Receptors and Signal Transduction

PO-168 IKBKE REGULATES TSC1 FOR THE ACTIVATION OF MTOR/S6K PATHWAY IN TUMOUR CELLS

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Introduction IKBKE (IKKε) has emerged as an oncogenic protein which has diverse substrate selectivity in tumorigenic pathways in multiple malignancies. mTOR signalling is one potential signalling pathway that affects the tumour development and angiogenesis. Constitutive activation of mTOR signalling is required for protein synthesis upon stressful conditions including cancer. In the upstream PI3K/AKT may phospho-regulate antagonistic TSC1/TSC2 complex which otherwise inhibits mTOR/S6K activation.

Material and methods By using IKKε knockdown in various cancer cell lines, we have observed a unique phenotype of reduction in S6K phosphorylation and TSC1 stabilisation. Then we addressed TSC1 and IKKε interaction by co-immunoprecipitation and finally we performed in vitro kinase assay to show unique phoso-regulation of TSC1 by IKKε.

Results and discussions Previous studies has shown PI3K/AKT signalling, which potentially regulates angiogenic mTOR/S6K signalling cascades, are directly regulated by IKBKE phosphorylation. However, here we propose a new control mechanism by the IKBKE, which directly interacts and phosphorylates TSC1 to restore mTOR/S6K activiation and proliferation in cancer cells.

Conclusion These findings collectively suggest that IKKε may provide an alternative regulatory circuit for mTOR/S6K activation. Therefore, IKKε regulatory network in cancer cells may be more elaborate than previously thought and these results point out that IKBKE can be more than a potential target for cancer therapy.

PO-169 INVOLVEMENT OF NEUROTENSIN AND NEUROTROPHINS PATHWAYS IN HUMAN COLORECTAL CANCER CELLS 5-FLUOROURACIL TREATMENT RESISTANCE

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Introduction Colorectal cancer (CRC) is one of the leading causes of cancer-related deaths. Surgical resection is the only curative treatment. Depending on disease stage, chemotherapies and targeted therapies are available. Despite considerable progress, recidive cases remain frequent. Studies lead in our laboratory on different cancer models, such as CRC, highlighted the importance of neuropeptides in cancer cells survival and progression: neurotrophines (NT) and neurotensin (NTS). NT is a family of four growth factors, BDNF, NT4/5, NT3 and NGF respectively binding a specific Tropomyosin Receptor Kinase (Trk A, B or C), tyrosin kinase receptors, with a strong affinity. NT can all bind another receptor, p75NTR, with low affinity, as well as the Sortilin, a TRK co-receptor, a NT and NTS intracellular trafficking regulator and also known as Neurotensin Receptor 3 (NTR3). Neurotensin can also bind two other receptors: NTR1 with strong affinity and 2 with low affinity; both are G protein coupled receptors. Very few is known about NT and NTS pathways in CRC cells resistance to chemotherapies, 5-Fluorouracil (5FU) being the backbone of any chemotreatments. The aim of my project is to understand if and how these pathways could be involved in 5FU CRC cell resistance and survival.

Material and methods Two human CRC cell lines from different disease stages (WiDr, SW620) were treated with 8 µM of 5FU to obtain stably 5FU resistant cell lines. Both cell lines were also xenografted in Nude mice which were treated with 5FU. Protein expressions were assessed by western blot. Cell activation was assessed by flow cytometry. Stable knockdown cell lines (NTR3) were obtained by shRNA transfection. Exosomes were purified from culture supernatants by ultracentrifugation.

Results and discussions Preliminary results show that NTSR3 protein expression is increased after 5FU treatment both in whole cell lysates and exosomes especially in the most agressive cell line (SW620). The same results were obtained in vitro and in vivo. Moreover, 5FU treatment induces a decrease of tumour size only for the least agressive line (WiDr). Indeed, the 5FU induces a quiescence state of these cells.

Conclusion It is the first time that, in CRC, the NTSR3 seems to be overexpressed in 5FU-resistant tumour cells (from primary and advanced stages), in vitro and in vivo. This receptor could constitute a new potential therapeutic target.

PO-170 DISSECTING THE ROLE OF IFITM1 IN RESPONSE TO ANTICANCER TREATMENT

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Introduction Constitutive expression of interferons (IFNs) and their downstream signalling pathways play a critical role in host responses to cell transformation in the tumour microenvironment. Induction of IFNs initiates the transcription of a variety of genes, so called IFN- stimulated genes (ISGs). Although expression of ISGs is classically associated with tumour suppression, a subset defined as the IRDS (Interferon - Related DNA- Damage Resistance Signature), is elevated in response to endogenous IFN, self-DNA and RNA exposure in the tumour microenvironment acquiring radiation and chemotherapy resistance. INF induced transmembrane receptor (IFITM1) is thought to participate in proliferating signalling and oncogenesis. Three out of five IFITM genes (IFITM1,2,3) share high amino-acid homology, however, IFITM1 has a unique C-terminal domain, a 13 amino-acid extension, and a shorter N-terminal amino-acid sequence.

Material and methods To analyse the impact of IFITM1 loss, or mutation, on signalling and phenotypic events such as growth, viability and drug resistance, knock – out, knock – in and domain mutants were generated using Crisp/Cas9 mediated gene – editing. To identify the effects of radiation in the growth of wild type and mutant IFITM1 cells, proliferation assays were conducted while immunoassays were used to check for activation of the IRDS pathway. The gene edited cell lines were used in Mass Spectrometry proteomic approaches to discover IFITM1 interacting proteins under
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 chemotherapeutic 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 localize 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 overexpression 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 (ISGs). 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). Isoformic 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 MS.

**Results and discussions** How IFITM1 regulates oncogenic cell signalling or viral restriction is not mechanistically defined. A cytosolic association between IFITM1 and SRSP family of splicing factors was identified as possible dominant protein-interaction in interferon treated cells. SRSP1 isoform detected is associated to be in the cytoplasm. As such, we focused on understanding whether IFITM1 is required for protein synthesis in response to interferon signalling.

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 synthetized 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 stabilized 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.