colorectal adenocarcinoma cells using alamar blue and trypan blue exclusion assay and determined their IC50. We also proposed to illustrate the anticancer mechanism of these natural compounds by studying cell cycle response, proteome profile and response of cell cycle specific genes in HT29 and DLD1 colorectal cancer cells. natural inhibitor of high mobility box 1 protein (HMGB1).We studied the anticancer activity of glycyrrhizic acid and quercetin in SW480, HT29 and DLD1 colorectal adenocarcinoma cells using alamar blue and trypan blue exclusion assay and determined their IC50. We also proposed to illustrate the anticancer mechanism of these natural compounds by studying cell cycle response, proteome profile and response of cell cycle specific genes in HT29 and DLD1 colorectal cancer cells.

Material and methods Cell cycle analysis was performed using a propidium iodide based staining assay in a Muse flow cell analyzer. For proteomics response, R and D proteome-profiler-antibody-arrays for oncology panel for 84 oncoproteins were assayed. A SYBR green based approach for the quantification of selected genes was considered in qPCR analysis.

Results and discussions Both glycyrrhizic acid and quercetin showed altered protein and gene expression in HT29 and DLD colorectal adenocarcinoma cells.

Conclusion As evident from their low IC50s (80 and 160mcg/ml) against the colorectal cells, both quercetin and glycyrrhizic acid showed a promising scaffold for medicinal chemistry to advance anticancer drug discovery.

PO-034 STRUCTURAL AND FUNCTIONAL STUDIES OF β2-GLYCOPROTEIN I IN SUPPRESSION OF MELANOMA CELL MIGRATION BOX1 INHIBITORS IN COLORECTAL CANCER CELLS

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Introduction β2-glycoprotein I (β2-GPI) is a human plasma protein which has been reported to inhibit human aorta endothelial cell migration in our previous study. Migration of endothelial cells has been found to be involved in tumorigenesis, however, whether β2-GPI regulates tumor cell migration or tumor growth is still unknown. In this study, we investigated the biological function and the structural characteristics of β2-GPI in suppression of melanoma cells.

Material and methods Different lengths of cDNA encoding β2-GPI gene has been constructed and cloned into the pFastBac vector using a Bac-to-Bac baculovirus expression system. This recombinant β2-GPI genes were transfected into insect Sf9 cells to generate recombinant β2-GPI peptides. Expression and purification of the recombinant proteins were performed to identify the function domain of β2-GPI in anti-melanoma cell migration. Cell migration was determined by wound healing and transwell migration assays. Cell proliferation was determined by cell counting assay. Effects of purified β2-GPI and recombinant β2-GPI domains on tumor growth in vivo were determined in the melanoma cell-implanted mice. The structural and functional relationships among specific peptide domains and regression of tumor growth were investigated using amino acid sequence alignment, solvent accessible surface area, and 3-D structure of human β2-GPI.

Results and discussions We found for the first time that purified β2-GPI and several recombinant peptides of β2-GPI were able to suppress melanoma cell migration and proliferation. Furthermore, the purified β2-GPI and some specific recombinant domains of β2-GPI inhibited tumor growth in vivo. To highlight the structural and functional relationship between specific peptide motif and regression of melanoma growth, we compare the amino acid sequences among the DI to IV domains. The sequences of D1 contain more different amino acid residues compared to other β2-GPI domains. We thus investigate more structural characteristics of β2-GPI D1 peptide, which may shed light on its function in anti-melanoma growth.

Conclusion This study sought specific sites within β2-GPI functional domain which may interact with melanoma cell membrane and play essential roles in the suppression of melanoma growth.

PO-035 LKB1 DEFICIENCY RENDERS NON-SMALL-CELL LUNG CANCER CELLS SENSITIVE TO ERK INHIBITOR

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Introduction Lung Cancer is the first cause of cancer-related death in the world. The alterations in KRAS oncogene are very frequent (25%), but, unfortunately, this protein is at present undruggable. KRAS mutations determine over-activation of important pathways of growth and proliferation (PI3K-AKT-mTOR and MAPK). KRAS-mutated tumours are also frequently co-mutated in LKB1 (50%), an important regulator of metabolic homeostasis and oxidative stress in the cells. LKB1 modulates catabolic processes through AMPK-mediated mTOR inhibition. Thereafter, the inactivation of LKB1 causes in KRAS mutated-tumours further activation of PI3K-AKT-mTOR and MAPK pathways, making them particularly aggressive. The possibility to specifically target tumours with both KRAS and LKB1 alterations represent an important medical need.

Material and methods We generated from the NSCLC cell line NCI-H1299 clones over-expressing KRAS WT or KRAS G12C forms. These clones have been subsequently modified through CRISPR-CAS9 system to obtain deletions in LKB1 gene. We successfully generated isogenic cells differing only for the status of KRAS and LKB1 ($KRAS^{wt}/LKB1^{wt}$, $KRAS^{mut}/LKB1^{wt}$, $KRAS^{wt}/LKB1^{mut}$, $KRAS^{mut}/LKB1^{mut}$). These clones were treated with a panel of inhibitors of MAPK and PI3K pathways. Viability was evaluated with MTS assay. Molecular characterizations were performed by western blot analysis. *In vivo* antitumor activity was determined after subcutaneous injection of NSCLC cells in immunodeficient mice.

Results and discussions Using the isogenic system generated we tested the activity of several inhibitors of MAPK and PI3K pathways. The results highlighted a strong response of the clones with deletion in LKB1 to ERK inhibitor, independently from the KRAS status. These results were confirmed '*in vivo*', where tumours with LKB1 deletion showed a significant sensitivity to ERK inhibitor, compared to LKB1 WT tumours. At molecular level we tested the activation of proteins related to MAPK and PI3K pathway such as p70, S6, 4-EBP1, ERK. The results showed that the response to ERK inhibitor was mainly due to mTOR signalling inhibition.

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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

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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 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 minimum 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

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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

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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