NEUROTENSIN RECEPTOR TYPE 2 PROTECTS B-CELL CHRONIC LYMPHOCYTIC LEUKAEMIA CELLS FROM APOPTOSIS

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Abstracts

SPOT-008

Introduction B-cell chronic lymphocytic leukaemia (B-CLL) cells are resistant to apoptosis, and consequently accumulate to the detriment of normal B cells and patient immunity. Because current therapies fail to eradicate these apoptosis-resistant cells, it is essential to identify alternative survival pathways as novel targets for anticancer therapies.

Material and methods B-cells were isolated from the peripheral blood of healthy donors and CLL patients (n=34) using the MACSxpress human B-CLL Cell Isolation Kit. Gene expression was assessed by RT-qPCR, protein expression and signaling pathway activation was assessed by Western Blot. Protein–protein interaction was confirmed by both Indirect Immunofluorescence and co-immunoprecipitations. Cell death was assessed by assayed Cell Death as well as Flow Cytometry. Studied receptors were inhibited either by RNA interference or pharmacological inhibitors.

Results and discussions In this study, we identified neurotensin receptor 2 (NTSR2) as an essential driver of apoptosis resistance in B-CLL. NTSR2 was highly expressed in B-CLL cells, whereas expression of its natural ligand, neurotensin (NTS), was minimal in both B-CLL cells and patient plasma. Surprisingly, NTSR2 remained in a constitutively active phosphorylated state, caused not by a mutation-induced gain-of-function but rather by an interaction with the oncogenic tyrosine kinase receptor TrkB. Functional and biochemical characterisation revealed that the NTSR2–TrkB interaction acts as a conditional oncogenic driver requiring the TrkB ligand BDNF, which unlike NTS is highly expressed in B-CLL cells. Together, NTSR2, TrkB and BDNF induce autocrine and/or paracrine survival pathways that are independent of mutation status and indolent or progressive disease course. The NTSR2–TrkB interaction activates survival signalling pathways, including the Src and AKT kinase pathways, as well as expression of the anti-apoptotic proteins Bcl-2 and Bcl-xL. When NTSR2 was downregulated, TrkB failed to protect B-CLL cells from a drastic decrease in viability via typical apoptotic cell death.

Conclusion Together, our findings demonstrate that the NTSR2–TrkB interaction and the sustained activation of the signalling pathways under the control of these two actors plays a crucial role in B-CLL cell survival, suggesting that inhibition of NTSR2 represents a promising targeted strategy for treating B-CLL malignancy.

SPOT-009

IDENTIFICATION OF NOVEL HIPPO PATHWAY REGULATORS USING A GENOME WIDE CRISPR SCREEN

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Introduction The Hippo pathway regulates tissue growth and organ size and its dysregulation/inactivation leads to a host of cancers such as breast, colon and bladder cancer. YAP/TAZ are transcriptional regulators which among other functions enhance cell growth. The core kinase cassette consisting of LATS1/2, MST1/2, SAV and Mob phosphorylate YAP and TAZ promoting their cytoplasmic localization and degradation. Currently much remains to be learned about the upstream regulators that modulate the pathway. The purpose of this study was to carry out a genome wide positive selection CRISPR screen to uncover novel upstream regulators of the Hippo pathway. For the screen it is necessary to generate stable cell lines with a reporter responsive to YAP/TAZ. This was carried out by expressing a fusion protein where the catalytic domain of Caspase-9 is fused to a modified FKBP (FK506 binding domain) under the transcriptional control of YAP/TAZ. This protein is homodimerized by the addition of a drug AP20187 which triggers an apoptotic pathway leading to cell death. When YAP/TAZ activity is disrupted, cells are expected to survive, despite addition of AP20187. The cell lines are also designed to express Cas9 which will be used to knockout the genes in the human genome using a 90K guide RNA library targeting over 17,500 human genes.

Material and methods T24 bladder cancer cells were maintained in McCoy’s 5A medium with 10% FBS. Lentiviral constructs of Cas9 and FKBP-Caspase9 were obtained (courtesy Dr. Angers) and the YAP/TAZ responsive FKBP-Caspase9 was constructed by restriction digestion and ligation. Using lentivirus T24 cells were transduced with both constructs and single clones were isolated expressing both FKBP-Caspase9 and Cas9 to obtain the screen cell line. The dimerizer AP20187 was obtained from Clontech. The lentiviral library TKO-v1 (gift from Dr. Jason Moffat) was applied to the T24 screen cell line using lentiviral transduction. After 3 days of puromycin selection, the pool of surviving cells were split into replicates and AP20187 was added to one set of replicates while the other set was treated with ethanol as control. Once the AP20187 treated plates were confluent, the cells were collected, genomic DNA was extracted and sequenced by next generation sequencing.

Results and discussions The top genes enriched in the surviving cells included well known members of the Hippo pathway including YAP and TAZ along with new proteins.

Conclusion These new proteins are currently being investigated for their role in the Hippo pathway.

SPOT-010

A ROLE FOR P53 IN THE ADAPTATION TO GLUTAMINE STARVATION THROUGH THE EXPRESSION OF SLC1A3

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Introduction Cancer cells are frequently exposed to nutrient and oxygen limited environments, resulting from poor vascularity in the developing tumour mass, and there is a growing interest in understanding the metabolic plasticity that supports cancer cell survival and proliferation under these conditions. Glutamine is the most abundant amino acid in serum, and glutamine levels are often severely depleted in
developing cancers. Successful tumour development is therefore likely to depend on the ability of tumour cells to withstand glutamine depletion. Analysis of a RNA sequencing experiment comparing the transcriptional profile of cancer cells grown in medium containing all amino acids, or without glutamine revealed that the top pathway induced by glutamine starvation in these cells was p53. We thus asked if the tumour suppressor p53 could play a role in the adaptation of cancer cells to glutamine deprivation.

**Material and methods** We carried out an extensive metabolomics analysis of p53-null HCT116 colorectal cancer cell lines and their corresponding controls exposed to glutamine-free medium and completed this analysis with different technics of cell biology.

**Results and discussions** We showed that p53 was transiently induced upon glutamine withdrawal and that p53-null HCT116 lines failed to proliferate and showed decreased viability under glutamine starvation. This pro-survival role of p53 correlates with its ability to maintain TCA cycle activity and mitochondrial respiration, promoting *de novo* glutamate and glutamine synthesis to improve cell viability and proliferation. Mechanistically, p53 appears to mediate this effect by supporting aspartate utilisation through the induction of the glutamate/aspartate transporter Scl1a3. Interestingly, this transporter is induced under glutamine withdrawal in a wide range of cancer cell lines and strongly supports their ability to grow in glutamine-free condition.

**Conclusion** This study reveals a new role for the duo p53 and its target Scl1a3 in helping cancer cells to survive glutamine deprivation, highlighting a new metabolic vulnerability of cancer cells exposed to glutamine limitation that might be targeted for therapy.

**SPOT-011 STUDYING THE IMPLICATION OF THE RAS/MAPK AND PI3K/AKT/TOR PATHWAYS IN PROSTATE CANCER THROUGH AN IN VIVO MODEL OF DROSOPHILA ACCESSORY GLANDS TUMORIGENESIS**

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**Introduction** Clinical studies have demonstrated that, in prostate cancer, Ras/MAPK and PI3K/PIK3CA/mTOR signalling pathways are often aberrantly co-activated in tumours, their activation levels increasing again in resistance phases. As these pathways are important for many fundamental processes (proliferation, cell growth or cell cycle), they are likely implicated in prostate tumorigenesis. However, the relative implication of each of these two pathways during prostate tumorigenesis, especially during the early phases, is not fully understood. Thus, we want to define the molecular mechanisms that are implicated in the co-deregulation of these pathways, and their possible implication in prostate cancer initiation and progression.

**Material and methods** We propose and alternative *in vivo* model of prostate tumorigenesis in drosophila, where accessory glands are a functional equivalent of the human prostate. In this model, we can cumulate molecular modifications for both the considered pathways, and so study the impact of one on tumorigenesis.

**Results and discussions** Our results show that only the hyper-activation of the Ras/MAPK pathway in accessory glands can promote tumorigenesis, with production of cell masses that recapitulate many cancer hallmarks including uncontrolled cell growth and proliferation, enhanced matrix metalloproteinases expression, loss of expression of epithelial markers, neovascularisation-like tractogenesis. However, both pathways are necessary to tumorigenesis, but they display different roles: the Ras/MAPK pathway is activated earlier and is able to recruit the PI3K/AKT/TOR pathway. Furthermore, we have shown that tumour formation in the accessory glands relies on an overproduction of Epidermal Growth Factor, EGF, which is known to be expressed at abnormally high levels in prostate cancer.

**Conclusion** We have developed a *Drosophila* model that displays many phenomena that are described in human prostate tumorigenesis. We now want to decipher which molecular mechanisms are implicated in the PI3K/AKT/TOR pathway activation. Then, we want to evaluate the pertinence of our findings in the human pathology, by the use of human biopsy samples: our aim is to transfer our results to new therapeutic applications, as the discovery of new mechanisms could allow definition of new biomarkers and eventually of new therapeutic targets.

**SPOT-012 LARGE-SCALE CRISPR SCREENING TO IDENTIFY ACTIONABLE CANCER DRUG TARGETS**

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**Introduction** In order to identify new putative oncology drug targets, we have developed a whole-genome CRISPR drop-out screening pipeline.

**Material and methods** The CRISPR drop-out library used in this pipeline is composed of ~19 000 genes (5 or 10 guides/gene). To facilitate interpretation of data generated by this pipeline, all cell lines have been extensively characterised by whole-exome sequencing, SNP6 copy number arrays, RNA-sequencing and drug sensitivity testing. A challenge of interpreting CRISPR drop-out data, is the high false-positive rates in detecting essential genes, particularly for those that are within copy number amplified regions of the genome. We have developed a computational tool, CRISPRRelcateR, which is capable of identifying and correcting gene-independent responses to CRISPR-Cas9 targeting.

**Results and discussions** We have identified >700 core pan-cancer essential genes using an adaptive computational method. These genes are members of a *priori* known essential gene sets, as well as being involved in essential biological processes such as cell cycle, DNA synthesis and DNA replication. Methods for associating gene essentiality with genomic features have been developed to understand cellular mechanisms underpinning differential gene essentiality and to identify potential biomarkers for patient stratification. Known dependencies have been identified e.g. PIK3CA is essential in PIK3CA mutant breast cancer cell lines, as well as promising novel associations. Identified associations are incorporated into a weighted prioritisation scoring system integrating clinical, experimental