PO-031 NAA INDUCES ANTITUMORAL EFFECTS IN BXPC3 PANCREATIC CANCER CELL LINE

[1]C Mazzoccoli*, [2]F Agriesti, [3]T Tataranni, [3]V Ruggieri, [4]C Pacelli, [4]N Capitanio, [4]C Piccoli. [1]*IRCCS CROB, Laboratory of Pre-clinical and Translational research, Rionero in Vulture, Italy*; [2]*IRCCS-CROB- Referral Cancer Center of Basilicata, Laboratory of Pre-Clinical and Translational Research-, Rionero in Vulture, Italy*; [3]*IRCCS-CROB- Referral Cancer Center of Basilicata, Laboratory of Pre-clinical and Translational Research, Rionero in Vulture, Italy*; [4]*University of Foggia, Department of Clinical and Experimental Medicine, Foggia, Italy*

10.1136/esmoopen-2018-EACR25.76

Introduction Pancreatic adenocarcinoma is a tumour with poor prognosis. Usually diagnosed at a late stage, the high mortality is linked to resistance to conventional chemotherapy. Combination therapy and targeted therapies proved to be not very effective. Thus, a better understanding of the molecular mechanisms underlying drug resistance in pancreatic cancer could lead to the development of more effective therapeutic strategies.

Material and methods BX-PC3 pancreatic tumour cells were treated with increasing doses of NAA (2,4,8 and 16 mM) for 72 hour and cell viability was assessed by xCELLigence system technology. The gene expression profile induced by NAA treatment in BX-PC3 cells was examined using Real-Time qPCR. Anti-proliferative and differentiating effects of NAA in BX-PC3 treated cells were evaluated by flow cytometric analysis. Acetyl CoA levels after 72 hour NAA treatment was mesured by HPLC/HRMS. The expression of proteins involved in acetylation mechanism were measured by Western Blotting. The metabolic analysis were performed by the Seahorse Bioanalyzer. The effects of NAA in 3D cultures were studied morphologically by inverted microscope.

Results and discussions NAA treatment in BX-PC3 pancreatic tumour cells elicited anti-proliferative and differentiating effects evident with the arrest of proliferation and decreased expression of specific stemness markers such as *cMyc*, *Klf4*, *Lin28* and *Oct4*. Exposure of cells to NAA induced down-regulation of CD133 and CD184 surface markers, arrest of cell cyle at G0/G1 phase, associated to increased levels of *p53*, *p21* and *p27* genes. Moreover, NAA-treated BX-PC3 cells showed decreased levels of the central metabolite Coenzyme A, which correlates with alterations in protein acetylation. In addition, an overall impairment of mitochondrial function was observed following NAA treatment, resulting in a revised feeding of metabolic substrates. Finally, NAA showed a strong effect on tumour spheroid growth, with reduction in colony size.

Conclusion To our knowledge, this is the first study that demonstrates the differentiating effects of NAA treatment in pancreatic tumour cells and its ability to reduce the size of 3D pancreatic carcinoma spheroids.

PO-032 THE KNOCK-DOWN OF FERRITIN HEAVY SUBUNIT INDUCES XENOBIOTIC-RESISTANCE IN K562 CELLS THROUGH THE ACTIVATION OF NF-KB PATHWAY

[1]I Aversa*, [1]R Chirillo, [2]F Biamonte, [2]M Perrone. [1]*Magna Graecia University, Department of Experimental and Clinical Medicine, Catanzaro, Italy*; [2]*University of Magna Graecia, Department of Experimental and Clinical Medicine, Catanzaro, Italy*

10.1136/esmoopen-2018-EACR25.77

Introduction The transcriptional factor NF-κB, composed by five subunits (RelA/p65, c-Rel, RelB, p50, p52), is largely involved in many facets of cellular physiology such as innate and adaptive immunity as well as inflammation. In addition, NF-kB play a central role in cancer cell survival and chemoresistance partly by its implication in cross-talks with redox-regulating proteins. Ferritin is the major iron storage protein; it is composed by a variable assembly of Heavy (FHC) and Light (FLC) subunits. FHC, in particular, has been widely demonstrated to be devoted in iron uptake and release thus controlling the redox homeostasis.

Material and methods K562 erythroleukemia cells were stably silenced for FHC by using the shRNA method. Then FHC reconstitution was achieved by transient transfection of a FHC specific expression vector. ROS were determined by incubating cells with the redox-sensitive probe 2'−7'-DCF. NAC was used to inhibit ROS production. MTT assay was performed to analyse cell viability. Increasing concentrations of Doxorubicin, ranging from 0 to 5 μM, were used to treat K562 cells.

Results and discussions The results of this study highlighted that FHC amounts negatively affect NF-kB activation in K562 cells. FHC silencing was accompanied by an increased expression of the nuclear NF-kB subunit p65, FHC rescue determined nuclear p65 decrease. FHC silencing is responsible for intracellular ROS production and ROS are implicated in NF-kB pathway. To elucidate the relationship between ROS amount and nuclear p65 content, we determined ROS amounts in our *in vitro* model and evaluated p65 nuclear expression after treatment with the ROS scavenger NAC. First, we observed that, as expected, ROS levels increased upon FHC silencing and return to basal levels upon NAC treatment. Interestingly, NAC was also able to decrease nuclear p65 amount in FHC-silenced K562 cells. Considering the effect of NF-kB activated pathway on cell survival, we analysed the effect of FHC silencing-mediated p65 increase in K562 cells upon treatment with increasing doses of Doxorubicin. Cell viability assay highlighted that FHC-silencing was accompanied by an increased resistance to the drug with an IC_{50} about doubled compared to that of the K562 control cells at each the time points. This resistance of FHC-silenced cells was reverted upon NF-kB inhibitor transfection.

Conclusion FHC silencing induced NF-kB activation in K562 cells through the modulation of intracellular ROS content. This regulatory axis can be used to modulate K562 chemoresistance.

PO-033 ANTICANCER ACTIVITY OF NATURAL HIGH MOBILITY GROUP BOX1 INHIBITORS IN COLORECTAL CANCER CELLS

[1]A Khan*, [2]MY Wani, [1]K Subramanian, [1]J Kandhavelu, [1]P Ruff, [1]C Penny. [1]*University of the Witwatersrand, Internal Medicine, Johannesburg, South Africa*; [2]*University of Jeddah, Chemistry Department, Jeddah, Saudi Arabia*

10.1136/esmoopen-2018-EACR25.78

Introduction Despite being significant advances in colon cancer treatments, recurrence and chemoresistance remain an important challenge in the treatment of patients. During the process of autophagy, cancer cells acquire anoikis resistance and escape chemotherapy. The High Mobility Group Box 1 (HMGB1) molecule is a key mediator of autophagy and can be exploited to develop effective targeted anticancer therapies. Glycyrrhizin and quercetin are natural inhibitor of high mobility box 1 protein (HMGB1).We studied the anticancer activity of glycyrrhizic acid and quercetin in SW480, HT29 and DLD1