or K-ras

Introduction PI3K/mTORC1- and Ras/MAPK-signalling pathways are aberrantly regulated in most cancers. Specific resistance to drugs targeting these pathways can emerge during tumour evolution. Preexisting, innate resistance mechanisms, such as stemming from feedback-loops, should ideally be known already during drug-target nomination. However, as the example of B-Raf-inhibitors that paradoxically activate MAPK-signalling has shown, feedback mechanisms may only become apparent at very late drug development stages.

Material and methods HEK cells expressing FRET-pairs of Ras proteins were used to study specific effects on Ras isoforms (nanoclustering). Breast cancer cells were grown in 2D for Western blotting of Ras and mTORC1-pathway proteins, or as spheres to analyse stemness traits.

Results and discussions Here, we describe two broad feedback loops from the mTOR-pathway back to the nanoscopic membrane signalling complexes (nanocluster) of H-ras and K-ras4B (hereafter K-ras). Increased nanoclustering typically correlates with increased Ras output. The first, upstream loop leads to an inadvertent rapalog induced promotion of stemness traits and tumorigenicity in Ras transformed cells. This is due to an induction of the H-ras nanocluster scaffold galectin-1, when FKBP12 levels are low. Surprisingly we find that rapalogs do not only bind to but induce a loss of FKBP12 protein. Thus, rapalog treatment induces galectin-1, which stimulates H-ras signal output and stemness traits.

Secondly, modulation of the activity in the mTORC1 pathway downstream of the major lipidome regulator SREBP1, oppositely regulates H-ras and K-ras nanoclustering. Thus, ablation of SREBP1 increases K-ras, but decreases H-ras nanoclustering and signal output. We show that altered levels of phosphatidic acid downstream of SREBP1 are sufficient for the opposite regulation of the two Ras isoforms.

Conclusion The described feedback loops may only become apparent in certain tumour settings. For example, tumour promotion during rapalog-treatment may only be relevant in H-ras mutant cancers, which make up a small portion of human cancers. In those cases, rapalog efficacy may be improved in combination with novel anti-galectin-1 drugs. Targeting the mTORC1 pathway downstream of SREBP1, may have opposite effects in H-ras and K-ras mutant cancers. Thus, care may have to be taken when targeting the mTORC1-pathway in a mutant Ras setting.

Lipocalin 2 Suppresses Metastases of Oral Cancer through miRNA-29a-Regulated Dipeptidyl Peptidase-4

Introduction Lipocalin 2 (LCN2), a secreted glycoprotein, is up- or downregulated in different human cancers and it has been found to play a different role in tumorigenesis. Dipeptidyl peptidase IV (DPP4), a membrane-bound peptidase on the cell surface of a wide variety of cell types and plays an important role through enzyme activity. Several recent studies have shown that DPP4 affects tumour progression and invasion in several human malignancies. However, how LCN2 and DPP4 involved in tumour progression and metastasis in oral cancer have not been studies yet.

Material and methods DPP4 expressed in LCN2 overexpression OSCC cells by protease array assay. MicroRNA array analysis and microRNA target prediction (TargetScan and Pita) reveal that DPP4 is one of the target gene of miR-29 family.

Results and discussions Overexpression of LCN2 in oral cancer cell lines reduced in vitro migration/invasion. Mechanistically, LCN2 inhibited the cell motility of oral cancer cells through transcriptional expression of the DPP4. Knockdown DPP4 in LCN2 overexpressed cell line significantly increased cell invasion and migration. Moreover, in oral cancer cells, LCN2 significantly decreased the levels of miRNA-29a, which increased the DPP4 expression. Overexpression of miRNA-29a significantly suppressed DPP4 expression and increased OSCC cell migration and invasion (p<0.05).

Conclusion We concluded that LCN2 suppresses oral cancer cell invasion and migration through miRNA-29a-regulated DPP4.
Moreover, deactivation of Myc resulted in immediate tissue resolution: excess cells were eliminated, proliferation halted, and liver function restored. We have identified CCl6 and CCl21 chemokine as Myc-dependent proximal signals that instruct the microenvironmental changes associated with tissue regeneration and resolution, respectively. Interestingly, during the resolution phase, liver was refractory to Myc re-activation (failure to induce proliferation) but responsive after resolution had completed suggesting the presence of a dominant resolution programme.

**Conclusion** We provide evidence that deregulated Myc is able to ‘hack’ the local tissue regenerative program and have identified some of the Myc-dependent molecular triggers responsible. Furthermore, we raise the possibility that novel cancer targets may be identified in the proposed ‘resolution programme’ in order to promote tumour regression.

**PO-095** PIM KINASES IN THE REGULATION OF PROSTATE CANCER CELL MOTILITY

1N Santio*, 2V Vainio, 3M Lång, 4Kl Mung, 5J Tuomela, 6P Härkönen, 7C Sahlgren, 6P Koskinen. 1University of Turku, Biology, Turku, Finland; 2University of Turku, Biotechnology, Turku, Finland; 3University of Turku, Biology, Turku, Finland; 4University of Turku, Biochemistry, Turku, Finland; 5University of Turku, Anatomy, Turku, Finland; 6Åbo Akademi, Cell biology, Turku, Finland

10.1136/esmoopen-2018-EACR25.137

**Introduction** Pim kinases are constitutively active serine/threonine kinases that are upregulated by the JAK/STAT pathway and are often overexpressed in advanced human haematological and solid cancers. There they have been observed to enhance cancer cell growth, survival and energy metabolism. Using both cell-based and animal models, we have shown that PIM kinases also efficiently promote cancer cell migration, invasion and metastasis formation. In addition, we have identified several substrates to mediate the PIM-dependent effects.

**Material and methods** Motility of PC-3 prostate cancer cells was analysed by cell-based assays, such as wound healing assays with single cell tracking method, Boyden chamber assays and adhesion assays. The effects of PIM kinases on tumour growth were studied using subcutaneous and orthotopic mouse models for human prostate cancer. The interactions of PIM kinases and their substrates were investigated by in vitro kinase assays as well as microscopic techniques such as proximity ligation assay and fluorescence lifetime imaging. To reveal PIM-dependent functions, we used PIM-selective chemical inhibitors, transient or stable PIM upregulation as well as PIM silencing by RNA interference or CRISPR/Cas9-based gene editing.

**Results and discussions** PIM upregulation was shown to promote prostate cancer cell motility both in cell-based wound healing assays and in the orthotopically inoculated xenografts. By contrast, inhibition of PIM kinase activity by the pyrrolotetrazole compound DHPCC-9 decreased cell migration, invasion and adhesion as well as tumour growth, angiogenesis, lymphangiogenesis and formation of metastases. Furthermore, the PIM-dependent effects were shown to be mediated by the ability of PIM kinases to phosphorylate several key substrates, such as CXCR4, FOXP3, GSK3B, NFATC1 and NOTCH1. More recently, we have observed PIM kinases to influence also the actin cytoskeleton and have identified actin-regulating proteins as novel PIM substrates.

**Conclusion** PIM kinases are promising targets for anti-cancer drug development, not only for their roles as pro-survival factors but also as pro-migratory factors. By inhibiting PIM kinases, it may be possible to simultaneously regulate many different PIM substrates that are essential for metastatic tumour growth.

**PO-096** DEFINING THE ROLE OF MYC IN THE PANCREATIC CANCER SUPER ENHANCER NETWORK

1T Campos*, 2N Hah, 3N Sodi, 4R Evans, 4T Littlewood, 4G Evan. 1University of Cambridge, Department of Biochemistry, Cambridge, UK; 3The Salk Institute for Biological Studies, Gene Expression Laboratory, La Jolla- California, USA

10.1136/esmoopen-2018-EACR25.138

**Introduction** Cell behaviour and identity are in great part determined by transcriptional switches present in the genome that are referred to as super-enhancers (SEs). The transcription factor Myc coordinates several physiological processes required for cell growth, proliferation and spread and its expression is deregulated in the majority of cancers. Our recent studies have shown that Myc deregulation in a KRas(G12D) driven mouse model drives immediate progression to aggressive and inflammatory pancreatic ductal adenocarcinoma (PDAC) accompanied by a profound desmoplastic response. Subsequent Myc de-activation triggers immediate regression of the tumour microenvironment and death of tumour cells. The complex phenotypic changes that accompany the Myc-driven transition to PDAC suggest that Myc plays a key role in driving the pancreatic cancer SE network. However, it remains unclear whether Myc establishes the PDAC SE or, instead, acts as the switch that activates a pre-configured SE network. To distinguish between these possibilities, we have addressed whether the phenotypic and gene expression changes that Myc engages in pancreatic epithelium are accompanied by modifications in the underlying tumour cell epigenome.

**Material and methods** Primary tumour cell lines were isolated from our mouse model p48-cre; LSL-Kras(G12D);Rosa-LSL-MycERTM, where Myc can be switch on and off at will solely in the pancreatic epithelium. ATAC-seq and ChIP-seq was used to identify Myc-dependent changes in the accessible chromatin and signature epigenetic markers, respectively.

**Results and discussions** We observe widespread Myc-dependent changes in gene expression and pro-tumorigenic signalling molecules, including PD-L1 (Programmed death-ligand 1), indicating rapid engagement of the SE network by Myc. Despite these changes in gene expression, we observe no significant accompanying qualitative changes in chromatin accessibility or the H3K27ac landscape. For instance, Myc binds the PD-L1 promoter and regulates its expression in a cell autonomous manner in the pancreatic tumour cell line in the absence of any changes in chromatin accessibility at the PD-L1 locus.

**Conclusion** Our data indicate that Myc does not configure the SE landscape but, instead, serves as a master switch to engage it. Therefore, abrogating Myc’s engagement of the SE network may provide a novel therapeutic strategy for the treatment of pancreatic cancer.