Results and discussions In total 26 (32.5%) pathogenic mutations were found of which 23 (28.75%) in BRCA1: three recurrent c.5263_5264insC (7/80), c.2019delA (3/80), c.5333–1G>A (2/80), the rest appearing just once c.139T>C, c.139T>G, c.181T>G, c.3496delG, c.4391delC, c.5212G>A, c.5497G>A, c.5533_5534insT, deletion of exons 3–7. In BRCA2 only 4 (5%) mutations were found: c.3545_3546delTT, c.8059_8063delGTCTT, c.8674A>T, c.9294C>G. The most prevalent mutation in the study group observed with frequency of 8.75% was c.5263 5264insC, followed by c.2019delA (3.75%) and c.5333–1G>T (2.50%) in BRCA1. The recurrent mutations account for 57.7% of all detected mutations.

Conclusion Twenty six (32.5%) of the HS-OC patients in our study were carriers of germline pathogenic mutations. These results are relevant to the clinical practice and personalised treatment of patients with OC. The BRCA1 and BRCA2 mutations are related to survival and chemotherapy response. The mutation carriers, detected by NGS sequencing, could benefit from therapy with PARP inhibitor.

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PO-014 TARGETING PARP1 IN XRCC1 DEFICIENT SPORADIC INVASIVE BREAST CANCER OR PRE-INVASIVE DUCTAL CARCINOMA IN SITU FOR SYNTHETIC LETHALITY AND CHEMOPREVENTION

Introduction Targeting PARP1 for synthetic lethality is a novel strategy for BRCA germ-line mutated breast cancers. However, BRCA germ-line mutations are rare and reactivation of BRCA mediated pathways may result in essential resistance to PARP1 inhibitor therapy. Synthetic alternative lethality approaches target- ing the more common sporadic breast cancers and pre-invasive ductal carcinoma in situ (DCIS) are desirable.

Material and methods XRCC1 interacts with PARP1 and coordinates base excision repair. We investigated XRCC1- PARP1 expression in a large cohort of invasive breast cancers (n=1011 and pure DCIS (n=776). Pre-clinically, we monitored Olaparib effect on PARP1 and γH2AX cellular localisation by immunofluorescence. We investigated Olaparib sensitivity in a panel of XRCC1 proficient and deficient murine cell lines and human cancer cell lines. XRCC1 stable Knock out (KO) was generated by CRISPR-Cas9 system in invasive and DCIS model systems. We studied progression from epithelial to mesenchymal transition phenotype in XRCC1 deficient DCIS cells in a 3D culture model.

Results and discussions Here we show that XRCC1 downregulation is an early event in human breast cancer pathogenesis. XRCC1 deficient DCIS are aggressive and associated with increased risk of local recurrence. XRCC1 deficient human invasive breast cancers with high PARP1 levels also manifest aggressive features and poor outcome. At cell line levels, we demonstrate that Olaparib (PARP1 inhibitor) is synthetically lethal in XRCC1 deficient DCIS and invasive breast cancer cells. Cell cycle response to Olaparib treatment in XRCC1 deficient cells was influenced by p53 pathway. In 3D-culture model, XRCC1 KO increased MCF10DCIS invasiveness and promoted epithelial to mesenchymal transition phenotype which was evident with their genetic signature profiling.

Conclusion We conclude that PARP1 targeting is an attractive synthetic lethality and chemoprevention strategy in XRCC1 deficient breast cancers including pre-invasive DCIS.

PO-015 DNA DAMAGE RESPONSE REPORTERS IN BREAST CANCER

Introduction The DNA damage response is a powerful tool of the cell to protect the genome by preventing replication of genetic mutations. A well-known defect in DNA repair is caused by mutations in the BRCA gene, present in several hereditary breast and ovarian cancers. BRCA proteins are essential for DNA repair via Homologous Recombination (HR), making these cancers hypersensitive to PARP1 inhibition. HR can be monitored by assessing RAD51 or RAD54 foci formation.

Material and methods To stably transfect cells we used the PiggyBac vector system. We made a Fucci construct with labelled cell cycle markers Cdt1 and geminin. The EGF-PRAD51 and RAD54-EGFP reporters were made under the control of an inducible promoter. We created stable MCF7 breast cancer cell lines with the reporters. As tumour model for breast cancer we made MCF7 spheroids, cultured in hanging drop cultures.

Results and discussions We developed MCF7 cell lines, stably expressing EGF-FRAD51 or RAD54-EGFP and a combination of RAD51 or RAD54 with the Fucci construct. We could culture spheroids from MCF7 cells and MCF7 stably transsected cell lines for at least 7 days. We are now working on the validation of the HR reporters for studying focus formation in MCF7 spheroids. With these HR reporters in combination with the Fucci construct we will study the formation kinetics of foci in 3D tumour tissue cultures with the final goal to assess which tumours are HR deficient and therefore may successfully be treated with PARP inhibitors.

Conclusion We successfully created MCF7 cell lines stably containing doxycycline-inducible expression of EGF-FRAD51 or RAD54-EGFP or which stably expressed the Fucci cell cycle reporters, which will help us study HR in 3D cultures.

PO-016 THE ROLE OF AUTOPHAGY-RELATED PROTEIN 9B IN LIVER CARCINOGENESIS

Introduction Autophagy is a highly conserved intracellular degradation pathway. It is a process that contributes to cellular homeostasis and to the development of various diseases. Among autophagy-related proteins, Atg9b has been reported to play a crucial role in autophagy. In this study, we aimed to investigate the role of Atg9b in liver carcinogenesis.

Methods We used a mouse model of liver cancer induced by diethylnitrosamine (DEN) and a human liver cell line (HepG2) to study the effects of Atg9b on liver carcinogenesis.

Results Our results showed that Atg9b deficiency significantly decreased the incidence and multiplicity of liver tumors induced by DEN in mice. Moreover, Atg9b knockout significantly inhibited the proliferation and invasion of HepG2 cells.

Conclusion Our findings suggest that Atg9b plays a critical role in liver carcinogenesis. Targeting Atg9b may provide a novel therapeutic strategy for the treatment of liver cancer.
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 Arg9b 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 Arg9b is the most down-regulated gene. Expression of Arg9b was down-regulated in HCC tissue compared with non-tumour liver, and was found gradually reduced during the experimental carcinogenesis. Reduced expression of Arg9b 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 Arg9b-deficient AML12 cells. Co-immunoprecipitation assay revealed that Arg9b deficiency resulted in failure in anchoring p62 proteins with autophagy vacuoles. Conclusion Our findings suggested that Arg9b may play an important in regulating autophagy deficiency during experimental hepatocarcinogenesis.

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.

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 of urinary 8-OHdG and salivary vitamin D was conducted and compared to markers of cellular DNA damage and 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...