Conclusion AKT protein is a prospectively valuable recurrence predictive biomarker of epithelial ovarian cancer.

**PO-111**

CAPICUA SUPPRESSES HEPATOCELLULAR CARCINOMA PROGRESSION BY CONTROLLING ETV4-MMP1 AXIS

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**Introduction** Hepatocellular carcinoma (HCC) is developed by multiple steps accompanying progressive alterations of gene expression, which leads to increased cell proliferation and malignancy. Although environmental factors and intracellular signalling pathways that are critical for HCC progression have been identified, gene expression changes and the related genetic factors contributing to HCC pathogenesis are still insufficiently understood. In this study, we identify a transcriptional repressor Capicua/CIC as a suppressor of HCC progression and a potential therapeutic target.

**Material and methods** We used human HCC patients samples, tissue microarray, and TCGA database to check CIC levels between normal and HCC patients. We used various HCC cell lines to check cell proliferation, migration, and invasion activity by using CIC knockdown, CIC overexpression, or ETV4 knockdown cells and so on. Also we used two different mouse models, Xenograft and liver specific CIC knockout mice to evaluate tumour progression, metastasis, or survival.

**Results and discussions** Expression of CIC is posttranscriptionally reduced in HCC cells. CIC levels are correlated with survival rates in patients with HCC. CIC overexpression suppresses HCC cell proliferation and invasion, whereas loss of CIC exerts opposite effects in vivo as well as in vitro. Levels of polyoma enhancer activator 3 (PEA3) group genes, the best-known CIC target genes, are correlated with lethality in patients with HCC. Among the PEA3 group genes, ETS translocation variant 4 (ETV4) is the most significantly up-regulated in CIC-deficient HCC cells, consequently promoting HCC progression. Furthermore, it induces expression of matrix metalloproteinase 1 (MMP1), the MMP gene highly relevant to HCC progression, in HCC cells; and knockdown of MMP1 completely blocks the CIC deficiency–induced HCC cell proliferation and invasion.

**Conclusion** Our study demonstrates that the CIC–ETV4–MMP1 axis is a novel regulatory module controlling HCC progression.

**PO-112**

THE ROLE OF TRANSCRIBED ULTRACONSERVED REGIONS UC160 AND UC346 IN COLORECTAL CANCER PROGRESSION

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**Introduction** Expression of Transcribed Ultra Conserved Regions (Transcribed Ultra Conserved Regions, T-UCRs) is often deregulated in many types of cancer, including colorectal cancer (CRC). Our previous results showed that T-UCRs Uc160 and Uc346 are methylated in CRC. Additionally, their tumour methylation is associated with time to disease progression (TTP) and appears to be a promising biomarker for CRC. However, their role in CRC progression has not been elucidated to date.

**Material and methods** Aim of the study was to investigate the role of Uc160 and Uc346 in proliferation, motility and migration in colon cancer cells. For that purpose, Uc160 and Uc346 were cloned into plasmids and three colon cancer cell lines (HT-29, Caco-2 and DLD-1) were transiently transfected. After overexpression of Uc160 and Uc346, proliferation (MTT assay), motility (scratch wound healing assay) and migration (transwell migration assay) rates were evaluated.

**Results and discussions** Proliferation rates, 48 hour after overexpression, were higher in the transfected cells in all cell lines, compared with the control cells (mock transfected). The most significant differences in proliferation rates were noticed for Uc160 overexpression in Caco-2 (p=0.008) and Uc346 overexpression in DLD-1 cells (p=0.033). Similar results were observed in motility assay, with cells overexpressing Uc160 or Uc346 having higher motility rates compared to control cells in all cell lines. More specifically, most significant differences in motility rates were observed in HT-29 and DLD-1 cells overexpressing Uc160 or Uc346 (p=0.017, p=0.041 and p=0.023, p=0.004 respectively). Further analysis of DLD-1 cells migration confirmed the above results, with higher number of Uc160 or Uc346 overexpressing cells migrating compared to the control cells (p=0.005 for both T-UCRs).

**Conclusion** T-UCRs Uc160 and Uc346 appear to affect the proliferation, motility and migration rates of colon cancer cells, implicating a complex role in CRC progression.
approach to discover the upstream modulators of IRF4 expression in melanoma cells. In parallel, we have performed localization (ChIP-seq) and transcriptomic (RNA-seq) assays in order to identify the genome-wide targets of IRF4 in melanoma cell lines.

Results and discussions As upstream regulators of IRF4 expression, we identified a known melanoma master transcription factor and a major signalling pathway with therapeutic targeting options. For downstream targets of IRF4, together with The Cancer Genome Atlas (TCGA) data analyses, our data point to a role of IRF4 in epigenetic regulation of melanoma cells, among other cancer- and development-related pathways. In addition, our preliminary studies implicate IRF4 as a critical factor in melanoma cell proliferation and survival.

Conclusion Overall these studies on IRF4 in melanoma cells are complementing mechanistically the available genetic findings in melanoma patient samples, and describing a novel pathway in melanoma cells, with the potential to point to new therapy approaches for melanoma.

Introduction T-ALL is a malignancy characterised by aberrant Notch signalling, sustained by activating mutations in Notch1 as well as over-expression of Notch3, a Notch paralog physiologically subjected to lysosome-dependent degradation in human cancer cells. Given some limitations of existing drugs blocking Notch signalling, it is important to get new insights into the biology of Notch3 to further stimulate the development of Notch-targeted therapies in cancer.

Material and methods T-ALL cell lines and cells obtained from patient-derived xenografts (PDX) were treated in vitro with HDAC inhibitors and other drugs or HDAC6-specific shRNA and effects on Notch expression and activity were investigated by standard methods. Confocal microscopy was used for intracellular localization studies. PDX models were also utilised to investigate effects of HDAC6 silencing on leukaemia growth.

Results and discussions We initially found that treatment with the pan-HDAC inhibitor Trichostatin A (TSA) strongly decreases Notch3 full-length protein levels in T-ALL cell lines and primary human T-ALL cells xenografted in mice without substantially reducing NOTCH3 mRNA levels. Moreover, TSA markedly reduced the levels of Notch target genes, including pTa, CR2 and DTX-1, and induced apoptosis of T-ALL cells. We further observed that Notch3 was post-transcriptionally regulated following TSA treatment, with reduced Notch3 surface levels and increased accumulation of Notch3 protein in the lysosomal compartment. Surface Notch3 levels were rescued by inhibition of Dynemin with Cilobrevin D. Pharmacologic studies with HDAC1, 6 and 8-specific inhibitors disclosed that these effects were largely due to inhibition of HDAC6 in T-ALL cells. HDAC6 silencing by specific shRNA was followed by reduced Notch3 expression and increased apoptosis of T-ALL cells. Finally, HDAC6 silencing impaired leukaemia outgrowth in mice, associated with reduction of Notch3 full-length protein in vivo.

Conclusion These results connect HDAC6 activity to regulation of total and surface Notch3 levels by a mechanism involving endosomal sorting and suggest HDAC6 as potential novel therapeutic target to lower Notch signalling in T-ALL and other Notch3-addicted tumours.

PO-115 HER2-AMPLIFIED TUMOURS OVERCOME THE REQUIREMENT FOR HER3

Introduction Amplification and overexpression of HER2 underlies the pathogenesis of a large subset of cancers including more than 20% of breast cancers. A considerable body of experimental evidence strongly suggests that HER2 is the oncogenic driver of these cancers, important in the genesis and progression of these tumours. Current evidence suggests that HER2 driven tumorigenesis requires HER3, the preferred heterodimerization partner of HER2. This is felt to be due to the unique ability of HER3 to activate PI3K/Akt pathway signalling, not directly accessible to HER2. We have been interested in developing deeper insights into the role of HER3 in HER2-amplified cancers. In particular, we have been interested in understanding why HER3 is required, whether the requirement is absolute or perhaps conditional or transient.

Material and methods Using CRISPR-Cas9 technology, we knocked-out HER3 (HER3KO) in HER2-amplified HCC1569 cells and assessed the transforming and tumorigenic properties of HER3KO cells in vitro and in vivo. Co-immunoprecipitation assays in HER3-KO cells and stable doxycycline-inducible HER3 shRNA cell lines were performed to detect the binding of HER2 to the regulatory subunit of PI3K through direct and indirect mechanisms. Site-directed mutagenesis in the C-terminal of HER2 was used to identify the tyrosine involved in this interaction.

Results and discussions By genetic elimination of HER3 or shRNA knockdown of HER3 in HER2-amplified cancer cells we find residual HER2-driven activation of PI3K/Akt pathway signalling that is driven by HER2 through direct and indirect mechanisms. Indirect mechanisms involve second messenger pathways including Ras or Grb2. Direct PI3K binding occurs through Y1139 which has a weak affinity for PI3K that becomes significant at very high expression and phosphorylation. Mutation of Y1139 impairs the tumorigenic competency of HER2. The total elimination of HER3 expression in HER2-amplified cancer cells significantly impairs tumorigenicity, but the impairment is transient and overcome with further increases in HER2 expression and phosphorylation with binding and activation of PI3K. Effective treatment of these cancers must focus on much more potent inhibitors of HER2.

Conclusion This study shows for first time the intrinsic ability of HER2 to activate PI3K that becomes more relevant with increased HER2 expression, and can supplant the requirement for HER3 in the growth of HER2-amplified cancers.