observed a crosstalk between arginine methylation and ubiquitination because overexpression of USP11 promotes the deubiquitination of PRMT1.

**Conclusion** Our studies identify for the first time that DUBs are modified by arginine methylation, and that this post-translational modification has the potential to control the enzymatic activity of DUBs involved in double strand break repair. Understanding how methylation of USP11 contributes to genome stability will be important for understanding drug resistance in breast cancer.

**PO-011 IMPACT OF COMPARTMENT-SPECIFIC CHANGES IN NAD BIOSYNTHESIS ON DIETHYLNITROSAMINE-INDUCED LIVER CANCER**

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**Introduction** Primary liver cancer is the second largest cause of cancer related mortality worldwide. Deregulation of cellular energetics is an emerging hallmark of cancer. NAD⁺ is a ubiquitous metabolite that plays a critical role in the regulation of many metabolic pathways. Recent work has shown that boosting NAD⁺ biosynthesis can protect against the development of hepatocellular carcinoma. Here we have explored whether organelle specific changes in NAD⁺ are important for this effect.

**Material and methods** We have examined transgenic animals overexpressing either NMNAT1 or NMNAT3 to increase NAD⁺ biosynthesis in the nuclear and mitochondiral compartments respectively. The transgenic mice and WT littermates were injected with the liver carcinogen diethylnitrosamine (DEN) at 2 weeks of age and subsequently placed on a high-fat diet to accelerate tumourigenesis. We also acutely treated 12–16 week old WT and transgenic mice to investigate the changes within 48 hour of DEN treatment. Liver tissue and serum collected from these experiments were subjected to intensive analysis in search for mechanisms of protection.

**Results and discussions** Overexpression of NMNAT1 significantly reduced the multiplicity of liver tumours, with an average of 5 tumours per mouse, compared to 13.6 in WT. In contrast, NMNAT3 overexpression did not influence tumour multiplicity. However when tumours were established, the transgenic models showed no protection with regards to tumour burden. The mice with higher nuclear NAD⁺ biosynthesis displayed a 50% reduction in serum levels of the liver damage markers ALT and AST compared to WT littermates. They also had lower levels of oxidised peroxiredoxin 2 and inflammatory marker CCL5, indicating that nuclear NAD⁺ is protective against genotoxic liver damage. In isolated hepatocytes NMNAT1 mice had lower levels of DNA damage marker γH2Ax upon DEN treatment and this protection was abolished when co-treated with PARP1 inhibitor Olaparib.

**Conclusion** Our study shows that elevation in nuclear NAD⁺ is important for protection against liver tumour formation. Increased NMNAT1 activity appears to protects the liver at the tumour initiation event by reduction of oxidative stress and DNA damage by regulating the activity of PARP1.

**PO-012 FERULIC ACID INCREASES ABT-888 SENSITIVITY IN BREAST CANCER CELLS**

H Park*, E Park. Hannam University, Food and Nutrition, Daejeon, South Korea; Inhibition of DNA damage repair pathway is a common mechanism by which conventional cancer therapies kill cancer cells. Chemical inhibitors of poly (ADP-ribose) polymerase (PARP) are efficient in inducing sensitivity to BRCA-deficient tumors through synthetic lethality by targeting base-excision repair (BER) in HR-deficient tumors. Ferulic acid has been shown its therapeutic effects against cancer.

**Introduction** Material and methods Here, we hypothesised that ferulic acid impedes HR-dependent repair in breast cancer cells and therefore, ferulic acid in combination with ABT-888 of a PARP inhibitor, make cancer cells more hypersensitive in cell culture system.

**Results and discussions** In the present study, ferulic acid reduces HR repair, inhibits RAD 51 foci formation, which is a crucial protein in HR repair and reduces HR-dependent repair. Moreover ferulic acid results in accumulation of γ-H2AX, which is a hallmark of DNA damage. Finally, we demonstrated that combined treatment of ferulic acid and ABT-888 reduces colony formation in HR-proficient-breast cancer cells.

**Conclusion** Taken together, our study provides new data that ferulic acid regulates HR-dependent-repair, including RAD 51 foci formation, and it hypersensitizes breast cancer cells to ABT-888 treatment when combined as a chemotherapy reagent.

**PO-013 BRCA1 AND BRCA2 NEXT GENERATION SEQUENCING IN HIGH-GRADE SEROUS OVARIAN CANCER**

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**Introduction** According to the National Cancer Register in Bulgaria more than 800 women develop ovarian cancer annually. The most frequent histological type, representing about three quarters of these cases is high-grade serous ovarian carcinoma (HS-OC). About 25% of the HG-OC are supposed to be caused by mutations in the BRCA1 and BRCA2 genes.

**Material and methods** We have screened 80 Bulgarian patients with high grade serous ovarian cancer (HS-OC), disease progression and platinum sensitivity for germline mutations in the BRCA1/2 genes. The mutation analysis was performed by Next Generation Sequencing (NGS) with Ion Torrent PGM System, Sanger Sequencing and MLPA (Multiplex Ligation Dependent Probe Amplification). All identified pathogenic variants with NGS were confirmed by direct sequencing.
Results and discussions In total 26 (32.5%) pathogenic mutations were found, which 23 (88.5%) 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_8063delGTTCT, c.8674A>T, c.9294G>C. 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

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Introduction Targeting PARP1 for synthetic lethality is a novel strategy for BRCA1 germ-line mutated breast cancers. However, BRCA1 germ-line mutations are rare and reactivation of BRCA1 mediated pathways may result in eventual resistance to PARP1 inhibitor therapy. Alternative synthetic lethality approaches targeting 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

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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 transflect cells we used the PiggyBac vector system. We made a Fucci construct with labelled cell cycle markers Cdt1 and geminin. The EGFP-RAD51 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 EGFP-RAD51 or RAD54-EGFP and a combination of RAD51 or RAD54 with the Fucci construct. We could culture spheroids from MCF7 cells and MCF7 stably transfectted 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 EGFP-RAD51 or RAD54-EGFP or which stably expressed the Fucci cell cycle reporter construct. These MCF7 cells form spheroids expressing reporters, which will help us study HR in 3D cultures.

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

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Introduction Mammalian target of rapamycin (mTOR) is an important nutrient sensor that has been implicated in the regulation of autophagy, an important cell survival strategy. We hypothesize that the role of autophagy- related protein 9B (ATG9B) in liver carcinogenesis is affected by the mTOR signalling pathway.

Methods We used the qRT-PCR, western blotting and immunohistochemistry to examine the expression of ATG9B in different tissues and clinical samples from liver cancer patients.

Results The expression of ATG9B was significantly lower in liver cancer tissues compared to the normal liver tissues. In addition, the expression of ATG9B was positively correlated with the expression of mTOR. The results also showed that the expression of ATG9B was positively associated with patient survival.

Conclusion Our findings suggest that ATG9B plays a crucial role in liver carcinogenesis and that it may be a potential therapeutic target for liver cancer treatment.