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 disintegrated 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 vacuolar 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 (NH_4Cl) (10–50 mM), Bafilomycin A1 (BafA1) (10 nM) and the ERK inhibitor PD98059 (10 uM) Cell Invasion 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 subunit ($V1G1^{HIGH}$ 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.

PO-081 NOTCH SIGNALLING PATHWAY PLAYS A CRUCIAL ROLE IN MAINTAINING THE CANCER STEM CELL POPULATION IN LUNG AND COLORECTAL CANCER

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Introduction There is increasing evidence that cancer stem cells (CSCs) could mediate resistance to chemo- and radiotherapy, metastasis and recurrence in a wide range of solid tumours. Notch signalling pathway is involved in CSCs maintenance. Notch influences intra- and intercellular communications of various cell types in embryogenesis and adult organs. The oncogenic role of Notch signalling in survival, proliferation, self-renewal, differentiation and migration of cancer cells is demonstrated in most solid tumours and leukaemia. This study aims to explore the effect of Notch1 suppression on CSCs in human lung (A549) and colorectal (HCT116) adenocarcinoma cell lines.

Material and methods Knockdown of Notch1 by shRNA was confirmed by PCR and western-blot analyses. PCR was used for estimation of stemness, epithelia-to-mesenchyma transition (EMT) markers and proto-oncogene *c-MYC* expression. The proportion of $CD133^+$ cells in different subpopulations was identified by Flow cytometry. Cell migration potential was examined by Boyden chamber assay. The effect of Notch1 inhibition on CSCs was established using classical detection methods of CSCs in heterogeneous cancer cell cultures: tumourisphere formation, tumourigenicity *in vivo* and quantitative assessment of ABC-transporter activity assays.

Results and discussions Notch1 inhibition resulted in stemness (*SOX2, OCT3/4, NES*), EMT (*TWIST1, HES1, SNAIL1*), CSCs (CD133) markers, *c-MYC* expression decrease and *CDH1* (E-cadherin) expression increase suggesting that Notch pathway is involved in EMT. Loss of Notch1 did not influence cell proliferation *in vitro* but reduced migration potential. It was confirmed that Notch signalling interruption leads to reduction of stemness features: decreased drug resistance (A549 but not HCT116), ability to form tumourispheres and tumourigenic potential *in vivo*. ABC-transporter activity in HCT116 could be upregulated by TAZ/YAP signalling pathway and not by Notch. Moreover, reduced Notch1 expression increased the