**PO-072**  
**α3-POLYUNSATURATED FATTY ACIDS INHIBIT CELL GROWTH AND INVASION OF HUMAN HYPOPHARYNGEAL CARCINOMA CELLS**

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**Introduction** According to the latest statistics from the United States National Cancer Institute, it is estimated that about 17,000 Americans will be diagnosed with pharyngeal cancer in 2017. Although α3-polyunsaturated fatty acids (α3-PUFAs) have anti-tumorigenic properties in the several cancers, the anti-cancer effect of α3-PUFAs on hypopharyngeal carcinoma has not been known yet. In this study, we report inhibitory mechanisms of α3-PUFAs on cell growth and invasion in human hypopharyngeal carcinoma.

**Material and methods** FaDu and SNU1041 hypopharyngeal carcinoma cell lines were treated with α3-PUFAs, and cell viability, apoptotic parameters as well as autophagic activity were examined. Additionally, to investigate the effect of α3-PUFAs endogenously, fat1 stably expression cells of FaDu (fFaDu-sc), which express Caenorhabditis elegans α3-desaturase (α3-desaturase converts α6- to α3-PUFAs endogenously), were generated by stably transfection.

**Results and discussions** DHA and EPA inhibited the cell growth of FaDu and SNU1041 in a dose- and time-dependent manner. Moreover, DHA-induced apoptotic cell death was confirmed by TUNEL assay, and the induction of cleaved PARP and caspase-3 in DHA-treated FaDu and SNU1041. Colony formation was also inhibited after DHA treatment. Furthermore, treatment of hypopharyngeal carcinoma cells with DHA resulted in a significant increase in autophagic activity, as revealed by increased LC3-II levels, GFP-LC3 puncta, and autophagic flux activation. EGF-induced phosphorylation of EGFR, which is frequently overexpressed in hypopharyngeal carcinoma, was also suppressed after DHA pretreatment, and levels of p-Akt (p-Akt(Thr308 and Akt(Ser473), was also inhibited. The invasiveness of cells was significantly inhibited by DHA treatment. The MMP-2 and MMP-9 promoter activities were inhibited after DHA treatment. Furthermore, the proliferation of fFaDu-sc was more attenuated than that of cells expressing control vectors (fFaDu-cc). fFaDu-sc showed reduction of cell invasion compared with fFaDu-cc in transwell chamber. The MMPs promoter activities were also suppressed in fFaDu-sc.

**Conclusion** These findings provide evidence that α3-PUFAs may inhibit invasion as well as cell growth through suppression of p-EGFR and MMPs expression in hypopharyngeal carcinoma cells, indicating that the utilisation of α3-PUFAs may represent a potential effective therapy for the chemoprevention and treatment of human hypopharyngeal carcinoma.

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**PO-074**  
**METFORMIN-INDUCED APOPTOSIS FACILITATES DEGRADATION OF THE C-FLIPL PROTEIN THROUGH A CASPASE-DEPENDENT PATHWAY IN HUMAN RENAL CELL CARCINOMA A498 CELLS**

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**Introduction** Recent reports indicate that simultaneous targeting different signalling pathways regulating cell growth with combinations of small molecule inhibitors could be a useful strategy to combat cancer and prevent the development of drug resistance. In this study, we identified a dual effect of the pyridinyl imidazoles, widely used p38 MAPK inhibitors, on the growth of malignant melanoma cells bearing BRAF V600E mutation.

**Material and methods** Experiments were performed in NRAS (Mel-Juso) and BRAF V600E mutant melanoma cells (A375, G361). All cell lines were cultured in RPMI-1640. For the treatment of the cells we used p38 inhibitor BIRB796, MEK inhibitor PD184352, and pyridinyl imidazole compounds SB202190 and SB203580. As pyridinyl imidazoles induce vacuolization of melanoma cells, we used electron and fluorescence microscopy to analyse the origin of the vacuoles. Fluorescent protein-tagged proteins served as markers of the endosomal pathway. We performed western blot analysis to study the effect of pyridinyl imidazoles on the activity of mTOR and ERK pathways. Immunoprecipitations and in-vitro kinase assays were used for the detection of changes in BRAF interacting partners and activity of MEK-ERK signalling, after exposure to pyridinyl imidazoles.

**Results and discussions** Our study reveals mechanisms, by which the pyridinyl imidazoles affect two critical pro-proliferative pathways, stop cell cycle progression, and decrease the viability of melanoma cells. We found that the compounds can disrupt endocytosis in BRAF-mutant melanoma cells, rather than inhibiting autophagy as previously reported. We show that the resulting accumulation of endocytic vacuoles leads to the deactivation of mTORC1 signalling, that is independent of p38 inhibition. Apart from the effect of these drugs on mTOR activity, they cause a substantial decrease in levels of active ERK kinase. Mechanistically, pyridinyl imidazoles directly bind to the mutated BRAF kinase and inhibit the activation of its downstream targets MEK and ERK.

**Conclusion** This study identified pyridinyl imidazoles as a class of small molecule compounds capable of dually targeting central proliferative pathways in BRAF-mutant melanoma cells. Our findings could lead to the development of new drugs with anti-melanoma activity.

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**PO-073**  
**DUAL TARGETING OF PROLIFERATIVE PATHWAYS IN BRAF-MUTANT MELANOMA CELLS BY PYRIDINYL IMIDAZOLE COMPOUNDS**

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**Introduction** Renal cell carcinoma (RCC) is one of the most common cancers in adults. Previous studies have reported that the survival rate was significantly lower for renal cancer patients with diabetes than for those without diabetes. Metformin is a well-known anti-diabetic agent used for the treatment of type 2 diabetes mellitus (T2DM). It also inhibits cell proliferation and angiogenesis, and is known to have anti-tumour effects. However, the molecular mechanism for metformin-induced apoptosis in renal cell carcinoma is not understood. In the present study, treatment with metformin induced apoptosis in A498 cells in a dose-dependent manner.
Material and methods Effect of metformin on A498 human renal cell carcinoma cells was studied. Morphological changes were visualised using a LM. Cellular DNA was stained by applying PI and the relative DNA contents of the stained cells were analysed using FACS. Proteins such as anti-c-FLIP, anti-PARP, anti-Bcl-2, anti-Bcl-xL, anti-Mcl-1, anti-cIAP-2, anti-XIAP and anti-actin antibodies were detected using by Imaging System. c-FLIP mRNA expression was determined by RT-PCR. ROS generation was assessed by the dichlorofluorescence in fluorescence intensity of the cells using flow cytometer. Data were analysed using one-way ANOVA followed by post-hoc comparisons using the SPSS 8.0.

Results and discussions We found that degradation of cellular FADD-like interleukin-1-converting enzyme (FLICE) inhibitory protein (c-FLIP) and activation of procaspase-8 were associated with metformin-mediated apoptosis. In contrast, treatment with metformin did not affect the mRNA level of c-FLIP in A498 cells. Treatment with benzylxoycarbonyl-Val-Ala-Aspfluoromethyl ketone (z-VAD-fmk, a pan-caspase inhibitor) almost completely blocked metformin-induced apoptosis and degradation of c-FLIP protein. However, N-acetyl-l-cysteine (NAC), a reactive oxygen species (ROS) scavenger, did not inhibit metformin-mediated apoptosis in A498 cells.

Conclusion These results demonstrated that metformin-induced apoptosis was mediated by the degradation of c-FLIP protein via activation of caspase-8 in A498 human renal cell carcinoma cells. This suggests that metformin can play the role of a chemotherapeutic agent for diabetes, as well as an anti-cancer agent.

PO-075 METHANOL FRACTION OF CALLIANDRA PORTORICENSIS INDUCES APOPTOSIS AND GROWTH ARREST IN PROSTATIC TUMOUR CELL LINES

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Introduction Apoptosis is down regulated in most forms of cancer. Mitochondria are central to the apoptotic process and are targeted in cancer therapy by novel drug candidates. Calliandra portoricensis (CP) is used in the management of prostate enlargement in folk medicine. This study was designed to investigate the effects of CP on mitochondrial-mediated apoptosis and cell proliferation using prostate cancer cells.

Material and methods Prostate cancer cells were treated with methanol fraction of CP (MFCP), cell cycle analysis was evaluated by flow cytometry and levels of pro-apoptotic Bax, anti-apoptotic Bcl-2, Cytochrome C Release and activation of caspases 3 and 9 were determined using ELISA kits.

Results and discussions The MFCP inhibited (p<0.05) proliferation of prostatic cancer cells. The growth inhibition by MFCP (10 μg/mL) correlated with a 3-fold decreased expression of Bcl-2 and a 4-fold increase in Bax levels in LNCaP cells. The MFCP (10 μg/mL) activated C3 and C9 at ≥4.2 and 5.1 folds over control, respectively which prompted cancer cells to arrest at S phase. The LC-MS analysis revealed the presence of polyphenols in MFCP.

Conclusion Taken together, MFCP- induced cell death is mediated by alteration of mitochondrial integrity and cell cycle arrest. Hence, MFCP may be effective for cancer pharmacotherapy.

PO-076 HMNQ INDUCES APOPTOSIS AND AUTOPHAGY DEPENDENT ON REACTIVE OXYGEN SPECIES THROUGH ACTIVATION OF JNK SIGNALLING PATHWAY

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Introduction 8-hydroxy-2-methoxy-1,4-naphthoquinone (HMNQ), a natural compound isolated from bark of Juglans sinensis Dode, has been previously reported to possess cytotoxic activity toward various human cancer cells. However, the molecular mechanism of its anticancer effect remains unknown.

Material and methods In this study, the anticancer activity and molecular mechanism of HMNQ were investigated using cell viability/colony formation assay and wound healing assay. In addition, apoptosis analysis and measurement of mitochondria membrane potential were performed.

Results and discussions Our results showed that HMNQ reduced cell viability, decreased colony formation, and inhibited cell migration in breast, lung, and colon cancer cells. HMNQ effectively induced apoptosis by upregulating the expression of pro-apoptotic protein Bax, cleaved PAR, and downregulating the expression of anti-apoptotic protein Bcl-2 in A549 and MCF7 cells. In addition, HMNQ also induced reactive oxygen species (ROS) production through the decreased mitochondrial-membrane potential and this effect was attenuated by ROS scavengers, N-acetyl cysteine (NAC) and l-glutathione (GSH). Furthermore, HMNQ increased the expression of JNK phosphorylation and JNK inhibitor SP600125 suppressed HMNQ-induced decrease in cell viability.

Conclusion Taken together, our findings suggest that HMNQ exhibits anti-proliferative activity through induction of ROS-mediated apoptosis in human cancer cells, indicating that HMNQ as a potential anticancer agent.

PO-077 IDENTIFICATION OF DHX30 AS AN INHIBITOR OF THE TRANSLATION OF PRO-APOPTOTIC MRNAs AFTER P53 ACTIVATION BY NUTLIN

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Introduction The transcription factor p53 can be efficiently activated by the small molecule Nutlin-3 without inducing genotoxic stress. Treatment of different cell lines with this small molecule can result in different phenotypes, ranging from cell cycle arrest to apoptosis. HCT116 (colon cancer-derived cells) and SJS1 (osteosarcoma-derived cells) were used to model the opposite behaviour respectively, by