**PO-066**

**KNOCKDOWN OF CADHERIN 17 INACTIVATES WNT SIGNALLING PATHWAY AND INDUCES APOPTOSIS IN COLORECTAL CANCER CELLS**

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Introduction Large scale sequencing studies have identified that 93% of the colorectal cancer (CRC) patients carry at least one mutation in genes implicated in Wnt signalling pathway. Notably, majority of the CRC patients (88%) carry either APC or β-catenin mutations that can activate the Wnt signalling pathway. Recent evidences suggest that Wnt/β-catenin signalling activity is regulated by CDH17 in hepatocellular carcinoma. As CDH17 is exclusively expressed in the intestine and overexpressed in CRC, we hypothesised that CDH17 could be utilised as a therapeutic target to treat CRC patients.

Material and methods RNA interference-based stable knockdowns were established in a panel of CRC cells with varying mutations in APC and β-catenin. Wnt signalling activity of the cells were measured by TOPflash assay. Apoptosis studies were performed using fluorescence activated cell sorting. Cells were further subjected to immunoprecipitations with anti-CDH17 and anti-β-catenin antibodies followed by label-free quantitative proteomics analysis. A monoclonal antibody was developed to block CDH17 and sensitize CRC cells to chemotherapeutic drugs.

Results and discussions Knockdown of CDH17 in CRC cells downregulated β-catenin and attenuated Wnt signalling activity irrespective of APC and/or β-catenin mutations. Furthermore, CDH17 silencing induced apoptosis and sensitised CRC cells to the chemotherapeutic drugs 5-Fluorouracil. Immunoprecipitations using anti-CDH17 and anti-β-catenin antibodies followed by label-free quantitative proteomics analysis highlighted no direct interaction between CDH17 and β-catenin hence implying an indirect regulation of β-catenin expression and Wnt signalling pathway by CDH17. The analysis revealed E-cadherin and FAT1 as common interactors of CDH17 and β-catenin. Quantitative proteomic analysis of cell lysates revealed the upregulation of FAT1, a negative regulator of Wnt signalling pathway, upon knockdown of CDH17. Monoclonal antibodies developed against CDH17 were able to increase apoptosis and sensitivity of CRC cells to 5-Fluorouracil.

Conclusion Overall, these findings suggest that CDH17 can attenuate Wnt signalling pathway and induce apoptosis irrespective of the APC and β-catenin mutational status. As Wnt signalling pathway is aberrated in 93% of CRC patients, the membrane protein CDH17 can be exploited as therapeutic target to treat CRC.

**PO-067**

**AUTOPHAGY MODULATES RESPONSE TO CISPLATIN IN THYROID CANCER CELLS**

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Introduction Thyroid cancer is the most common endocrine malignancy and, although the death rate of thyroid cancer is relatively low, the recurrence rate of this disease is high. One of the most prevalent mutation in thyroid cancer is V600EBRAF. This mutation causes hyperactivation of the ERK-MAPKs pathway, which leads to a greater cell proliferation, survival and invasion. Nevertheless, the treatment with BRAF inhibitors lacks of efficacy in thyroid cancer, due to development of resistance and important adverse effects in other tissues. Thus, new therapies are required to improve treatment efficiency and specificity in this type of tumours. On the other hand, autophagy is a process whereby the cells recycle their own cellular components and obtain energy, but its role in thyroid cancer is not clarified. In some cases, autophagy is related to a chemotherapelig response in cancer cells. We sought to characterise the role of autophagy on survival of anaplastic thyroid cancer cells with V600EBRAF, and to determine the effects of its inhibition on chemosensitivity to cisplatin.

Material and methods Human anaplastic thyroid carcinoma cell line BHT-101 was treated with cisplatin in the presence of the autophagy-specific inhibitors bafilomycin A1 (BafA1) and 3-methyladenine (3MA). Cell viability was measured by MTT assay. Apoptosis and cell cycle were detected by flow cytometry. Western blotting assay was used to investigate autophagy markers levels before cisplatin treatment.

Results and discussions Our results show that autophagy plays a protective role in these cells, since its inhibition with BafA1 and 3MA increases cell death. Cisplatin also inhibits cell proliferation and induces cell death, but at lower rate. In addition, cisplatin modulates the expression of autophagy-related proteins LC3-II and p62 in BHT101 cells, indicating an increase of autophagic flux, compatible with a self-protection mechanism. Finally, the combined treatment with cisplatin and autophagy inhibitors shows higher effects on cell viability than the individual treatments.

Conclusion All this data, suggest that autophagy is a pro-survival mechanism in anaplastic thyroid cancer cells and that its inhibition enhances cisplatin treatment response. Thus, we establish the inhibition of this process as an effective approach in treating of V600EBRAF-expressing thyroid tumours.
distinct extrinsic signals that could offer a survival advantage to leukemic cells.

**Material and methods** We aim to assess in both ETP-ALL (Loucy cell line) and typical T-ALL (CEM-CCRF cell line) if distinct microenvironments affect the BCL-2 family proteins expression and the sensitivity to BH3 mimetics. To study the anti-apoptotic BCL-2 dependencies we will use the cutting-edge technology mitochondrial BH3 profiling. In brief, BH3 profiling is a functional assay that measures the response of mitochondria to exposure of known concentrations of synthetic BH3 peptides by measuring loss of mitochondrial membrane potential or cytochrome c release.

**Results and discussions** We found that ETP-ALL cell line was BCL-2 dependent and sensitive to ABT-199 (BCL-2 specific BH3 mimetic). While the Typical T-ALL cell line was BCL-XL dependent and sensitive to both ABT-263 (BCL-2,BCL-XL and BCL-w) and WEHI 539 (BCL-XL) BH3 mimetics. Interestingly, when the ETP–ALL cell line Loucy was co-cultured with the human splenic fibroblast (HSF) cell line we found a reduction in BCL-2 dependency, as assessed by BH3 profiling, and a reduced sensitivity to ABT-199 (BCL-2 specific BH3 mimetic). Remarkably, we found the inverse in the typical T-ALL cell line co-cultured with the HSF. We found an increase in BCL-2 dependence and an increase in sensitivity to ABT-199.

**Conclusion** Currently, we are trying to identify the signalling pathways which are activated to cause this switch in BCL-2 family dependence in the different subtypes of T-ALL. In addition, we are assessing the BCL-2 family dependence of a xenograft cell line isolated from the blood and spleen of animals to determine if this switching of BCL-2 dependence occurs *in vivo*.

**Conclusion** The calculated IC$_{50}$ value was low enough for decreasing the viability of breast cancer cells in 24 hours. This result underline the potential cytotoxic effect of carmofur for MCF-7 cells and is a initial further investigation in manner of drug developing for cancer treatment.

**PO-071 SILENCING OF CIRCRNA004582 SENSITISES GASTRIC CANCER CELLS TO APATINIB VIA MODULATING AUTOPHAGY BY TARGETING MIR-3657 AND ATG7**

**Introduction** Gastric cancer (GC) is the fourth most common cancer and the third-leading cause of cancer-related deaths worldwide. Despite certain advances in chemotherapy regimens and targeted therapies, the 5 year survival of patients with advanced GC is disappointing. The positive results of the apatinib phase III trial have cast new light on treatment for patients with advanced gastric cancer (GC) refractory to two or more lines of prior systemic chemotherapies. However, a dose reduction or treatment interruption is required to prevent apatinib toxicity such as hand-foot skin reaction (HFSR), proteinuria, and hypertension. In addition, suitable biomarkers are not accessible for identifying the patients who are most likely to benefit from apatinib. Therefore, predictive biomarkers are urgently needed to reduce toxicity and identify patients that will benefit from apatinib treatment.

**Material and methods** Clonogenic assay, cytotoxicity assay, apoptosis assay, qRT-PCR, western blot were conducted in GC cells to evaluate the effectiveness of apatinib *in vitro*. Confocal microscopy assay was performed to examine apatinib-induced autophagic flux in GC cells. Transmission electron microscopy assay was conducted to show the formation of autophagic vesicles. Xenograft models in mice were conducted and six tumour tissues were sent for miRNA-seq and circRNA-seq. Sanger sequencing was applied to determine the full length of the amplification products.

**Results and discussions** In this study, we found that apatinib inhibited the growth of GC cells and xenograft tumours. Apatinib promoted autophagy activation via upregulation of ATG7 expression, and autophagy inhibition enhanced apatinib-induced apoptosis. With miRNA-seq and circRNA-seq analyses of GC xenograft models, we demonstrated that circRNA004582 functioned as an endogenous sponge for miR-3657 to inhibit its activity and further upregulate ATG7 expression. Silencing of circRNA004582 inhibited apatinib-induced autophagy, which was rescued by miR-3657. Moreover, knockdown of circRNA004582 promoted apatinib-induced apoptosis in GC cells. These findings provided the first evidence that the circRNA004582-miR-3657-ATG7 axis mediates a regulatory pathway critical for the regulation of autophagy and apatinib sensitivity in GC cells and xenografts.

**Conclusion** In conclusion, specific blockade of circRNA004582 may be a potential therapeutic target for autophagy inhibition in the context of apatinib treatment in human GC. Prospective clinical studies are warranted to examine these biomarkers.