POTENTIAL ROLE OF P53 PROTEIN IN CELL DEATH INDUCED BY PHOTODYNAMIC THERAPY IN HUMAN CELLS

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Photodynamic Therapy (PDT) involves the activation of photosensitizers (PS) by visible light in the presence of molecular oxygen which results in formation of reactive oxygen species (ROS). PDT leads to cell death with morphological features of necrosis, apoptosis and autophagic cell death. The tumor suppressor protein p53 modulates both apoptosis and autophagy, in addition to several other cellular processes. This study aims to verify if p53 modulates cell death in response to PDT. We examined the effects of PDT on HEK293T control and p53 knockdown (KD) cells using the PS 1,9-dimethylmethylene blue (DMMB) photoactivated at 633 nm 11J/cm². In a previous study from our laboratory DMMB was reported to be accumulated and induce mitochondrial and lysosomal damage. We measured mitochondrial DNA (mtDNA) integrity of by a quantitative PCR-based assay. We investigated the PS phototoxicity by clonogenic assay. Acridine orange and propidium iodide staining were used for detection of acidic compartments and membrane-compromised cells by fluorescence microscopy, respectively. As expected, PS exhibited high phototoxicity with minimal dark toxicity. Significant mtDNA damage was observed immediately after irradiation. The viability curves indicated that cells expressing low levels of p53 were more resistant to the treatment than control cells. Furthermore, the treatment resulted in intense cytoplasmic vacuolization with no morphologic evidence of either apoptosis or necrosis. The accumulation of acidic compartments seemed to be more expressive in the control cells. Our results indicate that autophagy clearly contribute to cell death in response to DMMB photosensitization, likely due to mtDNA damage. The different responses between the two cell lines are consistent with our hypothesis that p53 protein may play a cytoprotective role against photoinduced damage. We are grateful for the support provided by CAPES and FAPESP.

Key Words: cell death, photodynamic therapy, p53 protein.