average 16 years after the orchectomy. Whole genome DNA methylation profiles were measured with Illumina’s MethylationEPIC BeadChip and analysed with the R package minfi. We used a linear regression model adjusting for smoking, age and cell type composition to identify CBCT differentially methylated CpG sites. Additionally, genes with p-value<0.1, were used for gene enrichment analyses (GSEA) based on Fisher’s exact test using KEGG and Reactome pathways.

**Results and discussions** Out of the initial 866,836 CpG sites, 8,624,400 were included in our analysis. Thirty-three and 14 differentially methylated CpG sites were associated with CBCT after adjusting for multiple testing with False Discovery Rate (FDR) and Bonferroni correction, respectively. Eighty-five genes had a FDR adjusted p-value<0.1 and were used for GSEA. The ‘PTK6 regulates RHO GTPases, RAS GTPase and MAP kinases’ pathway was significantly (adjusted p-value<0.05) enriched with differential DNA methylated sites in three out of 26 genes. This pathway is part of signalling transduction related to several cell functions including growth, differentiation, division, survival and apoptosis. This is in concordance with the cytotoxicity of CBCT, and with the observation that it can be measured in serum many years after application in CBCT-treated patients.

**Conclusion** Our results suggest that CBCT has long-term effects on the epigenome. Overall these results can contribute to elucidate what cellular mechanism are behind later consequences for TC survivors, like organotoxicity and metabolic syndrome. Our results should be further explored in a larger study of TC survivors treated with CBCT.

**PO-362 IMPULSIVE EPIGENETIC AGENTS ON MOUSE TERATOMA DEVELOPMENT IN VITRO**

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**Introduction** Testicular Germ Cell Tumours (TGCT) are the most frequent malignancies in young male population and believed to be initiated by epimutations, i.e. aberrant epigenetics. Among various, teratoma is the most differentiated TGCT type encompassing all three germ layer derived tissues. Mouse teratoma is a well-established in vitro model obtained by cultivating mouse embryos. It represents an ideal system to investigate the effect of most prominent epigenetic drugs and agents.

**Material and methods** After isolation, embryos were treated for two hours with 5-azacytidine, Trichostatin A, Valproate, esiNanog, esiOct3/4 and esiTrrap, respectively. The embryos/teratomas were measured at day 0 and for the consequent 7 days of culturing in MEM enriched with rat serum. For analysis of proliferative and apoptotic activity, immunohistochemistry on paraffin embedded embryos/teratomas was performed using anti-Ki-67 and anti-Caspase-3, respectively. Signal intensity was measured by morphometric analysis. For gene expression analyses, embryos/teratomas were pooled into experimental groups from which specific gene related RNA quantity was analysed by both qPCR and ddPCR.

**Results and discussions** Epigenetic modulators reduced significantly embryo/teratoma growth. Most prominent decrease was detected in 5-azacytidine and esiOct3/4 treated embryos/teratomas. Furthermore, 5-azacytidine almost completely disrupted tissue architecture and cellularity. Proliferative activity was not decreased by any epigenetic modulator. EsiNanog, esiTrrap and surprisingly 5-azacytidine, in fact, showed a slight increase in proliferation. Still, 5-azacytidine induced an increase in apoptotic activity as well. Even stronger incitement of apoptosis was found in Valproate treated embryos/teratomas, while other modulators had no effect on apoptotic activity at all. Expression of analysed stemness and differentiation genes panel was significantly disrupted by 5-azacytidine, Valproate and esiOct 3/4. Other modulators induced slighter decrease or no change in gene expression.

**Conclusion** This research presents a strong adverse influence of epigenetic modulators on experimental germ cell tumour development. It seems that this effect is consequent to induced change in stemness and differentiation genes expression.

**PO-363 IMPLICATION OF GLOBAL HISTONE METHYLATION (H3K4ME) LEVELS IN THE PROGNOSIS OF CLEAR CELL RENAL CELL CARCINOMA**

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**Introduction** Histone modifications play an important role in setting up the epigenomic profile of a cell during the process of tumorigenesis. Previous studies documented the role of several histone modifications in predicting clinical outcome in patients of various tumours. In the present study, we evaluated the prognostic potential of histone 3 lysine 4 mono- methylation (H3K4me1), -di-methylation (H3K4me2) and -tri-methylation (H3K4me3) in clear cell renal cell carcinoma (ccRCC).

**Material and methods** Histone 3 lysine 4 mono- methylation (H3K4me1), -di-methylation (H3K4me2) and -tri-methylation (H3K4me3) in ccRCC were determined on isolated histones from 50 histo-pathologically confirmed cases of ccRCC and adjacent normal renal tissues by ELISA assay.

**Results and discussions** The global H3K4me levels were increased in ccRCC as compared to adjacent normal renal tissues. Further, lower cellular levels of all the three modifications viz H3K4me1, H3K4me2 and H3K4me3 were found to be associated with higher TNM stage and Fuhrman grade of the tumour. Also, there was an increase of H3K4me levels with the degree of its methylation.

**Conclusion** The present study provides the prognostic potential of global H3K4me methylation in ccRCC.

**PO-364 LNCNRA-P23154 PROMOTES THE INVASION-METASTASIS POTENTIAL OF ORAL SQUAMOUS CELL CARCINOMA BY REGULATING GLUT1-MEDIATED GLYCOLYSIS**

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Introduction The dysregulation of glycolysis has been suggested to lead to the alteration of cell drug resistancesignals, proliferation and metastasis. Emerging evidence indicates that IncRNAs play a key role in the cellular processes of tumour cells, including glycolysis, growth, and movement. However, the role of IncRNAs in glycolysis-mediated metastasis and the potential mechanism has not been explored.

Material and methods First, microarrays were performed to explore the IncRNA, mRNA and miRNA profiles in 4 pair OSCC and adjacent non-tumour tissue samples. qRT-PCR and bioinformatic analysis were used to confirm the expression and coding capability of Inc-p23154 in OSCC cell lines. Then, functional experiment, the nude mouse model and RNA sequence were performed to demonstrate that Inc-p23154 may act as a metastasis driver in OSCC and a potential biomarker for OSCC diagnosis and treatment. Furthermore, we verified Inc-p23154 promoted OSCC metastasis via Glut1-mediated glycolysis through a rescue assay. At last, luciferase assay, FISH assay, RNA immunoprecipitation and RNA pull down was utilised to prove that Inc-p23154 influenced miR-378a-3p transcription by interacting with its promoter region, then regulated miR-378a-3p targeted gene Glut1 expression and promoted Glut1-mediated OSCC metastasis.

Results and discussions In this study, we identified a novel IncRNA, Inc-p23154, which is up-regulated in oralsquamous cell carcinoma (OSCC) tissues and cell lines, is associated with OSCC patientmetastasis and promotion of OSCC cell migration and invasion in vitro and in vivo. Furthermore, we found that Inc-p23154 also participates in OSCC glycolysis by facilitating Glut1 expression. Rescue of Inc-p23154 reversed the suppression of OSCC cell migration and invasion induced by Glut1 knockdown. More importantly, Inc-p23154 is mainly located in the nucleus and binds to thpromoter region of miR-378a-3p, which represses Glut1 expression by targeting to its 3’UTR directly.

Conclusion In summary, we described a novel mechanism of Inc-p23154-miR-378a-3p/Glut1 axis in Glut1-mediated glycolysis and OSCC metastasis regulation. Meanwhile, we provided evidence that overexpression of Inc-p23154 is significantly associated with higher metastasis tendency in both OSCC cells and patients with OSCC. These results indicated that Inc-p23154 may act as a metastasis driver in OSCC and a potential biomarker for OSCC diagnosis and treatment.

**PO-366** LONG NON-CODING RNAS TINCR AND DANCR IN UROTHELIAL CARCINOMA SUBTYPES

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**Introduction** Long non-coding RNAs (lncRNAs) are a diverse set of transcripts spanning more than 200 nucleotides. Many show a tissue-specific expression pattern and are involved in fine tuning of cell fate determination by regulating gene expression. Two lncRNAs, TINCR and DANCR, are known as important factors in keratinocyte differentiation. Upregulation of TINCR is required for terminal epidermal differentiation whereas DANCR maintains the undifferentiated state of keratinocytes. We wondered whether these lncRNAs could be involved in aberrant differentiation of urothelial carcinoma (UC) and contribute to the development a specific molecular UC subtypes with squamous features, termed Basal-Squamous-like-subtype (BASQ). We analysed lncRNA expression in a large set of UC tissues and a smaller set of bladder squamous cell carcinomas (B-SCC) and compared the results with TCGA RNA-Seq data.

**Material and methods** LncRNA expression was measured by qRT-PCR in 161 UC, 8 normal and 12 B-SCC samples. RNA-Seq data from the TCGA cohort was accessed via the TANRIC database. Statistical comparison for differential expression between each group was done by Wilcoxon rank sum test in R. Correlation of histopathological patient data with lncRNA with the epigenetic pathway(s) response in order to induce apoptosis by modifying the expression of key apoptotic genes in an *in vitro* model of human malignant melanoma.

**Material and methods** Our *in vitro* human malignant melanoma model consists of (i) immortalised normal keratinocyte (HaCaT) cells; (ii) malignant melanoma (A375) cells and epidermoid carcinoma (A431) cells subjected to the following ITCs: R, S-Sulforaphane (SFN), Phenethyl Isothiocyanate (PEITC), Benzyl Isothiocyanate (BITC), Allyl Isothiocyanate (AITC) and Iberin (IBN) over different concentrations and time points of exposure. Apoptotic induction was confirmed by TaqMan qPCR gene expression profiling arrays while involvement of the epigenetic machinery was assessed by western immunoblotting for determining protein expression levels of histone deacetylases (HDACs), histone acetyltransferases (HATs) and various other histone modification tags.

**Results and discussions** Our results showed that all ITCs were capable of inducing apoptosis as evident by the differential expression of key target genes in a manner where PEITC and IBN were involved primarily in up-regulation compared to SFN and AITC both of which were involved in down-regulation of the majority of these apoptotic genes. Finally, differences in HDAC and HAT protein expression levels, among ITC treatments, were evident in addition to their differential compartmentalization between nucleus and cytosol.

**Conclusion** Overall our results suggest an ITC-dependent cytotoxicity effect which is mediated via apoptotic induction and is underlined, at least partially, by epigenetic pathway(s) response mechanism(s). Our data support the notion that ITCs may be promising candidates in the context of epigenetic therapy for the treatment of human malignant melanoma.

**PO-365** ISOTHIOCYANATES AS POTENT EPIGENETIC REGULATORS IN HUMAN MALIGNANT MELANOMA CHEMOPREVENTION

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**Introduction** Glucosinolates (GLs) are phytochemicals abundant in cruciferous vegetables, which are hydrolysed, by myrosinase, to a range of isothiocyanates (ITCs). These molecules are biologically active metabolites capable of mediating a plurality of anticancer effects including cell cycle arrest, inhibition of proliferation and apoptotic induction. Their wide range of biological properties may be reflected by their ability to interfere with the epigenetic machinery at both DNA and histone levels. The aim of the current study is to investigate how ITCs interact with the epigenetic machinery at both DNA and histone levels. Emerging evidence indicates that lncRNAs play a key role in the cellular processes of tumour cells, including glycolysis, growth, and movement. However, the role of lncRNAs in glycolysis-mediated metastasis and the potential mechanism has not been explored.

**Material and methods** First, microarrays were performed to explore the lncRNA, mRNA and miRNA profiles in 4 pair OSCC and adjacent non-tumour tissue samples. qRT-PCR and bioinformatic analysis were used to confirm the expression and coding capability of lnc-p23154 in OSCC cell lines. Then, functional experiment, the nude mouse model and RNA pull down was utilised to prove that lnc-p23154 inhibited miR-378a-3p transcription by interacting with its promoter region, then regulated miR-378a-3p targeted gene Glut1 expression and promoted Glut1-mediated OSCC metastasis.

**Results and discussions** In this study, we identified a novel lncRNA, lnc-p23154, which is up-regulated in oralsquamous cell carcinoma (OSCC) tissues and cell lines, is associated with OSCC patientmetastasis and promotion of OSCC cell migration and invasion in vitro and in vivo. Furthermore, we found that lnc-p23154 also participates in OSCC glycolysis by facilitating Glut1 expression. Rescue of lnc-p23154 reversed the suppression of OSCC cell migration and invasion induced by Glut1 knockdown. More importantly, lnc-p23154 is mainly located in the nucleus and binds to thpromoter region of miR-378a-3p, which represses Glut1 expression by targeting to its 3’UTR directly.

**Conclusion** In summary, we described a novel mechanism of lnc-p23154-miR-378a-3p/Glut1 axis in Glut1-mediated glycolysis and OSCC metastasis regulation. Meanwhile, we provided evidence that overexpression of lnc-p23154 is significantly associated with higher metastasis tendency in both OSCC cells and patients with OSCC. These results indicated that lnc-p23154 may act as a metastasis driver in OSCC and a potential biomarker for OSCC diagnosis and treatment.