Conclusion The results obtained highlight a possible strategy to target NSCLC with KRAS-LKB1 co-mutations, that at moment are those with a worse prognosis. The sensitivity to ERK inhibitor is remarkable, also in presence of KRAS WT, therefore this strategy could be applied to all LKB1-mutated lung tumours, that represent 30% of all NSCLC. These studies are being confirmed in other NSCLC backgrounds and mouse models.

PO-036 TRADITIONAL CHINESE MEDICINE ZE-QI-TANG FORMULA INDUCES APOPTOSIS AND S PHASE ARREST VIA ROS-DEPENDENT JNK AND ERK ACTIVATION IN LUNG CANCER

Introduction Ze-Qi-Tang (ZQT) is a classic Chinese herbal formula, consisting of nine different herbs, which has been used to effectively cure respiratory tract diseases for thousands of years in China. In the present study, we aimed to elucidate the anti lung cancer effect of ZQT in tumor-bearing mouse and the underlying antineoplastic mechanism of action.

Material and methods CCK-8 and colony formation assay were used to investigate the cell growth. Flow cytometry analysis was used to evaluate the cell cycle and cell apoptosis. The peroxide-sensitive fluorescent probe DCFH-DA was used to measure the intracellular ROS levels. Western blot assay was used to detect the levels of cell cycle and apoptosis related proteins. Xenografts in nude mice were used to evaluate the effect of ZQT on lung cancer cell in vivo. ELISA assay was used to test the liver and kidney function post ZQT treatment.

Results and discussions Lung cancer cells were significantly killed by ZQT, and it inhibited the proliferation of lung cancer cells and induced cell cycle arrest at S phase and mitochondrial-related apoptosis. Moreover, ZQT induced a sustained activation of the phosphorylation of ERK and JNK. Moreover, ZQT provoked the generation of reactive oxygen species (ROS) in lung cancer cells. In vivo, ZQT suppressed tumour growth in mouse xenograft models. Besides, ZQT was safe, showing minimal toxicity in liver and kidney.

Conclusion These findings suggest Traditional Chinese medicine Ze-Qi-Tang formula is promising to be a novel, potent and safe anti tumour drug candidate for lung cancer.

PO-037 ROLE OF MARCH8 IN ESOPHAGEAL SQUAMOUS CELL CARCINOMA

Introduction E3 ubiquitin ligases are potential targets for cancer treatment. Increasing amounts of evidence strongly suggest that the abnormal regulation of some E3 ligases is involved in cancer development. In this study, for the first time, we detected the aberrant expression of MARCH8 mRNA and protein, an E3 ubiquitin ligase, in human esophageal squamous cell carcinoma. Initial studies have primarily focused on its immunomodulatory role but its relevance in cancers remains unknown.

Material and methods Quantitative Real Time PCR and immunohistochemistry were carried out to examine the levels of MARCH8 mRNA and protein in esophageal squamous cell carcinoma tissues. The roles of MARCH8 in proliferation, migration/invasion and apoptosis of esophageal cancer cells was evaluated through MARCH8 gene knockdown, western blot analysis, colony formation assay, matrigel assay and flow cytometry.

Results and discussions MARCH8 mRNA expression was found to be significantly upregulated in esophageal squamous cell carcinoma as compared to distant matched non-malignant tissues (p=0.024, AUC=0.654). Immunohistochemical analysis revealed overexpression of MARCH8 protein in 86% of esophageal squamous cell carcinoma tissues (p<0.001, AUC=0.908). Interestingly, intense nuclear staining of MARCH8 protein expression was detected in both cytoplasm and nucleus of cancer cells. Knockdown of MARCH8 inhibited proliferation, migration, invasion and clonogenic potential of esophageal cancer cells. In addition to this, cell cycle analysis showed increase in subG0 and G2/M populations and decrease in S-phase population post-MARCH8 silencing. Interestingly, MARCH8 silencing resulted in a significant increase in the number of cells in early apoptotic phase.

Conclusion Our results indicate that silencing of MARCH8 suppresses proliferation, migration/invasion and promotes apoptosis of esophageal cancer cells.

PO-038 PKC ISOFORMS DISTINCTIVELY MODULATE TELOMERASE EXPRESSION AND AFP SECRETION IN HEPATOCELLULAR CARCINOMA

Introduction Despite its role as a diagnostic and prognosis marker for Hepatocellular carcinoma (HCC), alpha fetoprotein (AFP) plays a key role in advancing tumorigenesis and tumour expansion. Telomerase, an enzyme elongating telomere length, is upregulated in 80% of cancers including HCC. Our team had elucidated a modulation of AFP by telomerase and protein kinase C (PKC). PKC family is formed of several isoforms, each with a specific cellular function and expression. The aim of this study is to investigate the interrelation between PKC isoforms, telomerase and AFP in HCC.

Material and methods PKC isoforms were quantified by RT-qPCR in two AFP secretory cell lines, HepG2/C3A and PLC/PRF/5 and two non-secretory AFP cell lines SNU-387 (hTERT +) and SKOV-3 (hTERT-). According to the results, the expression of four isoforms was suppressed by si-RNAs in HepG2/C3A and PLC cells. AFP and telomerase mRNA levels were quantified in transfected cells by q-PCR, and AFP secretion by ELISA. Toxicity and cell proliferation were assessed by WST-1. In order to examine the effect of the dual presence of AFP and hTERT on PKC isoforms, SNU-387 and SKOV-3 were transfected with AFP expression plasmid pCMV3-AFP, then PKC isoforms mRNA was assessed by qPCR.

Results and discussions Four PKC isoforms, alpha, beta, delta and epsilon exhibited the highest expression levels in all cells
compared to the remaining isoforms. An increase in telomerase expression, AFP expression and secretion and cell proliferation reaching a maximum of two folds was observed after individual gene silencing of the four isoforms in HepG2/C3A cells; however, an inverse pattern was noticed when using PKC pan inhibitor Go6983. Similar results were observed in PLC cell line. The expression of the four isoforms increased in SNU-387 and SKOV3 cells after 24 hour transfection. The effect persisted after 48 hour in SNU-387, contrary to SKOV3 where the levels decreased returning to the controls expression levels.

Conclusion Taken together, decreased individual PKC isoforms rise telomerase expression and AFP secretion. However, PKC isoforms overexpression requires the presence of hTERT in AFP secretory cells. Thus, these results show for the first time the possible inter relation linking PKC isoforms to both AFP and hTERT in HCC.

PO-039 SOPHORIDINE INDUCES APOPTOSIS AND S PHASE ARREST VIA ROS-DEPENDENT JNK AND ERK ACTIVATION IN HUMAN PANCREATIC CANCER CELLS

Introduction Pancreatic cancer is generally acknowledged as the most common primary malignant tumour, and it is known to be resistant to conventional chemotherapy. Novel, selective antitumor agents are pressingly needed.

Material and methods CCK-8 and colony formation assay were used to investigate the cell growth. Flow cytometry analysis was used to evaluate the cell cycle and cell apoptosis. The peroxide-sensitive fluorescent probe DCFH-DA was used to measure the intracellular ROS levels. Western blot assay was used to detect the levels of cell cycle and apoptosis related proteins. Xenografts in nude mice were used to evaluate the effect of Sophoridine on pancreatic cancer cell in vivo.

Results and discussions Sophoridine killed cancer cells but had low cytotoxicity to normal cells. Pancreatic cancer cells were particularly sensitive. Sophoridine inhibited the proliferation of pancreatic cancer cells and induced cell cycle arrest at S phase and mitochondrial-related apoptosis. Moreover, Sophoridine induced a sustained activation of the phosphorylation of ERK and JNK. In addition, Sophoridine provoked the generation of reactive oxygen species (ROS) in pancreatic cancer cells. Finally, in vivo, Sophoridine suppressed tumour growth in mouse xenograft models.

Conclusion These findings suggest Sophoridine is promising to be a novel, potent and selective antitumor drug candidate for pancreatic cancer.

PO-040 CHARACTERISATION OF CDK12 KNOCKED OUT OVARIAN CANCER CELL LINES

Introduction While cyclin-dependent kinases (CDKs) have a key role in promoting/controlling transition between the different phases of the cell cycle, transcriptional kinases, like CDK12, are mainly involved in gene transcription. CDK12 has been shown to regulate the expression of genes involved in DNA damage and to maintain genomic stability. Impairment of CDK12 activity is synergic with PARP inhibitor and cisplatin treatments in different cellular systems. We here aimed to generate ovarian cancer cell lines knocked out (KO) for CDK12 to understand its role in ovarian cancer and in response to chemotherapy.

Material and methods A2780 and SKOV3 CDK12 KO clones were generated with CRISPR/Cas9 technology. Cell cycle analysis was evaluated by standard flow cytometric methods and DNA repair genes levels by Real Time PCR. Caspase 3 activity was measured to detect apoptosis with a luminescence-based assay. Cytotoxicity experiments were performed treating cells with different drug concentrations and evaluating cell survival after 72 hours by MTS assay. For in vivo studies, 7.5 millions of cells were transplanted subcutaneously in nude mice and animals were monitored for tumour appearance and growth.

Results and discussions We obtained 2 CDK12 KO ovarian cancer clones, A2780 KO and SKOV3 KO, out of more than 300 clones screened. The cell growth of both A2780 KO and SKOV3 KO cells is slower than the wild type (WT) cells, they have a less clonogenic ability and a tetraploid DNA content. Both CDK12 KO clones have a higher basal caspase activity than the WT cell lines, indicative of higher basal induction of apoptosis, while no increase in autophagy or senescence is observed. Both CDK12 KO clones show a decreased expression in BRCA1 and FANC2 DNA repair genes than the WT cells. Cytotoxic experiments with anticancer agents with different mechanism of action show that both KO clones are less sensitive to ATM, CHK1 and WEE1 inhibitors treatment as compared to WT cells, while platinum and PARP inhibitors show similar cytotoxic activity in KO and WT cells. Interestingly enough, when KO clones were transplanted in nude mice, no tumour take was observed.

Conclusion We were able to obtain CDK12 KO cells. We think that these models could help in disclosing new roles of CDK12 in ovarian carcinoma and may represent a useful tool to study new combination therapies for tumours with CDK12 mutations.

PO-041 TNF PATHWAY IN METASTATIC COLORECTAL CANCER ACCORDING TO RAS STATUS AND IMPLICATION OF POTASSIUM CHANNELS

Introduction Tumour necrosis factor α (TNF) is a key player in onco-inflammatory context and their implication in the progression of colorectal cancer (CRC) is still controversial. It is equally established that potassium channels contribute to tumour progression but their link to TNF-dependent microenvironment remains poorly described. Our aim is to investigate the effects of TNF on cellular models of colorectal cancer and

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