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Effect of s on mitochondrial DNA copy number

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Radiation and oxidative stress contribute substantially to multiple pathological processes in the nervous system. Mitochondrial DNA (mtDA) is particularly susceptible to damage due to the lack of protective histones. Aberrations in the mitochondrial DNA (mtDNA) copy number has been reported in broad range of primary human cancers. Alteration of mtDNA content may be a pivotal factor in eliciting mitochondrial deficient activities and contributing the cancer pathogenesis. Therefore, the present study aims at investigating mtDNA copy number changes in human astrocytes after treatment with various dosages of radiation and oxidative-stress inducing chemical sodium dichromate. Cells were exposed to proton radiation (0.5Gy, 1.5Gy, and 3Gy) and X-rays (0.5Gy, 1.5Gy) at Willis-Knighted Cancer Center, Shreveport, LA. To induce oxidative stress, the astrocytes were treated with 10mM NaCr₂ and 50mM NaCr₂ for 16 hours. Following radiation treatment, the cells were incubated for 24 hours in humidity incubator. The total DNA was purified and the quality and quantity of the nucleic acid was assessed using NanoDrop 2000c instrument. Changes in mtDNA copy number were determined using real-time qualitative PCR (qPCR) couple with high-resolution melt analysis. Two pairs of primers were used in the two steps of the relative quantification of mtDNA content. One primer pair was used for the amplification of the ATP synthase(ATP) gene in mtDNA. Another primer pair was used for the amplification of the nuclear beta-2- microglobulin (B2M) gene. Baseline levels of mtDNA numbers were obtained using nontreated cell line. The relative quantification of mtDNA was determined based on the difference of the cycle threshold values (Ct) between the two genes. The results from this study showed that exposure to radiation and oxidative-stress inducing chemicals is associated with increase in mtDNA copy number that is indicative of stress response to mitochondria dysfunction.