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Cold atmospheric plasma selectively affects Retinoblastoma viability *In Vitro*

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Retinoblastoma is responsible for over 3% of all childhood malignancies in the United States. Treatments include chemotherapy followed by enucleating the eye. However, the risk of remnant tumor stem cells and progression of the disease is dependent on the chemotherapeutic drugs. More recently cold atmospheric plasma (CAP) has gained importance due to selective ablation of tumor cells shown in in vitro cultured cells from head and neck squamous cell carcinoma, glioblastoma, lung, liver and metastatic breast cancer. We therefore aimed to study the effect of CAP induced cell stress and cell death in retinoblastoma cells (Y79) and retinal pigment epithelial cells (ARPE-19) and compared it with other tumor cell lines in vitro.

ARPE-19 and Y79 cells were treated with cold atmospheric plasma (CAP) using the Canady Helios™ Cold Plasma Scalpel at 0.2W and a time course, as well as a dose dependent study, was carried out. In vitro cultured cells were tested for viability using MTT and trypan blue dye exclusion assays. Mitochondrial cell stress was detected using mitosox for reactive oxygen species (ROS) and reactive nitrogen species (RNS) was detected using a fluorogenic probe. Both intracellular and extracellular ROS/RNS were also measured. The proportion of ROS positive cells and ROS induced cell death was determined using Zeiss confocal microscopy and spectroscopy.

We report for the first time that CAP induces cell death selectively in all tumor cells studied. Almost 100% cell death was observed in the Y79 cells 48 hours after CAP treatment while the control cells were not affected. These results suggest CAP as a possible and new therapeutic intervention in ocular biology that can replace or reduce the chemotherapeutic drugs for retinoblastoma patients.

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