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 μM 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.

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**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 anti-cancer 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-VDAM-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, pE1F2α, 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.

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**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 anti-tumour 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, IC50 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
IC_{50} concentration of nano-formulation for 24 hours and cells were double stained with phalloidin and acridine orange. Changes on the cell morphology were photographed under a confocal microscope.

**Results and discussions** The viability of the treated cells decreased with the increase of the applied concentration. IC_{50} value of 3.20 μM for 24 hours. On the confocal micrographs of A549 cells exposed to IC_{50} value of escin nano-formulation for 24 hours was seen many morphological alterations as dis-integrated and deformed nuclei, chromatin and cytoskeleton, chromatin condensation also cell shrinkage and holes on cytoskeleton.

**Conclusion** According to our laboratory studies and results, escin nano-formulation has been shown that escin-loaded solid lipid nanoparticles induced apoptosis in human lung adenocarcinoma (A549) cells and caused morphological changes on these cells. As escin nano-formulation doses increased the viability of the treated A549 cells decreased. Escin nano-formulation caused holes on the skeleton of these cells and caused cells to shrink. It also triggered apoptosis of these cells. We suggest this drug as an alternative agent in the treatment of cancer but further investigations are needed.

## Cancer Initiating Cells – Cancer Stem Cells

**PO-080** V-ATPASE G1 EXPRESSION IN HUMAN GLIOMA STEM CELLS CORRELATES WITH ERK ACTIVATION

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**Introduction** The vacular ATPase (V-ATPase) is a multisubunit proton pump acting in multiple processes in eukaryotic cells. Alteration of V-ATPase activity is associated to a wide range of human diseases including cancer. We have recently showed that V-ATPase expression has a central role in glioma stem cells (GSC) maintenance. Therefore we aimed to get insights into the signalling associated with V-ATPase expression in GSC.

**Material and methods** Signalling survey was performed using phospho-specific antibodies and the Cancer 10-pathway Reporter Luciferase Kit. GSC were treated with Ammonium chloride (NH₄Cl) (10–50 mM), Bafilomycin A1 (BafA1) (10 nM) and the ERK inhibitor PD98059 (10 μM) Cell Invasión through collagen matrix and sphere formation were evaluated after 48 hour of drugs treatment, while cell cycle, apoptosis and ROS production were evaluated by flow cytometry after 24 hour. Mitochondrial depolarization and activity were evaluated by flow cytometry after TMRE staining and western blot, respectively. Autophagy was analysed by western blot using an antibody to p62. All experiments were performed using primary GSC cultures with high and low levels of V-ATPase G1 subunits (V1G1^{HIGH} and V1G1^{LOW}, n=3 each).

**Results and discussions** The MAPK/Erk pathway was significantly upregulated in V1G1^{HIGH} GSC and V-ATPase impairment by BafA1 reduced Erk phosphorylation, besides decreasing lysosomal acidification. Therefore we investigated if this effect was specific for the pump activity or if it was related to lysosomal dysfunction or to MAPK/Erk signalling.

The comparison of the three drugs revealed that only BafA1 treatment induced cells death, reduced clonogenicity and invasion ability and decreased the phosphorylation level of proteins involved in proliferation and pro-apoptotic processes. Moreover BafA1 was the only drug that, at not lethal dosage, impaired cell cycle progression.

This effect was associated with an increase in ROS production and mitochondrial depolarization and using a ROS inhibitor the effects of BafA1 were reverted.

**Conclusion** Taken together these results indicate that the V-ATPase play a central role in GSC viability that goes beyond lysosomal activity or ERK phosphorylation. Further studies are needed to elucidate the roles of the proton pump in GSC and to target this molecule for innovative anti-cancer strategies.