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**Acquired DNA damage in adolescent obesity – a promoter and predictor of cancer?**

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**Introduction** Carcinogenesis in the liver involves a series of pathological and biomedical changes including disorders of autophagy regulation. Deficiency of autophagy during hepatocarcinogenesis has been widely reported. However, the regulatory mechanism underlying autophagy deficiency has not yet been fully unveiled. The aim of this study is to understand the role and function of autophagy-related protein 9b (Atg9b) in autophagy deficiency and endoplasmic reticulum (ER) stress-induced cell death.

**Material and method** To establish the experimental hepatocarcinogenesis model, we feed mice with choline deficiency, amino acid-defined (CDAA) diet for 56 and 72 weeks. Initiation of liver tumour was assessed by macroscopic and microscopic analysis. PCR array on autophagy-related gene was conducted to understand the expression profile of autophagy-related genes during hepatocarcinogenesis. Murine hepatocyte cell line AML12 was applied as an *in vitro* model to assess the ER stress and cell death under Atg9b deficiency.

**Results and discussion** Mice fed with CDAA diet initiated liver tumour at 56 weeks while completely being observed with liver cancer after 72 weeks. Livers of CDAA-fed mice showed significant autophagy deficiency, ER stress and hepatocyte death. PCR array analysis showed that a few autophagy-related gene was differentially regulated during CDAA-induced hepatocarcinogenesis, among which Atg9b is the most down-regulated gene. Expression of Atg9b was down-regulated in HCC tissue compared with non-tumour liver, and was found gradually reduced during the experimental carcinogenesis. Reduced expression of Atg9b by RNA interference in AML12 suppressed autophagy induction by ER stress inducers, and increased ER stress marker expression as well as cell death when exposed to ER stress inducers. This may be associated with the failure of degradation of protein aggregates by autophagic flux in Atg9b-deficient AML12 cells. Co-immunoprecipitation assay revealed that Atg9b deficiency resulted in failure in anchoring p62 proteins with autophagy vacuoles.

**Conclusion** Our findings suggested that Atg9b may play an important role in regulating autophagy deficiency during experimental hepatocarcinogenesis.

#### PO-017 IS RECQL4 A NOVEL PLAYER IN GLIOBLASTOMA PATHOGENESIS?

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**Introduction** Glioblastoma (GBM), the common and aggressive human primary brain tumour (WHO grade IV), is highly resistant to standard radio- and chemotherapy. This is partly due to numerous genetic alterations in oncogenes and DNA damage repair systems. Despite progress in understanding the molecular background of GBM and advances in treatment modalities, survival of GBM patients is only 14 months post-diagnosis. RECQL4 belongs to RecQ family of ATP-dependent DNA helicases and plays an important role in genomic integrity and stability maintenance via involvement in DNA replication, repair, recombination, transcription and telomere maintenance. Mutations in the human *RecQ* genes are linked with cancer predisposition and/or premature ageing. Of all five human helicases, only RECQL4 is found in mitochondria. We explored if alterations of RECQL4 expression or functions contribute to pathogenesis of human GBM.

**Material and methods** We determined the RECQL4 expression in various tumour specimens (tumour samples, human primary and established glioma cell cultures) by qPCR and Western Blotting. We determined the effect of RECQL4 depletion on cell viability, proliferation and GBM sphere formation using MTT metabolism, BrdU incorporation and tumour sphere forming assays, respectively.

**Results and discussions** We found the upregulated expression of RECQL4 in GBM at mRNA and protein levels when compared to non-transformed human astrocytes. This finding was corroborated by TCGA data analysis. Fractionation of mitochondrial and cytosolic fractions from human glioma cells revealed the presence of RECQL4 in mitochondria. Downregulation (by siRNA) or genetic depletion of *RECQL4* (by CRISPRCas9 knockout) in human glioma LN18 and U87-MG cells impaired cell viability and proliferation. We found upregulation of RECQL4 expression in GBM sphere cultures, enriched in glioma stem cells. Transient knock-down of RECQL4 significantly affected tumour sphere formation as evidenced by decreased numbers and sizes of cultured spheres.

**Conclusion** These data indicate that deregulation of RECQL4 expression or function may play an important role in GBM pathobiology. Our results provide a rationale for further studies of RECQL4 role in gliomagenesis.

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#### PO-018 ACQUIRED DNA DAMAGE IN ADOLESCENT OBESITY – A PROMOTER AND PREDICTOR OF CANCER?

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**Introduction** Epidemiological evidence linking obesity with increased risk of cancer is steadily growing, although the causative aspects underpinning this association are only partially understood. Obesity coincides with deficiencies in micronutrients such as Vitamin D, a key player in DNA repair processes. As a result, vitamin D deficiency in obesity may have a marked impact on DNA stability and integrity. 8-hydroxyguanosine (8-OHdG) is a well-established marker of oxidative DNA damage that has been identified in higher concentrations in cancer patients. Here we report, preliminary, unpublished findings from our study on acquired DNA damage in childhood obesity.

**Material and methods** Body Mass Index (BMI), Waist to Hip ratio (WHR) and body fat percentage via bioelectrical impedance was assessed in over 70 participants, aged 11–18 and recruited from National Health Service (NHS) obesity clinics and schools in London. A non-invasive, integrated evaluation of urinary 8-OHdG and salivary vitamin D was conducted using ELISA based methods and compared to markers of adiposity.

**Results and discussions** A BMI percentile >99 was found to be associated with decreased salivary vitamin D and increased urinary 8-OHdG when compared to healthy weight controls (BMI=5th-85th percentile). Vitamin D levels in saliva were found to be inversely correlated with BMI and body fat percentage. Urinary 8-OHdG positively correlated with body fat

percentage and WHR. Most importantly, an inverse correlation between vitamin D in saliva and 8-OHdG in urine was also identified.

Recent evidence has suggested vitamin D in obesity to be a consequence of altered behaviour, reduced intestinal absorption, and sequestration of vitamin D into adipose tissue. As a result, DNA repair processes against oxidative DNA damage in obesity may be impaired, resulting in the excess of lesions including 8-OHdG. The effects of excess 8-OHdG lesions have been well researched to include various mutations that can drive carcinogenesis.

**Conclusion** Our results suggest Vitamin D deficiency in obese adolescents may play a significant role in triggering oxidative DNA damage, thus increasing the likelihood of cancer later in life.

**PO-019 PHOSPHOPROTEOMICS TO CHARACTERISE DNA DAMAGE RESPONSE IN MOUSE MAMMARY TUMOURS OF DIFFERENT PARP INHIBITOR SUSCEPTIBILITY**

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**Introduction** Triple negative breast cancers show very poor prognosis, with only limited treatment options. A subgroup of these tumours is deficient in homologous recombination (HR) and therefore sensitive to DNA damaging drugs or PARP inhibitors (PARPi). Unfortunately, resistance to PARPi is often observed. Therefore, the identification of biomarkers for PARPi response or status of HR are of importance for personalised therapy. The DNA damage response is regulated via post-translational modifications such as phosphorylation. We thus study differential phosphorylation events in the context of different PARPi susceptibility.

**Material and methods** We used a collection of biopsies from either untreated or irradiated (15 Gy, 2 hour) HR-deficient mouse mammary tumours and matched PARPi resistant tumours which restored HR. Changes in phosphorylation were examined via titanium dioxide based phosphopeptide enrichment or protein expression profiling using single-shot LC-MS/MS (label-free).

**Results and discussions** In total, we identified 14 695 phosphopeptides and 11 138 high-confidence phosphosites (PS) from 48 individual tumours samples that were measured in duplicates. As proof of concept, we analysed changes in PS in PARPi naive tumours upon induction of the DNA damage response via ionising irradiation. We detect known events of the DNA damage response such as increased phosphorylation of proteins involved in DNA damage checkpoint or double strand break repair. Further evaluation of these PS, like sequence motif analysis, prediction of upstream kinases and kinase substrate enrichment analysis revealed activation of ATM/ATR in irradiated tumours as expected. To further proof the validity of our approach, we analysed samples with known PARPi resistance mechanism – loss of 53 BP1 expression – and observed downregulation of PS specific to 53 BP1. We are now extending our analysis to additional samples sets with known and unknown PARPi resistance mechanisms.

**Conclusion** Our data point to the feasibility of using phosphoproteomics as a tool to study DNA damage response in BRCA1 deficient tumours with different status of HR and thus contrasting susceptibility to PARPi.

**PO-020 FUNCTIONAL CHARACTERISATION OF VARIANT OF UNKNOWN SIGNIFICATE IN FAMILIAL BREAST CANCER**

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**Introduction** Familial breast cancer (BC) cases account for 5%–10% of all BC cases, and are essentially correlated with the prevalence of pathogenic *BRCA1* and *BRCA2* mutations in cancer patients. Recent advances in next generation sequencing (NGS) have enabled panel gene testing, or simultaneous testing for mutations in multiple genes for a clinical condition. With more widespread genetic testing, an increased detection of alleles with moderate penetrance without established clinical guidelines and of variants of unknown significance (VUS) as either benign or disease-causing will occur. Functional analyses on VUS may identify pathogenicity, and clearly categorise their mutational status. We carried-out a proof-of concept *in vitro* functional analysis in peripheral blood lymphocytes of VUS-harboring individuals and healthy controls assessing the cellular response to  $\gamma$ -radiation.

**Material and methods** Four samples were collected, one BC patient with a pathogenic *ATM* mutation; one *BRCA2* VUS detected without diagnosed disease, and two controls (A, B, no variant detected). All samples were previously sequenced by NGS to confirm the presence of pathogenic mutations or VUS detected in a genetic testing panel. Several methodologies were selected to evaluate the cellular response to genetic lesions induced by  $\gamma$ -radiation (2Gy): chromosomal aberrations (CA), micronuclei (MN) and comet assay.

**Results and discussions** Comet assay results revealed a higher DNA damage in the patient with a pathogenic *ATM* mutation (14% DNA in tail) after irradiation, which might be related with the non-optimal function of the *ATM* protein. The *BRCA2* VUS sample also showed a higher%DNA in tail (6.5%), contrasting with the damage quantified for control samples (2.8% and 0.9% DNA in tail for control A and B, respectively). These results revealed that the presence of such variants might be correlated with their biological function, being crucial to categorise the mutational status. Regarding CA and MN, these assays were not sensitive enough to discriminate pathogenic from control samples.

**Conclusion** Results obtained for comet so far suggest a significant difference between variant carriers and controls, which may indicate an increased susceptibility to ionising radiation. However, more sensitive assays (e.g. H2AX) will be necessary allowing more informative results. As this study is a proof-of concept approach, in the near future the sample size will be incremented.