TARGETED METABOLOMIC ANALYSIS OF HUMAN BRONCHIAL EPITHELIAL CELL (BEAS-2B) SPHEROIDS PRE-EXPOSED TO A NAD^+ PRECURSORAND SUBMITTED TO BENZO[A]PYRENE-INDUCED CARCINOGENIC TRANSFORMATION

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INTRODUCTION: Lung cancer is one of the most prevalent and lethal neoplasms worldwide, strongly associated with smoking and exposure to air pollution, both rich in polycyclic aromatic hydrocarbons (PAHs). Among PAHs, benzo[a]pyrene (B[a]P) is a potent carcinogen known to induce metabolic reprogramming. Meanwhile, NAD+ depletion may foster cancer development, giving rise to the hypothesis that restoring NAD+ levels may be a cancer chemoprevention strategy. Among NAD+ precursors, nicotinamide riboside (NR) stands out for its efficient profile. OBJECTIVES: To investigate whether pre-exposure to NR can protect human bronchial epithelial cell (BEAS-2B) spheroids against B[a]P-induced dysfunctions. MATERIALS AND METHODS: BEAS-2B cells were seeded in monolayer culture (2×10^4 cells/well) in 96-well plates and cultured in LHC-9 medium supplemented with NR (1 µM) for 168 h, with daily exposure replacement. After this pre-treatment, spheroids were formed on 1.5% agarose-coated plates (2×10^4 cells/well). Following five days of spheroid formation, the structures were exposed daily to B[a]P (2 µM) for 168 h. Metabolic profiling was performed using ion trap mass spectrometry coupled with HPLC, while spheroid morphology was assessed by optical microscopy and analyzed using Cell Profiler software. RESULTS AND CONCLUSION: Spheroids exposed to B[a]P exhibited intercellular disaggregation. Pre-exposure of cells to NR mitigated this deleterious effect, preserving the compact spheroidal architecture observed in the control group. However, spheroids exposed to B[a]P exhibited energetic stress, as indicated by the ATP/ADP ratio, regardless of pre-exposure to NR. Despite the persistence of energetic stress, pre-exposure of cells to NR protected against decreases in glutamate, glutamine, NAD+, NADP+, nicotinamide, nicotinamide mononucleotide, citrate, and argininosuccinate in spheroids exposed to B[a]P for 48 h. NR proved to be a potent metabolic modulator, attenuating metabolic reprogramming and promoting healthy growth of B[a]P exposed spheroids. The effects induced by NR in the monolayer culture persisted for several days after the end of the exposure, since spheroids themselves were not treated with NR. The knowledge of the persistent beneficial effect of low concentrations of NR on cellular metabolism may help in establishing doses for cancer chemoprevention.

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