PO-084 ASCORBIC ACID SELECTIVELY TARGETS GLUCOSE METABOLISM OF OSTEOSARCOMA STEM CELLS

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Introduction Osteosarcoma (OS) is the most common primary bone sarcoma that mainly occurs in children and adolescents. The existence of drug resistant cancer stem cells (CSCs) with progenitor properties is responsible for OS relapse and metastasis. Thus, development of specific therapies targeting OS-CSCs is necessary to increase the long-term survival rate. Although ascorbic acid (AA) has controversial history as anticancer agent, recently it has been re-evaluated revealing more cytotoxic effect to cancer than normal cells. The aim of the study was to analyse AA as potential therapeutical for selective targeting of OS-CSCs.

Material and methods To establish primary tumour cultures, tumour samples were mechanically dissected and enzymatically digested. Sarcosphere assay was used to isolate OS-CSCs. The cytotoxic effect of AA was determined by MTT assay as well as relationship between cell concentration and AA. OS-CSCs were treated with different concentrations of AA (2.5–55 μg/ml) during 72 hour. Concentrations of AA used for further experiments were 30 μg/ml and 40 μg/ml, respectively. Effect of AA on sarcosphere-forming ability was measured under low-attachment condition during 28 days. Cell death type was determined by Annexin V/PI staining using flow cytometry. Levels of GAPDH were determined by western blot while ROS were measured by DCFH-DA assay. Seahorse XF analyzer was used to measure glycolysis and oxidative phosphorylation.

Results and discussions While AA did not have any effect on hMSCs, U2OS and Hek 293, respectively, AA efficiently induced dose-dependent viability reduction of OS-CSCs. Further, it can be concluded that IC50 values of AA depend on the number of seeded OS-CSCs. AA successfully reduced sarcosphere formation on 6th day. High cytotoxicity of AA was further confirmed by Annexin V/PI staining. Prevalent death mode induced by AA was apoptotic since more than 70% of Annexin V-positive cells were detected. In addition, AA inhibited the activity of the key glycolytic enzyme GAPDH and induced ROS levels. Following the treatment with AA, extracellular acidification rate as a measure of glycolysis, was reduced significantly. Moreover, AA increased metabolic potential of OS-CSCs implying cells’ ability to meet an energy demand via respiration and glycolysis.

Conclusion Based on the obtained results, it can be concluded that AA selectively targets OS-CSCs. The death mechanism is based on the blockage of glycolytic cycle and increased intracellular levels of ROS.

PO-085 MIR-424(322)/503 REGULATES WNT/B-CATENIN PATHWAY AND RESISTANCE TO TREATMENT IN BREAST CANCER

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Introduction The female mammary gland is a very dynamic organ that undergoes continuous tissue remodelling during adulthood. Although it is well established that the number of menstrual cycles and pregnancy increase the risk of breast cancer, the reasons are unclear. Clinical and experimental evidence indicates that improper involution plays a role in the development of this malignancy. Recently, we described the miR-424(322)/503 cluster as an important regulator of mammary epithelial involution after pregnancy and that miR-424 (322)/503 is commonly lost in a subset of aggressive breast cancers. Through the use of a knockout mouse model, we demonstrated for the first time that loss of miR-424(322)/503 promotes breast tumorigenesis in vivo. Remarkably, we found that loss of miR-424(322)/503 promotes chemoresistence due to the up-regulation of two of its targets: BCL-2 and insulin-like growth factor-1 receptor (IGF1R). Importantly, targeted therapies blocking the aberrant activity of these targets restore sensitivity to chemotherapy.

Material and methods Patient data were assessed from METABRIC and TCGA corresponding to breast cancer samples with available whole-genome DNA CNAs, mRNA expression data, and clinicopathological data.

Results and discussions Tumours generated in the miR-424 (322)/503 knock out mouse model present morphological and molecular characteristics of metastatic squamous cell carcinoma (SSC) of the breast. These include the presence of a non-glandular component, spindle cells and expression of cytokeratin 6. Primary SSC of the breast is a very aggressive tumour with high metastatic activity and refractory to treatment. It has been reported that nearly all the primary metastatic carcinomas present activation of Wnt pathway, and about 40% of them carry mutations in genes of the Wnt pathway. By analysing the expression data of over 3000 breast tumours, we have determined that the loss of miR-424(322)/503 contributes to the activation of Wnt/b-catenin pathway in SSC. We also show that the miR-424(322)/503 directly targets the Wnt receptor LRP-6, preventing the translocation of b-catenin to the nucleus and inhibiting the transcription of b-catenin-regulated genes.

Conclusion MiR-424(322)/503 is a tumour suppressor in breast cancer and provide a link between mammary epithelial involution, tumorigenesis, and chemoresistance.

PO-086 BCL11A INTERACTS WITH SOX2 TO CONTROL THE EXPRESSION OF EPIGENETIC REGULATORS IN LUNG SQUAMOUS CELL CARCINOMA

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Introduction Lung cancer accounts for the highest rate of cancer related diagnosis and mortality worldwide. Small cell lung cancer and non-small cell lung cancer (NSCLC) make up the
major subtypes of lung cancer. NSCLC patients only have a 15% five-year survival rate. The major subtypes of NSCLC are lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC). LUSC accounts for more than 40 000 deaths each year and unlike LUAD there are limited targeted therapies. Therefore, a great deal of work still needs to be done to understand the drivers for this cancer.

Material and methods shRNA was utilised to modulate the levels of BCL11A and SOX2 in LUSC cell lines. In vitro 3D colony assays and xenograft mouse models were employed to understand the role of BCL11A in driving LUSC tumorigenesis. To explore the role of BCL11A in vivo, a Cre-inducible BCL11A overexpression mouse model was used. To further investigate BCL11A in LUSC, a mouse tracheal basal cells organoid assay was employed. CHIP-seq, immunoprecipitation and immunoblotting assays were designed to dissect the mechanism by which BCL11A elicits its function in LUSC. Drug dose response assays were used to test the efficacy of SETD8 inhibitors and cisplatin on an array of lung cancer cell lines.

Results and discussions Analysis of TCGA has revealed BCL11A to be upregulated in LUSC but not LUAD. Subsequently reducing BCL11A levels in LUSC cell lines results in diminished xenograft tumour growth. Inversely, its overexpression in vivo led to lung airway hyperplasia and the development of reserve cell hyperplastic lesions which is a precursor to squamous metaplasia. Moreover, deleting Bcl11a in mouse tracheal basal cells abolished the development of tracheosphere organoids while its overexpression led to solid tracheospheres expressing markers of squamous cells.

At the molecular level we found BCL11A to be a target of SOX2 and we show that it is required for the oncogenic role of SOX2 in LUSC. Furthermore, we showed that BCL11A and SOX2 interact at the protein level and that together they co-regulated the expression of several transcription factors. We demonstrate that pharmacological inhibition of SETD8, a gene co-regulated by BCL11A and SOX2, alone or in combination with cisplatin treatment, shows significant selectivity to LUSC in comparison to LUAD cells.

Conclusion Collectively, these results indicate that the disruption of the BCL11A-SOX2 transcriptional program provides a future framework for the development of targeted therapeutic intervention for LUSC patients.

**PO-087**

**OXIDATIVE STRESS AS A SELECTION ANTI-CANCER AGENT: PRECLINICAL EVALUATION OF A TARGETED COMBINATION STRATEGY FOR MUTANT P53 NON-SMALL CELL LUNG CANCER**

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**Introduction**

Increased oxidative stress is a hallmark of cancer cells, which makes them more vulnerable to induction of reactive oxygen species (ROS). P53 plays a crucial role in sensing and removing oxidative damage to DNA, and inactivating mutations in the TP53 gene attenuate this function. In addition, it was shown that mutant p53 is able to suppress the function of major antioxidant factors. Therefore, mutant p53 renders cancer cells even more susceptible to the induction of oxidative stress. Besides p53, the poly (ADP-ribose) polymerase 1 (PARP-1) protein plays an important role in the repair of ROS-induced DNA-damage. This led us to explore the potential of combining oxidative stress induction with the targeted inhibition of the PARP-1 protein to selectively target mutant p53 NSCLC cancer cells.

**Material and methods** APR-246 and Auranofin (inhibition glutathione (GSH) and/or thioredoxin reductase 1 (TrxR1)) and Olaparib (PARP-1 inhibitor) were used. The cytotoxicity (SRB-assay) of these compounds was determined in a panel of NSCLC cell lines with different p53 status, including isogenic cell lines (p53 shRNA-knockdown, p53 knock-in). Total GSH content (GSH/GSSG-GloTM) and ROS content (CellROX) were determined. N-acetyl-l-cysteine (NAC) was used as a potent ROS-scavenger. Induction of apoptosis/cell death was determined by the AnnV/PI assay (FC) or the Cytotox Reagent (Incucyte). DNA-damage was assessed by g-H2AX foci (IF) and the Comet Assay. Synergism was determined using the Additive model.

**Results and discussions**

P53 Mut knock-down reduces the cytotoxic effect of APR-246, Auranofin and Olaparib, while p53 Mut knock-in sensitised cells for all three compounds. APR-246/Olaparib treatment reduced GSH levels and increased ROS content, resulting in a strong accumulation of DNA-damage and synergistic induction of cell death. Co-treatment with NAC or p53-knockdown significantly reduced this cytotoxic response. Similar synergistic effects were observed for Auranofin/Olaparib treatment in several cell lines with clinically relevant p53 mutations.

**Conclusion**

Mutant p53 protein expression renders NSCLC cells more susceptible to APR-246, Auranofin and Olaparib treatment. In addition, the combination of oxidative stress induction (APR-246, Auranofin) with PARP-1 inhibition (Olaparib) results in remarkable synergistic effects in the presence of mutant p53. Therefore, this combination strategy could be a promising and selective treatment option for mutant p53 NSCLC patients in which resistance to standard therapies often occurs.