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-cFLIPL, 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-FLIPL mRNA expression was determined by RT-PCR. ROS generation was assessed by the dichlorofluoresence 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-FLIPL in A498 cells. Treatment with benzyloxycarbonyl-Val-Ala-Aspfluoromethyl ketone (z-VAD-fmk, a pan-caspase inhibitor) almost completely blocked metformin-induced apoptosis and degradation of c-FLIPL 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-FLIPL 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 3and 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 by 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 measerement 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 PARP, 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-acetylcysteine (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 SJSA1 (osteosarcoma-derived cells) were used to model the opposite behaviour respectively, by

analysing the transcriptional and translational responses after Nutlin-3 treatment.

Material and methods Total and polysomal-bound mRNAs were collected and sequenced after 12 hour of 10 uM Nutlin-3 treatment. A bioinformatics analysis of the polysome-enriched mRNAs using Weeder allowed the identification of a 3'UTR motif ('CG-rich') which is enriched in the translationally upregulated genes of SJSA1. The effect of the motif on translation was evaluated after cloning its consensus sequence in the 3'UTR of the b-globin gene, which was put downstream the luciferase reporter. The activity of the construct was evaluated after 12 or 24 hours of Nutlin-3. The same consensus was used for a pull-down experiment followed by mass spectrometry to identify proteins interacting with it.

Results and discussions RNA-seq data indicate that HCT116 and SJSA1, although sharing almost completely the transcriptional program lead by p53, show almost no overlap at a translation level. SJSA1 present different pro-apoptotic translationally-upregulated genes after Nutlin-3, which have one or more instances of a CG-rich motif in the 3'UTR. The impact of the motif is to enhance the activity of the luciferase reported when cloned in two copies flanking the 3'UTR of the b-globin gene, but only in SJSA1. A pull-down experiment using an RNA bait with the consensus motif was used to identify interactors, among which DHX30 was deeply studied. DHX30 silencing in HCT116 causes: 1) enhanced the activity of the reporter construct after Nutlin; 2) polysomal association of selected mRNAs containing the motif; 3) induction of apoptosis as assessed by Annexin-V staining. In addition, silencing of DHX30 in U2OS cells decreased their survival after Nutlin-3 treatment.

Conclusion We show how a p53-dependent transcriptional program can be shaped at a translational level thanks to the action of a CG-rich motif which is enriched in the 3'UTR of some pro-apoptotic mRNAs and that can be bound by DHX30. This protein acts as a translational repressor of mRNAs containing the motif. The exact mechanism and the generalisation of the model are currently being investigated.

PO-078 ROLE OF THE ER STRESS-AUTOPHAGY AXIS AND MITOCHONDRIAL METABOLISM REPROGRAMMING IN THE APOPTOSIS INDUCED BY δ-TOCOTRIENOL IN PROSTATE CANCER

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Introduction Castration resistant prostate cancer (CRPC) is an aggressive tumour with still limited therapeutic outcomes. Tocotrienols (TT), vitamin E derivatives, were reported to exert anticancer activity in different tumours. The aim of this study was to assess the effects of δ-TT on human CRPC cells growth and the molecular mechanisms associated with its activity.

Material and methods In human normal prostate (RWPE-1) and CRPC (PC3 and DU145) cell lines the effect of δ-TT on cell viability was evaluated by MTT assay; in PC3 and DU145 cells Trypan blue exclusion and colony formation assays were also performed. The expression of apoptosis-, ER stress- and autophagy-related proteins was analysed by Western blot and immunofluorescence assays, and the cytotoxic effect of δ-TT was also assessed using specific inhibitors of these pathways. The effect on mitochondrial metabolism was evaluated analysing the expression of the OXPHOS complexes (Western blot), the mitochondrial activity and mass (flow cytometry), the oxygen consumption (Clark-type oxygen electrode) and the ATP production (colorimetric assay).

Results and discussions We demonstrated that δ-TT exerts a cytotoxic effect on PC3 and DU145 but not on RWPE-1 cells. In particular, δ-TT induces caspase 3 and PARP cleavage and cytochrome *c* release from mitochondria, and its cytotoxic effect is partially blocked by co-treatment with the pan-caspase inhibitor z-VAD-FMK, confirming that δ-TT exerts a pro-apoptotic effect on CRPC cells.

We also observed that δ-TT significantly increases the expression of ER stress (BiP, IRE1α, PERK, pEIF2α, ATF4 and CHOP) and autophagy mediators (LC3-II and p62). Using the ER stress inhibitors salubrinal and 4-phenylbutyrate (4-PBA) and the autophagic flux inhibitors 3-methyladenine and chloroquine, we confirmed that the effect of δ-TT is mediated by both these mechanisms. In addition, treatment with salubrinal or 4-PBA impairs δ-TT-induced LC3-II expression, demonstrating that this compound triggers the ER stress-autophagy axis.

Finally, we observed that δ-TT severely alters mitochondrial metabolism: the expression of the OXPHOS protein complexes, the mitochondrial activity/mass ratio, the oxygen consumption and the ATP production were significantly reduced after δ-TT treatment.

Conclusion These results demonstrate that δ-TT exerts a selective pro-apoptotic effect on human CRPC cells through the activation of the ER stress-autophagy axis and the rewiring of mitochondrial metabolism.

Cancer Cell Metabolism

PO-079 EFFECT OF A ESCIN NANO-FORMULATION ON HUMAN LUNG ADENOCARCINOMA CELLS

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Introduction Escin is the predominant active constituent of *Aesculus hippocastanum* seed extract. In addition, *Aesculus hippocastanum* seed contains flavonols, glycosides (Aesculin, esculin) and triterpenoid saponins (aescin, escin). The antioxidant biological effect of escin means ability to protect against free radicals and toxins production in lung cells. Also, several *in vivo* studies have shown that low doses of escin are antitumour and anti-inflammatory in different cell lines. In this study was investigated potential cytotoxic effects of escin-loaded solid lipid nanoparticles on the morphology of A549 cell line.

Material and methods The MTT method was used to determine the cytotoxic effects of the nano-formulation in A549 cells. Firstly, the nano formulation of escin was prepared and the A549 cells were exposed to this compound at different concentrations for 24 hours, then read on the ELISA reader at 570 nm wavelength. Based on these results, IC_{50} value was found. Morphological changes on the A549 cells caused by escin nano-formulation were examined under confocal microscope. For this manner A549 cells were incubated with the