POSTER

MITOCHONDRIAL DNA COPY NUMBER VARIATION AND ROS PRODUCTION IN ZEBRAFISH AND HUMAN CELL LINES EXPOSED TO HEAVY METALS

G.S. Calogero, M. Giuga, A. Mazzurco, G. Manganaro, V. Ferrito, A.M. Pappalardo.

[1]*Dept. of Biological, Geological and Environmental Sciences, University of Catania, Italy*

Mitochondrial DNA is highly susceptible to stress-induced changes, due to the lack of protective histones but especially because it is in the proximity of reactive oxygen species (ROS) generation sites. This feature has made mitochondrial DNA copy number (mtDNAcn) variation a potential biomarker of exposure to environmental contaminants and other stress-related disorders. The aim of this research was to conduct a comparative analysis using different types of cell lines: ZFL, zebrafish hepatocytes, NCTC (clone 2544) and A375, both of which are human cell lines. Cells were exposed to various concentrations of heavy metals (cadmium, arsenic, lead) and subsequently mtDNAcn variation was assessed. Here, the relative mtDNAcn was evaluated using real-time PCR, amplifying a fragment of mitochondrial and nuclear genes for both species (Danio rerio and Homo sapiens), while DCFH-DA assay was used to quantify ROS production. With regard to ROS production, this was found to be closely correlated with metal toxicity and the resulting level of cell mortality following exposure. The highest levels of ROS were recorded in ZFL cells, which, however, exhibited the lowest levels of mortality. Regarding variations in mtDNAcn, different responses were observed. Zebrafish cells showed a decrease in mtDNAcn after 24 hours of exposure, followed by a moderate increase after 48 hours and a further decrease after 72 hours. Cells from the two human cell lines showed, in almost all cases, a gradual increase in mtDNAcn during the exposure period. These results highlight how heavy metals can alter the mitochondrial genome and endogenous production of ROS, and how using these biomarkers in in vitro studies could help us understand the cellular changes that disrupt the organism's homeostasis.