is a new and recently developed non-invasive cancer diagnostic technique. This technique includes collection of blood or urine sample and diagnosis of cancer based on analysing molecular bits or cancer cells that are released from tumour tissue in to the blood or urine system. Circulating cell-free DNA (cfDNA) fragments is one those molecular bits that are released into the bloodstream after the rapid apoptosis or necrosis of the tumour cells in the cancer patients.

**Material and methods** Our goal is to do the comprehensive study between distinct types of glioma cancer tumours and cfDNA of the respective patients to elucidate the scope of cfDNA in liquid biopsy technique for glioma diagnosis.

**Results and discussions** We have successfully detected glioma specific mutations such as IDH1 and 2, PDGFRA, NOTCH1, PIK3R1 etc., on the cfDNA isolated from the plasma of glioma patients and could relate this mutations to the different tumour grades of glioma. We are also studying the dynamics of these mutations in response to the glioma drug treatment by collecting blood samples at different time intervals

**Conclusion** This study may help in developing liquid biopsy technique for glioma tumour diagnosis and in its prognosis for monitoring the glioma treatment by non-invasive approach, and will eventually help physicians to decide the right treatment on right time and will bypass the existing ‘wait-and-see’ approach of treatment monitoring.

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**THE ROLE OF SPARCL1 IN UPPER URINARY TRACT CANCER OF TAIWAN**

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10.1136/esmoopen-2018-EACR25.333

**Introduction** Upper urinary tract urothelial carcinoma (UTUC) in Taiwan is a relatively high prevalent cancer and locally advanced UTUC often carries a poor prognosis. This study is to analyse role of SPARCL1 in UTUC of Taiwan and analyse if advanced UTUC often carries a poor prognosis. This study is to

**Material and methods** We initially got fresh frozen cancer tissue from three high grade locally advanced UTUC (stage >2) and three fresh frozen normal urothelium tissue from low grade and stage 0 disease in Kaohsiung Chang Gung Memorial Hospital. In this study, we used DNA methylation assay and SPARCL1 was identified as most significant different DNA methylation in locally advanced cancer compared with normal urothelium tissue. From 2005 to 2012, this study included 78 patients with pT3N0M0 UTUC. Perioperative factors, pathological features, and SPARCL1 immunostaining were reviewed and prognostic factors were examined by multivariate analysis. Cell line study with BFTC909 and BFTC909-SPARCL1 overexpression was used for cancer behaviour observation. The sensitivity of both cell line to cisplatin and radiation was tested.

**Results and discussions** SPARCL1 was revealed as 20 fold change of methylation in locally advanced UTUC compared with normal urothelium. The patient demography revealed that all pT3N0M0 patient is each group have similar clinical and pathological factors. Systemic UTUC recurrence was significant more in UTUC with negative SPARCL1 presentation (p=0.042). Multivariate analysis found that negative SPARCL1 and non-papillary tumour architecture were the two significant prognostic factors associated with systemic UTUC recurrence (p=0.011 and 0.008 respectively). Cell line study revealed that BFTC 909-SPARCL1 overexpression is associated with less aggressive cancer cell migration/invasion and more sensitivity to cisplatin or radiation treatment.

**Conclusion** SPARCL1 is the most significant methylated gene in locally advanced tissue compared with normal urothelium. For those patients with pT3N0M0 UTUC, SPARCL1 is an independent prognostic marker. The presence of SPARCL1 interferes with UTUC cell line behaviour and sensitivity to cisplatin or radiation.

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**GERMLINE DETERMINANTS OF THE SOMATIC MUTATION LANDSCAPE IN 2642 CANCER GENOMES**

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10.1136/esmoopen-2018-EACR25.334

**Introduction** Cancers develop through somatic mutations; however, germline genetic variation contributes to cancer risk via diverse mechanisms including by modulating mutational processes.

**Material and methods** Within the Pan Cancer Analysis of Whole Genomes (PCAWG) project, we discovered and phased 88 million single nucleotide variants, short insertions/deletions, and large structural variants in whole genomes from 2642 cancer patients, and employed this resource to investigate germline determinants of somatic mutation across 39 cancer types.

**Results and discussions** We describe over 100 germline L1 retrotransposons mediating somatic retrotransposition activity in cancer. Furthermore, rare damaging germline mutations in genes involved in DNA repair, DNA replication, and cell cycle associate with a variety of somatic mutation processes. We implicate mutations in the DNA glycosylase MBD4 with an elevated rate of C>T mutations at CpG dinucleotides, resulting in the genetic modulation of a widespread mutational process. Genome-wide association analysis reveals common genetic variation within the APOBEC gene cluster modulating mutational processes. Analysis of somatic structural variation additionally exposed complex rearrangement patterns including duplications and template insertion cycles in BRCA1-deficient cancers.

**Conclusion** Our study underscores the notable impact rare and common germline variants have on cancer mutational landscapes.

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**CHROMATIN ACCESSIBILITY PROFILING IDENTIFIES AN UNDERLYING HNF4A-GATA6 REGULATORY MODULE IN OESOPHAGEAL ADENOCARCINOMA**

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10.1136/esmoopen-2018-EACR25.335

**Poster Presentation:** Cancer Genomics, Epigenetics and Genomic Instability
Introduction The chromatin landscape of mammalian cells underpins their transcriptional profiles, which then dictate fate and function. Mutations within chromatin remodelling genes have been implicated in early oesophageal adenocarcinoma (OAC) development, thus alterations in the chromatin landscape may pose an important molecular step in OAC development. OAC is often lethal, therefore genome-wide, basic research will provide foundations to develop new treatments and patient stratification methods.

Material and methods We have used Assay for Transposase-Accessible Chromatin using sequencing (ATAC-seq) to profile the accessible chromatin landscape of normal oesophagus and oesophageal adenocarcinoma tissue samples, in addition to representative cell lines. Downstream analysis has involved bioinformatic de novo motif analysis and ATAC-footprinting. Chromatin immunoprecipitation with sequencing (ChIP-seq) and siRNA-mediated knockdowns.

Results and discussions Our results revealed an altered chromatin landscape in cancerous tissue and identified ‘cancer-specific’ regions of accessibility. Using de novo motif discovery methods and footprinting analysis, we have identified enriched transcription factor motifs for GATA6 and HNF4A in differentially accessible regions and footprints. Also, comparison between the chromatin landscape of OAC cell-lines and OAC tissue identified the cell-line that best represents OAC tumours. HNF4A ChIP-seq and GATA6 ChIP-seq data, in oesophageal cancer cells, shows co-occupancy of HNF4A and GATA6 at 90% of sites and confirm motif enrichment and footprint observations. HNF4A and GATA6 knockdown experiments demonstrate overlap of gene regulation and also identify genes that are exclusively regulated by the presence of HNF4A and GATA6. Importantly, genes directly regulated by HNF4A or GATA6 are on average overexpressed in OAC, however genes regulated by both factors are more overexpressed by 2-fold.

Conclusion Our data demonstrate the power of ATAC-seq in genome-wide discovery methods and we have identified a novel HNF4A-GATA6 transcription network that is active in OAC.

PO-306 IDENTIFICATION OF CHROMATIN MODIFIERS REGULATING TEMOZOLOMIDE RESISTANCE IN Glioblastoma Multiforme

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10.1136/esmoopen-2018-EACR25.337

Introduction Glioblastoma multiforme (GBM) is the most common of all malignant brain tumours. Unfortunately, only 5.1% of patients survive five years post diagnosis. Therapy options for GBM patients are very limited, majority of the patients receive radiation therapy and the chemotherapeutic Temozolomide (TMZ). However, most tumours recur making therapy resistance and an extremely important issue. Cancers show aberrant global epigenetic alterations, yet, the epigenetic mechanisms that cause therapy resistance are not well-known. In this project, the role of chromatin modifying proteins in TMZ-resistance is studied by a chemical screen.

Material and methods We established TMZ resistant cell lines by two different methodologies, 1) dose escalation method and 2) high dose TMZ selection. For the dose escalation method, U373 cells were treated TMZ every two days, starting from 25 μM. Every two weeks, dosage was doubled (or when cells become resistant to that dosage) up to 250 μM. For high dose TMZ resistant selection, cells were treated with 250 μM TMZ for 15 days. Both cells lines have been kept under TMZ treatment for 3 months. To identify chemical inhibitors that target resistant cells, the TMZ-resistant cells and their parental controls were treated with a chemical library consisting of 90 epigenetic drugs against chromatin modifiers and cell viability was measured after 72 hours.

Results and discussions We successfully generated isogenic subpopulations of GBM cell lines that are resistant to TMZ. We observed that the high dosage TMZ treatment regimen caused a more sustainable resistant cell line compared to dose escalation regimen. Both resistant cell line models were stable, as they did not depend on prolonged TMZ treatment and remained resistant to TMZ after 3 months of drug holiday.

We then screened for epigenetic compounds that target TMZ-resistant cells. In addition to PARP inhibitors, Olaparib and Rucaparib, we identified Histone Deacetylase (HDAC) inhibitors as TMZ-sensitising agents. We are currently delineating the epigenetic alterations between parental and TMZ-resistant cells and assess the role of HDAC-mediated changes in TMZ-response by RNA-seq.

Conclusion Histone acetylation and deacetylation are interesting focus areas in drug resistance because of the central role of these modifications in many aspects of cell physiology and pathology. In our screen, we have shown that various HDAC inhibitors can sensitize GBM cells to TMZ in established cells as well as our newly generated in acquired TMZ resistance models.

PO-307 PARADOXICAL DECREASE IN H2AFX COPY NUMBER YET INCREASING MRNA EXPRESSION IN HUMAN BREAST CANCER

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10.1136/esmoopen-2018-EACR25.337

Introduction Histones are basic proteins responsible for DNA packaging in eukaryotic cell nuclei. H2AX is an H2A variant that plays an important role early in the DNA damage response. Although H2AX expression has been investigated, the direct contribution of this abundant chromatin protein in breast cancer is not well understood. The H2AFX gene encodes the same H2AX protein by both a short non-polyadenylated transcript and a longer polyadenylated transcript. We are characterising H2AFX copy number (CN), mRNA expression and protein abundance in breast cancer to discover if this foundation chromatin component contributing to genome stability could play a role in breast cancer.

Material and methods H2AFX mRNA abundance was analysed in a panel of breast cell lines by absolute quantitative PCR. The Molecular Taxonomy of Breast Cancer International