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01.013 **Nicotinamide Riboside Induced Energy Stress in BEAS-2B Cells.** Marzola EL[1], Cordeiro EWF[1] , Maekawa RS[1] , Santos MR[1] , Massafera MP2 , Di Mascio P[2] , Medeiros MHG[2], Ronsein GE[2], Loureiro APM[1] [1]FCF-USP São Paulo, Dpt of Clinical and Toxicological Analyses; [2]IQ-USP, Dpt of Biochemistry, São Paulo

Introduction: Nicotinamide adenine dinucleotide (NAD +) regulates molecular pathways important for cell survival, differentiation, growth, and death. Nicotinamide riboside (NR) is a promising NAD + precursor. Numerous studies point to the benefits of NR supplementation. *In vitro* cell culture can help to understand the mechanisms by which NR induces the beneficial effects. **Methods:** Survival and growth of monolayer-cultured BEAS-2B cells were assessed by crystal violet dye assay. Cells were exposed daily to 1 - 50 µM NR for 192 h. Viability analysis of BEAS-2B spheroid cells (3D cell culture) was performed using a BD FACSCanto II TM flow cytometer (BD, Biosciences). Nucleotide and metabolites quantification was performed using a Shimadzu UFLC system coupled to an ESI-Ion Trap mass spectrometer (amaZon Speed, Bruker Daltonics), or an Agilent 1200 series HPLC interfaced with a Linear Quadrupole Ion-Trap mass spectrometer (4000QTRAP, Applied Biosystems/MDS Sciex Instruments). Shotgun proteomics was performed on a Nano EASY-nLC 1200 coupled to an Orbitrap Fusion Lumos mass spectrometer (Thermo Fisher Scientific). **Results:** Daily exposure of the monolayer-cultured BEAS-2B cells to NR over 192 h led to cytotoxicity at concentrations above 1 µM. At 1 µM, NR induced energy stress and decreased mitochondrial respiration. Apoptosis was induced after 168 h of BEAS-2B spheroids exposure to NR (1 – 50 µM NR). The ATP/ADP ratios of the spheroids exposed to 10 and 50 µM NR for 96 and 168 h were decreased, characterizing energy stress. For proteomic analysis, monolayer cultured BEAS-2B cells were exposed daily to 1 µM NR for 144 h. Among 1911 proteins detected in the control and NR groups, 77 were differentially abundant. The increased protein abundance in the NR group pointed to glycolysis stimulation for ATP synthesis, increased generation and use of cytosolic NADH for energy production, increased antioxidant activity and fatty acid synthesis, modulation of amino acid metabolism, and increased ribose and deoxyribose phosphate generation for nucleotide and nucleic acid synthesis. In parallel, NR exposure led to decreased abundance of proteins involved in the folding, aggregation, or turnover of proteins, in L-serine biosynthesis, mRNA transcription, posttranscriptional gene silencing, double-strand break repair, and regulation of protein phosphorylation. Among the twelve downregulated proteins found after pathway enrichment analysis, eleven are shown to be upregulated in cancer. **Conclusion:** We can infer that BEAS-2B cells responded to the decrease in mitochondrial respiration and energy stress induced by NR with an increase in glycolysis to generate ATP. Downregulation of proteins involved in cancer development may have a role in slowing the growth and inducing apoptosis of the NR exposed cells, despite the shift to glycolytic metabolism. The role of a mild energy stress induced by NR in its beneficial effects deserves further investigation. On the other hand, strategies to increase the bioavailability of NR require attention to toxic effects that may arise. **Financial Support**: CAPES, FAPESP, CNPq, PRP USP, Redoxoma (INCT, NAP, CEPID). Acknowledgment: The Mass Spectrometry Resource at IQUSP and the Multi-user Mass Spectrometry Facility at FCFUSP (LMEM-FCFUSP) for the availability of the mass spectrometers; Prof. Sandro R. Almeida (FCFUSP) for providing the flow cytometer and Renata C. Albuquerque (FCFUSP) for the technical support in the flow cytometry analyses; Prof. Alicia J. Kowaltowski (IQUSP) for providing the Seahorse Analyzer and Camille Caldeira (IQUSP) for the technical support in the Seahorse analyses; and Prof. Fekadu Kassie (University of Minnesota, Minneapolis, MN, USA) for providing the cell line.