normal growth conditions as well as in INF treated and/or radiated cells.

Results and discussions Irradiation of glioma stem cells, results in a substantial increase in the expression of IFITM1 and an activation of the IRDS pathway which suggests a potential mechanism by which cancer stem cells escape chemotherapy. Deletion of IFITM1 in SiHa cells results in sensitivity to chemo- and radiation therapy, while loss of both IFITM1 and IFITM3 function generates chemoresistant cancer cells suggesting a potential interaction between IFITM1 and IFITM3. Structure-function analysis has shown that the C-terminal regulatory domain of IFITM1 is required for its ability to promote cell growth and to localise to the membrane.

Conclusion We have identified IFITM1 as an upstream regulator of the IRDS which promotes cancer cell survival and mediates chemoresistance. The C-terminal domain of IFITM1 is important for its proliferative activity in cancer and lay the foundation for future research aiming to determine IFITM1’s potential as a therapeutic target in cancer.

PO-171 IDENTIFYING IFITM1-DEPENDENT SYNTHETIZED PROTEINS IN INTERFERON GAMMA STIMULATED CELLS

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Introduction Interferon-induced transmembrane protein 1 (IFITM1) plays a dual role in restriction of RNA viruses and in metastatic cancer cell growth. IFITM1 expression has been extensively reported in many types of cancer and its high over-expression greatly correlates with tumour progression and leads to a poor outcome.

Interferons increase in response to a broad range of factors such as persistent viral infection or DNA damaging agents which activate the JAK kinase-STAT pathway. Ultimately, this signalling cascade will regulate the transcriptional synthesis of over 2000 interferon-stimulated genes (ISG). By contrast, the interferon resistance DNA-damage signature genes (IRDS), which is comprised of a subset of ISG, promote phenotypes that contribute to the tumour development such as resistance to DNA damage, metastasis, and EMT. IFITM1 is a pro-oncogenic receptor which is a component of the IRDS pathway.

Material and methods Affinity purification of isotopically labelled cells were analysed by mass spectrometry (MS). Isogenic cell panels were generated using CRISPR gRNAs. Validation of the protein-protein interactions were performed using PLA. Localization of IFITM1 to ribosomal protein and analysis of protein synthesis were analysed by MS.

Results and discussions How IFITM1 regulates oncogenic cell signalling or viral restriction is not mechanistically defined. A cytosolic association between IFITM1 and SRSF family of splicing factors was identified as possible dominant protein-interaction in interferon treated cells. SRSF1 isoform detected splicing factors was identified as possible dominant protein-interaction in interferon treated cells. SRSF1 isoform detected splicing factors was identified as possible dominant protein-interaction in interferon treated cells. SRSF1 isoform detected splicing factors was identified as possible dominant protein-interaction in interferon treated cells. SRSF1 isoform detected splicing factors was identified as possible dominant protein-interaction in interferon treated cells. SRSF1 isoform detected splicing factors was identified as possible dominant protein-interaction in interferon treated cells.

Our results are consistent with previous literature where STAT1 protein is mediated by interferon-dependent stimulus. We also detected new protein synthesis of IRF-1 and IN35 in IFITM1 independent mechanism. Strikingly, we specifically identified a small subset of IFITM1-dependent synthesized proteins upon interferon treatment. These signalling events that will be further investigated are important for anti-viral pathways as well as immune-cancer synapse.

Conclusion 1. The results suggested that IFITM1 modulates the ribosomal translation affecting the expression of certain target proteins.

2. We detected some stabilised proteins present in the IFN-pathway: STAT-1, IRF-1 and IN35.

3. Further analysis identified a regulatory effect on some target proteins modulated by IFITM1 as well as IFN-stimulation.

PO-172 ROLE OF LITHOCHOLIC ACID-INDUCED CELL SIGNALLING IN OESOPHAGEAL CANCER PROGRESSION MODEL

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Introduction A p53-checkpoint operates in the development of oesophageal adenocarcinoma (OAC) defined by prevalent TP53 gene mutations in patients with Barrett’s and high-grade dysplasia (HGD). The molecular genetic relationship between OAC and its precursor lesion, Barrett’s oesophagus, is poorly understood. The effective therapeutic targets should focus on early mutations of the disease, which become clonal in the later stage. Mutational context of some specific single nucleotide variants are common throughout the progression of the disease, suggesting exposure to common mutagens throughout the progression course.

Material and methods TP53 null or truncated cell lines were generated by targeting exon 4 of TP53 gene using CRISPR/Cas9 technology. Cell cycle analysis and Annexin V stained cells were analysed by FACS after treatment with lithocholic acid (LCA) to recapitulate the reflux of bile acids in OAC. Changes in proteins levels and signalling pathways upon LCA exposure were identified using mass spectrometry and validated with western blotting. Immunohistochemistry (IHC) analysis of OAC and normal tissues was performed to check the expression of identified bile acid-induced genes in tumours.

Results and discussions Oesophageal Barrett’s wt-p53 cell line CPA was used to define the effects of TP53 gene ablation on stress responses, cell survival, and mutation rates. FACS analysis revealed TP53 null cells sensitivity towards LCA via apoptotic pathway. Mass spectrometry analysis identified disrupted NDRG1 and TGFβ pathways. SMAD4 driver mutations have previously been reported exclusively to OAC, which provide a clear genetic boundary between OAC and HGD. Western blot analysis after LCA exposure showed increased levels of SMAD4-independent of p53 pathway. SMAD4 depletion in TP53 null cells stimulates cell-migration in the presence of inhibitory levels of LCA, corroborates the role of SMAD4 loss in metastasis. Loss of p53 also upregulates NDRG1 which increases migration in presence of LCA and was found to be a pro-invasive factor. IHC staining of OAC, lymph node and normal tissues showed heterogeneous expression of NDRG1 in tumour, mostly in membrane, suggesting its dynamic expansion on LCA exposure in vivo.
Conclusion The recurrent reflux of bile acids in Barrett’s cells, especially LCA, could sensitise cells to harbour further mutations. LCA induces p53-independent upregulation of SMAD4. Depletion of NDRG1 and SMAD4 in p53 null cells stimulates cell-migration in the presence of LCA, corroborating their role in metastasis.

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PO-174 ERBB2 GLYCOXYLATION LANDSCAPE IN GASTRIC CANCER CELLS – A NOVEL FUNCTIONAL TARGET?

Introduction Aberrant expression and hyperactivation of the human epidermal growth factor receptor 2 (Erbb2) constitute crucial molecular events underpinning gastric neoplastic transformation. Despite the extracellular domain of this cancer-relevant receptor tyrosine kinase (RTK) being a well-known target for extensive glycosylation, its detailed glycosylation profile and the molecular mechanisms through which it actively tunes ErbB2 towards malignancy in gastric cancer (GC) cells remain elusive.

Material and methods The expression of relevant glycosyltransferase-coding genes, and the expression and activation of the ErbB receptors were assessed in four GC cell lines. ErbB2-overexpressing NCI-N87 cells were selected for further glycan characterisation. ErbB2 was immunoprecipitated and validated by MALDI/TOF-TOF tandem mass spectrometry. Receptor’s glycosylation was confirmed by Peptide-N-Glycosidase F digestion and profiled with carbohydrate-binding lectins and monoclonal antibodies (mAbs). The expression of genes controlling the biosynthesis of cancer-associated glycans in association to ErbB2 status were studied. Expression and activation of ErbB2 were assessed in ErbB2-overexpressing cells submitted to in vitro deglycosylation and mAb-mediated glycan blocking. ErbB2-glycan in situ proximity ligation assay (PLA) was performed in tissue samples from ErbB2-positive GC patients.

Results and discussions Cellular- and receptor-specific glycan profiling of ErbB2-overexpressing NCI-N87 cells unveiled a heterogeneous glycosylation pattern harbouring the tumor-associated sialyl Lewis a (SLea) antigen. The expression of SLena and key enzymes of its biosynthetic pathway were strongly upregulated in this GC cell line. An association between the expression of ERBB2 and FUT3, a central gene in SLena biosynthesis, was additionally established in GC patients. Moreover, cellular deglycosylation and CA 19.9 antibody-mediated blocking of SLena drastically disrupted both receptor’s expression and activation in NCI-N87 cells. PLA staining disclosed ErbB2 as an in situ carrier of SLena in over 50% of the cases harbouring ErbB2 and SLena positive gastric carcinomas, further highlighting the crosstalk between ErbB2 and SLena expression.

Conclusion Our results show that the disclosed glycosylation profile of ErbB2 in GC cells has a major functional impact on receptor’s biology with potential clinical applications. Furthermore, NCI-N87 cell model constitutes an appealing in vitro system to study glycan-mediated regulation of ErbB2 in GC.

Introduction The homologous, ubiquitously expressed, members (Sos1 and Sos2) of the Sos family of RasGEFs participate in multiple signalling pathways but their specific cellular functions are not clearly defined yet.

Material and methods Using a tamoxifen (4OHT)-inducible, conditional Sos1 null mutation, here we generated and analysed wild type (WT), single Sos1-KO, constitutive Sos2-KO and double Sos1/2-DKO primary mouse embryonic fibroblasts (MEFs) in an effort to ascertain the functional specificity or redundancy of Sos1 and Sos2 at the cellular level.

Results and discussions Sos1-KO and Sos1/2-DKO MEFs exhibited distinct flat morphology, enlarged cell perimeter and altered cytoskeletal organisation that were not observed in WT and Sos2 KO counterparts. Sos1-KO and Sos1/2-DKO MEFs also displayed significant accumulation of cytoplasmic mitochondria by means of electron microscopy and specific markers. Consistent with a mitophagic phenotype, in vivo labelling using specific fluorophores revealed increased levels of mitochondrial oxidative stress in the Sos1-KO and the Sos1/2-DKO cells as compared to Sos2-KO or WT MEFs.

Treatment of the MEF cultures with antioxidants such as GSH and NAC corrected the altered perimeter size and proliferative rate of Sos1-KO and Sos1/2-DKO MEFs to levels similar to those of WT and Sos2-KO, but not recover oxidative stress. Furthermore, treatment with the specific mitochondrial superoxide scavenger mitoTEMPO recovered endogenous redox-homeostasis in Sos1-KO and Sos1/2-DKO to normal levels.

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