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