ESMO Open: first published as 10.1136/esmoopen-2018-EACR25.160 on 29 June 2018. Downloaded from http://esmoopen.bmj.com/ on 28 April 2019 by guest. Protected by copyright.

Results and discussions Our preliminary data showed that the CaSR was not expressed in the organoids cultured in stem cell media where even the differentiation markers were barely detectable. On the other hand, organoids cultured for 7 days in enterocyte-specific differentiating condition expressed both CaSR and FABP2, while in goblet cells CaSR expression remained undetectable. Our results not only show that inducing differentiation leads to higher CaSR levels, but also suggest that the CaSR is expressed in enterocytes rather than MUC2-expressing goblet cells.

Conclusion We conclude that, like poorly differentiated colorectal tumour cells, the colonic stem cells do not express the CaSR, which is expressed in mature intestinal cells. Further experiments will allow us to undoubtedly determine in which subtypes of differentiated cells the CaSR is preferentially localised.

PO-119 FERRITIN HEAVY SUBUNIT ENHANCES APOPTOSIS OF NON-SMALL CELL LUNG CANCER CELLS THROUGH MODULATION OF A MIR-125B/P53 AXIS

F Biamonte*, AM Battaglia, F Zolea, I Aversa, A Salatino, A Sacco, FM Perrone, R Chirillo. *'Magna Graecia' University of Catanzaro, Experimental and Clinical Medicine, Catanzaro, Italy*

10.1136/esmoopen-2018-EACR25.160

Introduction The ferritin molecule is a nanocage composed by the variable assembly of 24 heavy and light subunits. As major intracellular iron storage protein, ferritin has been studied for many years in the context of iron metabolism. However, recent evidences highlight its role, and particularly that of the heavy subunit (FHC), in pathways related to cancer development and progression, such as cell proliferation, epithelial-mesenchymal transition, cell death and angiogenesis. This new role of FHC is largely due to its ability to regulate a repertoire of oncogenes and oncomiRNAs. Moreover, the existence of a feedback loop between FHC and the tumour suppressor p53 has been observed in different cell types.

Material and methods FHC was overexpressed in A549 and H460 non-small cell lung cancer (NSCLC) cells through transient transfection of a specific expression vector. FHC, p53 and miR-125b levels were measured by q-PCR and TaqMan analyses in cancer cell lines as well as in tumour tissue specimens. The analysis of the methylation status of miR-125b promoter region was achieved by Methylation Specific PCR (MSP). FHC-overexpressing and control A549 and H460 cells were monitored for changes in proliferation and apoptosis through PI and Annexin/7-AAD flow cytometry assays. Intrinsic and extrinsic apoptosis biomarkers were measured by Western Blot. Statistical analysis was performed by Student t-test or non-parametric Wilcoxon signed-rank test.

Results and discussions The major finding of this study was that FHC is able to enhance p53 expression through the down-regulation of miR-125b in A549 and H460 NSCLC cell lines. Indeed, we found that FHC overexpression induces hypermethylation and thus the down-regulation of miR-125b which, in turn, is a direct repressor of the tumour suppressor p53. Absolute q-PCR highlighted a significant correlation among these three key molecules also in human tumour tissue specimens thus strongly suggesting the existence of a new regulatory axis In *vitro*, FHC overexpression also triggered p53-mediated cell apoptosis that is partially reverted by miR-125b reconstitution. Up-regulation of BAX, a pro-apoptotic member of Bcl-2 family, and the enhanced cleavage of caspase-9 demonstrated the activation of the intrinsic apoptotic pathway.

Conclusion Overall, the identification of a FHC/miR-125b/p53 regulatory axis may provide a novel molecular strategy for the regulation of the apoptotic cell death in non small cell lung cancer.

PO-120 PROLINE RICH HOMEODOMAIN PROTEIN IS REQUIRED FOR CHOLANGIOCARCINOMA TUMOUR GROWTH

[1]PS Jayaraman, KY Lee*[2], [2]P Kitchen, [3]D Clark, [4]J Lertsuwan, [5]A Sawasdichai, [6]S Oltean, [5]J Satayavivad, [7]SA Simon Afford, [8]K Gaston. [1]*U. Birmingham, Cancer and Genomic Sciences, Birmingham, UK*; [2]*U. Birmingham, Cancer and Genomic Science, Birmingham, UK*; [3]*U. Bristol, Biochemistry, Bristol, UK*; [4]*Chulabhorn Research Insitute, Cri, Bangkok, Thailand*; [5]*Chulabhorn Research Institute, Cri, Bangkok, Thailand*; [6]*U. Exeter, Institute of Biomedical and Clinical Sciences, Exeter, UK*; [7]*U. Birmingham, Institute of Immunology and Immunotherapy, Birmingham, UK*; [8]*U. Nottingham, Medicine, Nottingham, UK*

10.1136/esmoopen-2018-EACR25.161

Introduction Proline-Rich Homeodomain protein/Haematopoietically Expressed Homeobox (PRH/HHEX) is a transcription factor that regulates cell proliferation, migration, and differentiation in multiple tissues. PRH is essential for embryonic development of the liver and bile ducts and has tumour suppressor activity in hepatocellular carcinoma (HCC). Misregulation of PRH is associated with several cancers including HCC, breast cancer, prostate cancer, and leukaemia. Our objective is to determine whether PRH has a role in cholangiocarcinoma (cancer of the bile duct).

Material and methods PRH was over-expressed in cholangiocarcinoma (CCA) cell lines and in primary bile duct epithelial cells (BECs) using an adenovirus expressing myc-tagged PRH and knockdown of PRH was achieved by stable expression of PRH shRNA. Cell proliferation was measured by EdU incorporation. RNA sequencing (RNA-seq) was used to determine genes and pathways regulated by PRH in CCA. Quantitative RT-PCR (qPCR) was used to confirm these changes and Western blotting and immunohistochemistry was used to examine the expression and localisation of the corresponding proteins. Mining of TCGA transcriptomics and genomics data was performed using UCSC Xena.

Results and discussions Western blotting and immunohistochemical staining reveals that PRH protein is elevated in CCA compared to cholangiocytes and TCGA expression data indicate that PRH mRNA is commonly upregulated in CCA. Over-expression (OE) of PRH increases the proliferation of CCLP1 and CCSW1 CCA cell lines and BECs. Conversely, PRH KD decreases CCLP1 proliferation and alters the morphological phenotype of CCLP1 cells from mesenchymal to epithelial. Xenograft experiments with CCLP1 PRH knockdown (KD) cells compared to control cells shows that depletion of PRH significantly decreases tumour growth. Gene ontology and gene set enrichment analysis of RNA-seq data from CCLP1 PRH KD and OE cells indicates differential expression of genes involved in proliferation, adhesion, and migration including genes associated with Wnt signalling, and epithelial- mesenchymal transition (EMT). Western blotting confirms changes in protein expression in the KD cells in accord with the RNA-seq data. Examination of β-catenin