expression was performed using SPSS. The Broad institute Morpheus tool was used for hierarchical cluster analysis of the TCGA RNA-Seq data to identify associations of lncRNA expression with UC molecular subtypes.

Results and discussions Consistent with the TCGA RNA-Seq data, qRT-PCR analysis of our large tissue set revealed both TINCR and DANCRI to be upregulated in UC and in B-SCC compared to benign tissues. According to the qRT-PCR data, DANCRI expression was significantly elevated in non-invasive over invasive tumours. Kaplan-Meier analysis did not reveal associations of patient outcome with upregulated lncRNA expression, except that high TINCR expression was associated with worse metastasis-free survival. Hierarchical cluster analysis indicated that the BASQ subtype, which is defined by low expression of luminal marker genes (FOXA1, GATA3) and high expression of basal and squamous marker genes (KRT5, KRT6, KRT14), is also characterised by intermediate TINCR expression.

Conclusion Both TINCR and DANCRI expression are frequently upregulated in UC, but are not strongly associated with clinical parameters. Instead, our data support the emerging consensus that specific lncRNA expression patterns are associated with and may contribute to the characteristics of UC molecular subtypes.

PO-367 DFNA5 METHYLATION: A POTENTIAL BIOMARKER FOR BREAST CANCER, ON THE BASIS OF A LARGE SCALE ANALYSIS IN TCGA

Introduction Breast cancer is the most frequent cancer among women worldwide. Biomarkers for early detection and prognosis of these patients are needed. We hypothesised that DFNA5 (also known as Gasdermin E (GSDME)) may be a valuable biomarker, based upon strong indications for its role as tumour suppressor gene and its function in regulated cell death. In this study, we aimed to analyse DFNA5 methylation and expression in the largest breast cancer cohort to date using publicly available data from TCGA, in order to further unravel the role of DFNA5 as detection and/or prognostic marker in breast cancer.

Material and methods We analysed Infinium HumanMethylation450k data, covering 22 different CpGs in the DFNA5 gene (668 breast adenocarcinomas and 85 normal breast samples) and DFNA5 expression (Agilent 244K Custom Gene Expression: 476 breast adenocarcinomas and 56 normal breast samples; RNA-seq: 666 breast adenocarcinomas and 71 normal breast samples).

Results and discussions DFNA5 methylation and expression were significantly different between breast cancer and normal breast samples. Overall, breast cancer samples showed higher DFNA5 methylation in the putative gene promoter compared to normal breast samples, whereas in the gene body and upstream of the putative gene promoter the opposite is true. Furthermore, DFNA5 methylation, in 10 out of 22 CpGs, and expression was significantly higher in lobular compared to ductal breast cancers. An important result of this study was the identification of a combination of one CpG in the gene promoter (CpG07504598) and one CpG in the gene body (CpG12922093) of DFNA5, that was able to discriminate between breast cancer and normal breast samples (AUC=0.93). This model was externally validated in three independent datasets. Moreover, we showed that oestrogen receptor state is associated with DFNA5 methylation and expression. Finally, we were able to find a significant effect of DFNA5 gene body methylation on 5 year overall survival time.

Conclusion We conclude that DFNA5 methylation shows strong potential as detection and prognostic biomarker for breast cancer.

PO-368 EPIGENETIC REGULATION OF GLYCOSYLATION AND THE IMPACT ON CHEMORESISTANCE IN OVARIAN AND BREAST CANCER

Introduction Glycosylation is epigenetically regulated and is a fundamental post-translational modification altered in cancer. These alterations impact on tumour progression, and promote tumour survival. In the literature, there is a clear link between chemoresistance and hypoxia, hypoxia and epigenetics and more recently glycosylation and epigenetics. Our remit is to bring these paradigms together, to open up new avenues of approach for the detection, diagnosis and treatment of ovarian and breast cancer.

Material and methods Ovarian and breast cancer cells were treated with the DNA methyltransferase inhibitor, 5-AZA-2-deoxycytidine (5-AZA-dC). Cells were exposed to normoxia and differential hypoxic conditions. Methylation status of these alterations impact on tumour progression, and promote tumour survival. In the literature, there is a clear link between chemoresistance and hypoxia, hypoxia and epigenetics and more recently glycosylation and epigenetics. Our remit is to bring these paradigms together, to open up new avenues of approach for the detection, diagnosis and treatment of ovarian and breast cancer.

Material and methods Ovarian and breast cancer cells were treated with the DNA methyltransferase inhibitor, 5-AZA-2-deoxycytidine (5-AZA-dC). Cells were exposed to normoxia and differential hypoxic conditions. Methylation status of glycosylation and epigenetics and more recently glycosylation and epigenetics. Our remit is to bring these paradigms together, to open up new avenues of approach for the detection, diagnosis and treatment of ovarian and breast cancer.

Results and discussions Branching and sialylation known to aid in tumour survival, were increased on secreted N-glycans from chemoresistant cells compared to chemosensitive cells following treatment with 5-AZA-dC and in all cells under hypoxic conditions. These changes correlated with increases in MGAT5 and ST3GAL4 expression in demethylated ovarian cancer cells. GATA2/3 were identified in-silico, as possible TFs for these genes. Results show that there is a correlation between, ST3GAL4 and GATA2 and MGAT5 and GATA3, respectively. 5-AZA-dC-treated and hypoxia-exposed cells displayed increased migration, with a greater effect in chemosensitive
demethylated- and 0.5% hypoxia-exposed cells compared to chemoresistant cells. Apoptotic and senescence markers were increased in 5-AZA-dC treated cells.

Conclusion These results give insight into the effects epigenetic alterations have on cancer cell glycosylation and how these may impact on the overall fate of those cells. The GATA2/3 TFs are linked to cancer stage, increased invasiveness and are possible therapeutic targets. Our data show a correlation between GATA2/3 and the levels of glycosyltransferases involved in branching and sialylation which are involved in cancer cell survival and metastases.

PO-369 KDM3A/ETS1/MCAM AXIS IN RHABDOMYOSARCOMA AND OSTEOSARCOMA

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Introduction Rhabdomyosarcoma (RMS) and Osteosarcoma (OS) are the most common primary malignant tumours of soft tissue and bones, respectively, affecting children, adolescents and young adults. RMS can be divided into two major histopathological and molecular subtypes: driver oncofusion (PAX3/7-FOXO1) positive, alveolar (ARMS), and oncofusion-negative embryonal (ERMS). Oncofusion positive ARMS carries a worse prognosis, in part due to higher propensity for metastasis. OS is also an aggressive disease with high propensity for metastasis. Patients with metastatic RMS and OS have few therapeutic options, including currently no effective targeted therapies, and typically face poor outcomes. Our laboratory has previously identified a novel, potentially druggable, tumour and metastasis-promotional, axis in Ewing Sarcoma, another aggressive bone and soft tissue cancer of childhood, involving the histone demethylase KDM3A, the Ets1 transcription factor, and the cell adhesion protein MCAM. We have additionally observed KDM3A to be overexpressed in RMS, and in a subset of OS. The aim of this study was to examine whether the KDM3A/Ets1/MCAM axis is intact in RMS and OS, and what impact it has on the biology of these diseases.

Material and methods RMS and OS cell lines were analysed for KDM3A, Ets1 and MCAM expression, and selected cell lines were subjected to stable shRNA-mediated KDM3A, Ets1 and MCAM knockdown (KD). Phenotypic effects of knockdown were analysed by clonogenic and transendothelial invasion assays. Preliminary tumour xenograft studies were also performed.

Results and discussions KDM3A is uniformly overexpressed in ERMS and ARMS cell lines and a subset of OS cell lines. KDM3A KD inhibits colony formation and transendothelial invasion in ERMS and ARMS cells and a high-expressing OS cell line. In preliminary studies KDM3A KD also inhibits ARMS tumorigenesis in an orthotopic model. Strikingly, similar to our prior studies in Ewing Sarcoma, a KDM3A/Ets1/MCAM regulatory axis is intact in RMS and OS, and MCAM KD exerts similar phenotypic effects to those achieved with KDM3A KD.

Conclusion Our studies indicate that a KDM3A/Ets1/MCAM molecular axis is intact in RMS and OS, and regulates growth and invasive properties. Together with our previous studies in Ewing Sarcoma, these findings suggest that this may be an important tumour and metastasis promotional axis in all three common paediatric sarcomas, despite distinct cellular origins of these cancers.

PO-370 SUSTAINED OVER-EXPRESSION OF MIR-26A INDUCES CHROMOSOME INSTABILITY AND TUMORIGENESIS BY DYSREGULATION OF CHFR EXPRESSION

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Introduction MicroRNAs are small RNA molecules capable of inhibiting gene expression. Due to their ability to act as either tumour suppressors or oncogenes, miRNAs have been shown to play a vital role in cancer and metastasis. One such putative tumour suppressor is miR-26a. It has been shown previously that miR-26a reduces cell viability in several cancers, indicating that miR-26a could be used as a therapeutic option in patients. We decided to look at the long-term effects of miR-26a overexpression to draw conclusions about the feasibility of its use in therapy.

Material and methods We used a number of different breast cancer cell lines (MCF-7, MDA-MB-231, SK-BR-3, T-47D, ZR-75-1) to assess the effect of sustained overexpression of miR-26a on a number of cellular processes. Immunofluorescence experiment using DAPI, γ-tubulin and α-tubulin antibody was performed to assess nuclear and mitotic spindle morphology in cells overexpressing miR-26a. Metaphase chromosome spreads were used to investigate the effect of miR-26a on cellular ploidy. Luciferase assay was performed to show direct targeting of 3’ UTRs of CHFR, LARP1, MCL1 and YWHAE by miR-26a. A rescue experiment was performed by overexpression of each one of the targets in the presence and absence of exogenous miR-26a, followed by clonogenic assay as well as immunostaining to assess which putative target protein mediates the observed phenotype.

Results and discussions We demonstrated that miR-26a not only inhibits G1/S cell cycle transition and induces apoptosis in cancer cells, as shown previously, but it also plays a role in regulation of other cell-cycle checkpoints. We prove that sustained miR-26a expression in breast cancer cell line models as well as MEF cells, leads to formation of oversized cells with either a single large nucleus or two or more nuclei, implying aberrant mitosis and cytokinesis. Moreover, we have shown that miR-26a over-expression causes aneuploidy and centrosome defects, increasing the chances of tumorigenesis. We show that on a mechanistic level, it acts by targets G1-S transition genes as well as genes controlling mitosis and cytokinesis, such as CHFR, LARP1 and YWHAE. Importantly, we prove that re-expression of only CHFR, partially rescues normal mitosis and impairs tumorigenesis in miR-26a over-expressing cell indicating that CHFR is an important miR-26a target mediating regulation of the observed phenotypes.

Conclusion We propose that miR-26a delivery might not be a viable therapeutic strategy because of potential deleterious oncogenic activity.